Mutagenesis by Acrolein-Derived Propanodeoxyguanosine Adducts in Human Cells †

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Mutagenesis by Acrolein-Derived Propanodeoxyguanosine Adducts in Human Cells†

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ABSTRACT: Acrolein, which is widely spread in the environment and is produced by lipid peroxidation in cells, reacts with DNA to form two exocyclic 1,N^2-propanodeoxyguanosine (PdG) adducts. To establish their relative contribution to the acrolein mutagenicity, the genotoxic properties of α-OH-PdG and γ-OH-PdG together with their model DNA adduct, PdG, were studied in human cells. DNA adducts were incorporated site-specifically into a SV40/BK virus origin-based shuttle vector and replicated in xeroderma pigmentosum complementation group A (XPA) cells. Analysis of progeny plasmid revealed that α-OH-PdG and PdG strongly block DNA synthesis and that both adducts induced base substitutions with G → T transversions predominating. Primer extension studies, catalyzed by the 3′→5′ exonuclease-deficient Klenow fragment of *Escherichia coli* pol I, revealed limited extension from the 3′ primer termini opposite these two adducts. In contrast, γ-OH-PdG did not strongly block DNA synthesis or miscode in XPA cells. Primer extension from a dC terminus opposite γ-OH-PdG was much more efficient than that opposite α-OH-PdG or PdG. These results indicate that the minor α-OH-PdG adduct is more genotoxic than the major γ-OH-PdG. Furthermore, experiments using a HeLa whole cell extract indicate that all three DNA adducts are not efficiently removed from DNA by base excision repair.

Acrolein, the simplest member of an α,β-unsaturated aldehyde family, is found widely in the environment (*1*). Acrolein initiates urinary bladder carcinogenesis in rats (*2*) and is mutagenic in bacteria (*3*, *4*) and cultured cells (*5*−*7*). Acrolein reacts with dG residues in DNA to form two pairs of stereoisomeric exocyclic propano adducts (Figure 1): the *R* and *S* isomers of 3*H*-8-hydroxy-3-(β-D-2′-deoxyribofuranosyl)-5,6,7,8-tetrahydropyrido[3,2-*a*]purin-9-one (γ-OH-PdG)[1] and the *R* and *S* isomers of 3*H*-6-hydroxy-3-(β-D-2′-deoxyribofuranosyl)-5,6,7,8-tetrahydropyrido[3,2-*a*]purin-9-one (α-OH-PdG). γ-OH-PdG predominates over α-OH-PdG (*1*, *8*, *9*). These adducts have also been detected in DNA isolated from human and animal tissue (*1*, *10*). Lipid peroxidation is suspected to be the major endogenous source of acrolein (*1*).

The exocyclic ring of PdG adducts prevents normal Watson−Crick base pairing and may result in DNA synthesis block and miscoding during translesion synthesis. As a result, these adducts could contribute to spontaneous mutagenesis in mammalian cells and may play a significant role in aging and cancer. Studies of the γ-OH-PdG adduct unexpectedly revealed a high-fidelity translesion DNA synthesis in *Escherichia coli* (*11*, *12*) and human cells (*13*) and a much weaker blocking effect on DNA synthesis than 1,N^2-(1,3-propano)-2′-deoxyguanosine (PdG) (*14*), a synthetic structural analogue (Figure 1) that has been studied extensively as their model adduct (e.g., refs *15*−*20*). A recent structural study (*21*) has revealed that the exocyclic ring of γ-OH-PdG opens in duplex DNA (Figure 1) when paired to dC, permitting the pairing bases to assume the normal Watson−Crick conformation. This structure, which leaves the adducted moiety lying in the minor groove, is consistent with the marginal miscoding events and the weak blocking effect observed for γ-OH-PdG.

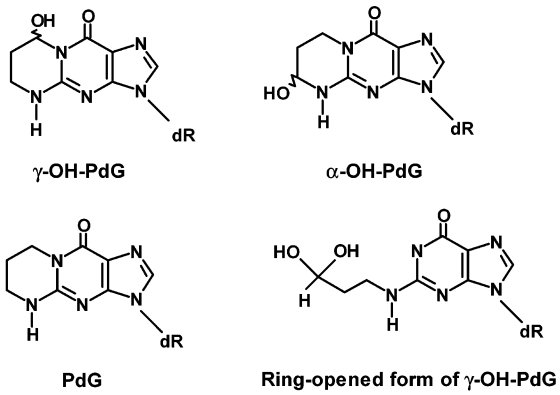

FIGURE 1: Structures of acrolein-derived exocyclic dG adducts and their model adduct, PdG.

Acrolein induces G → T and G → A substitutions when plasmid DNA modified randomly with this chemical is

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[1] Abbreviations: α-OH-PdG, *S* and *R* isomers of 3*H*-6-hydroxy-3-(β-D-2′-deoxyribofuranosyl)-5,6,7,8-tetrahydropyrido[3,2-*a*]purin-9-one); *bsd*, blasticidin S-resistance gene; ϵdA, 1,N^6-ethenodeoxyadenosine; γ-OH-PdG, *S* and *R* isomers of 3*H*-8-hydroxy-3-(β-D-2′-deoxyribofuranosyl)-5,6,7,8-tetrahydropyrido[3,2-*a*]purin-9-one; PdG, 1,N^2-(1,3-propano)-2′-deoxyguanosine; XPA, xeroderma pigmentosum complementation group A.

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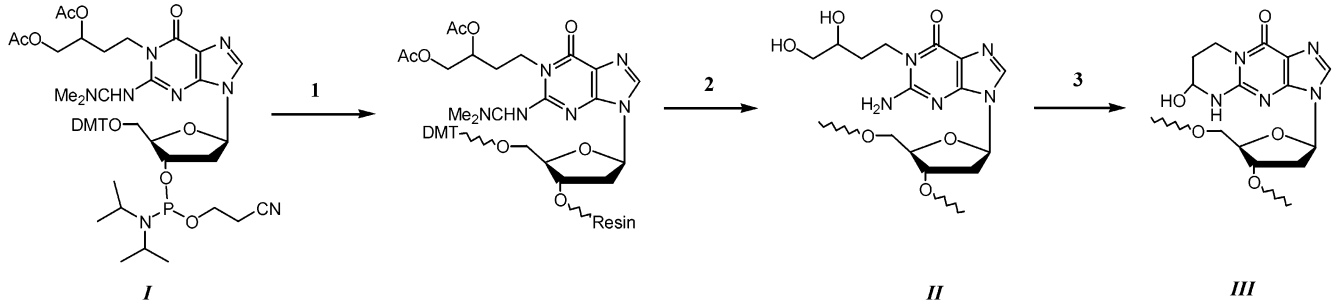

FIGURE 2: Incorporation of α-OH-PdG into oligonucleotide. (1) The dimethoxytrityl- (DMT-) capped phosphoramidite of 1-(3,4-diacetylbutyl)-N^2-(dimethylaminomethylene)-dG (**I**) is used in automated DNA synthesis to incorporate the precursor of α-OH-PdG. (2) The synthesized oligonucleotide is deprotected and cleaved from a glass support in 28% ammonia. The DMT cap is removed by 80% acetic acid. (3) The oligonucleotide containing the 1-(3,4-dihydroxybutyl)-dG moiety (**II**) is treated with 100 mM sodium periodate in 0.5 M sodium acetate buffer, pH 6.0, for 5 min at room temperature to generate α-OH-PdG (**III**).

replicated in human cells (*7*). The present study was undertaken to determine the relative contribution of the minor adduct, α-OH-PdG, to the genotoxicity of acrolein. Using a site-specifically placed single adduct, we compared in parallel the genotoxicity of α-OH-PdG, γ-OH-PdG, and PdG in human cells and also by in vitro primer extension studies.

MATERIALS AND METHODS

Oligonucleotides. A 13-mer, 5′ CTCCTCXATACCT, in which X represents a modified base, was used for studies in human cells. General procedures for the synthesis, purification, and characterization of site-specifically modified oligonucleotides were described previously (*11*, *14*, *22*). α-OH-PdG was incorporated into oligonucleotides according to the method of Huang et al. (submitted for publication), and the strategy is shown schematically in Figure 2. In brief, 1-(3,4-diacetylbutyl)-N^2-(dimethylaminomethylene)-dG was incorporated by automated DNA synthesis using standard phosphoramidite chemistry (Figure 2, step 1). The coupling time for the modified base was 5 min, yielding a coupling efficiency of 99.0%. Dimethoxytrityl-capped oligonucleotides were purified on a Luna 5 μm Phenyl-Hexyl (250 × 10 mm) column (Phenomenex, Torrance, CA) at a flow rate of 4 mL/min. A gradient of 16−36% acetonitrile over 35 min in 0.1 M triethylammonium acetate buffer, pH 6.8, was used; dimethoxytrityl-decapped oligonucleotides were purified with a gradient of 0−20% acetonitrile over 40 min. α-OH-PdG was generated postsynthetically by treating oligonucleotides containing the 1-(3,4-dihydroxybutyl)-dG moiety with 100 mM sodium periodate in 0.5 M sodium acetate buffer, pH 6.0, for 5 min at room temperature (*22*) (Figure 2, step 3). Final HPLC purification was performed under conditions described for the purification of decapped oligonucleotides. A single peak was observed at a retention time of ∼41 min, and the mass of this fraction was determined by electrospray ionization mass spectrometry. The observed *m*/*z* value for the 13-mer was 3900.25 ± 0.73; the theoretical value is 3901.5, and that for the precursor 13-mer is 3933.5. The oligonucleotide was subjected to further purification in denaturing 20% polyacrylamide gel and formed a single band in the gel following purification. The purified oligomer was treated with nuclease P1, snake venom phosphodiesterase, and bacterial alkaline phosphatase (*24*). HPLC analysis of the nucleoside mixture confirmed the presence of α-OH-PdG by comparing with authentic α-OH-PdG, which was synthesized as described previously (*23*).

For in vitro primer extension studies, 28-mer templates, 5′ CTG**CTCCTCXATACCT**ACACGCTAGAAC (the sequence in bold corresponds to that used in studies in human cells), and primers, 5′ GTTCTAGCGTGTAGGTATN (primer 1) and 5′ GTTCTAGCGTGTAGGTATCG (primer 2), were used; X represents dG, α-OH-PdG, γ-OH-PdG, or PdG and N in primer 1 is dA, dG, dC, or dT. For studies of base excision repair, 25-mers, 5′ GACCTG**CTCCTCXATAC-CT**CCACGG and its complementary oligonucleotide, were used; X represents α-OH-PdG, γ-OH-PdG, PdG, or 1,N^6-ethenodeoxyadenosine (εdA). All modified and unmodified oligonucleotides were purified by electrophoresis in denaturing 20% polyacrylamide gel. All purified oligonucleotides migrated as a single band in the gel.

Cell Lines. SV40-transformed xeroderma pigmentosum complementation group A cells (XPA) (GM04429) were obtained from Coriell Institute (Camden, NJ). HeLa cells were obtained from the Stony Brook tissue culture facility. Both cell lines were demonstrated to be free of mycoplasma. Cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37 °C in 5% CO_2.

Base Excision Repair Assay. Whole cell extracts were prepared from HeLa cells according to a published method (*25*). 5′-^{32}P-labeled modified 25-mer and its complementary 25-mer were hybridized by heating at 70 °C for 5 min followed by slow cooling and used as a substrate for a base excision reaction. The reaction mixture (50 μL) containing 20 mM Tris-HCl, pH 8, 1 mM EDTA, 1 mM dithiothreitol, 50 mM KCl, 1.5 pmol of DNA substrate, and 60 μg of crude cell extract protein was incubated at 37 °C for 30 min followed by treatment with 20 μg of proteinase K at 37 °C for 1 h in the presence of 0.5% SDS. The mixture was extracted with phenol/chloroform and the DNA precipitated with ethanol. DNA was dissolved in 2 μL of H_2O, 10 μL of 10 mM NaOH−95% formamide was added, and then the sample was heated at 90 °C for 3 min. Following electrophoresis in denaturing 20% polyacrylamide gel, radioactive bands were detected by a PhosphorImager (Molecular Dynamics).

Genotoxic Studies in Human Cells. The shuttle vector, pBTE, has been described (*13*). This vector is stably maintained in human cells and confers blasticidin S resistance to host human and *E. coli* cells. Expression of the blasticidin

Table 1: Coding Events Induced by Exocyclic Propano-dG Adducts in XPA Cells

no. of blasticidin-resistant colonies/ μg of construct	DNA adduct	no. of plasmid (DNA adduct → G, T, A, or C)				miscoding frequency (%)
		G	T	A	C	
1.7×10^5	dG	236	0	0	0	<0.43
1.1×10^5	γ-OH-PdG	278	0	0	0	<0.36
6.6×10^4	α-OH-PdG[a]	242	24	4	4	12.5
5.2×10^4	α-OH-PdG[a]	242	17	4	7	10.4
3×10^4, 4×10^4, 10×10^4	PdG[b]	454	17	2	3	4.6

[a] The results of two independent experiments are shown separately. [b] Data from three independent experiments were combined since the results were similar. The numbers of blasticidin-resistant cells per transfection are shown individually in the left-most column.

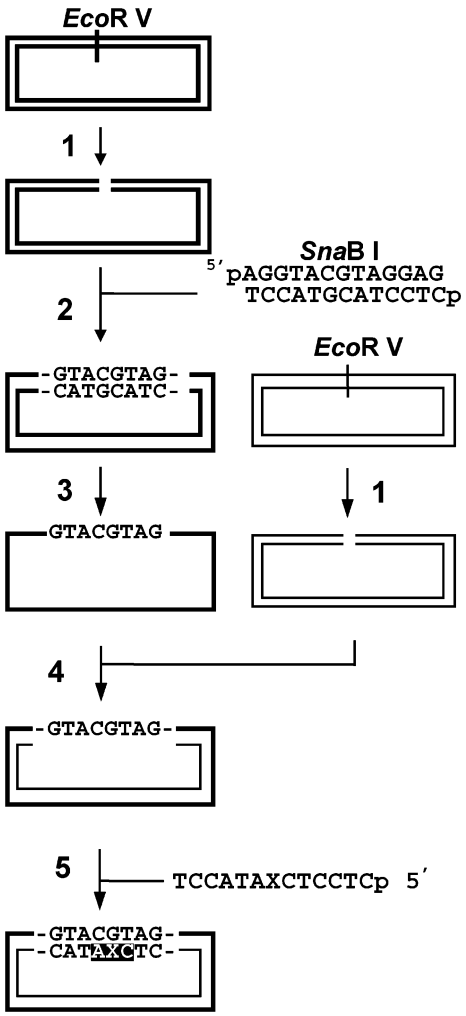

FIGURE 3: Construction of plasmid DNA containing a single adduct. (1) *Eco*RV digestion of pBTE. (2) Ligation of a duplex 13-mer containing a *Sna*BI site (5′ TACGTA). (3) Preparation of single-stranded DNA. (4) Preparation of gapped DNA. (5) Ligation of the modified 13-mer, in which X represents a DNA adduct. Note three contiguous base mismatches (highlighted) in the *Sna*BI site.

resistance gene (*bsd*) is driven by the SV40 early promoter in human cells and the EM7 bacterial promoter in *E. coli*.

The construction of double-stranded plasmid containing a site-specific DNA adduct has been described previously (*26*) and is shown schematically in Figure 3. The adduct was incorporated into the leading strand template. An important feature of this construct is that the adduct is located opposite a unique *Sna*BI site (5′ TACGTA) with a mismatch on both sides of the adduct (Figure 3); thus, only the unmodified complementary strand contains the *Sna*BI site. Progeny plasmids derived from the unmodified strand or from excision repair events are sensitive to *Sna*BI digestion while those derived from translesion synthesis are not. Hence, progeny derived from translesion synthesis are collected selectively by digesting with *Sna*BI prior to transformation of *E. coli* for fidelity analysis.

Human XPA cells were plated at 1×10^6 cells/25 cm^2 flask, cultured overnight, and then transfected overnight, using FuGENE 6 transfection reagent (Roche), with 1 μg of a DNA construct. Cells were detached by treating with trypsin−EDTA. Ninety-nine percent of the transfected cells were plated in a 150 cm^2 flask and 1% seeded on a 10 cm plate. The following day blasticidin S (Invitrogen) was added to the culture medium at 5 μg/mL. Resistant cells were collected after 5 or 6 days. Progeny plasmid was purified by the method of Hirt (*27*) and treated with *Dpn*I (2 units) for 1 h to remove residual input DNA. The plate containing 1% of the cells was incubated in the presence of blasticidin S for 8−10 days and stained with Giemsa to count resistant colonies. The results are included in Table 1.

To determine the efficiency of translesion DNA synthesis, the *Dpn*I-treated plasmid was used to transform *E. coli*. To determine coding events at the site of a DNA adduct, *Dpn*I-treated plasmid was digested with *Sna*BI prior to transformation. One-tenth to one-fifth of the recovered plasmid was electroporated into *E. coli* DH10B ElectroMAX (25 μL) (Invitrogen), after which 975 μL of YT (2×) medium (*11*) was added, and the bacteria were cultured for 40 min at 37 °C. Portions of the transformation mixture were plated onto YT (1×) plates containing blasticidin S (50 μg/mL) and ampicillin (100 μg/mL). After overnight incubation, *E. coli* transformants were subjected to differential oligonucleotide hybridization (*28*, *29*) to analyze for mutations in the adducted region. This method permits detection of specific sequences using oligonucleotide probes. G, T, A, C, and D probes (Figure 4) detect coding specificity at the site of the DNA adduct. The S probe hybridizes to the complementary *Sna*BI-containing strand. L and R probes confirm the presence of the 13-mer insert. Automated DNA sequence analysis was performed as necessary.

In Vitro Primer Extension Studies. Exonuclease-deficient Klenow fragment was purified as described (*30*). In vitro primer extension reactions were conducted as described earlier (*11*) with a slight modification. In brief, 5′-^{32}P-labeled primer and 28-mer template were mixed at a molar ratio of 1:2, heated in an annealing buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 100 mM NaCl) at 70 °C for 5 min, annealed by slow cooling, and then kept at 37 °C for 3 h. The reaction mixture (10 μL) contained 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 100 nM primer/template, 100 μM dNTP, and 0−33.8 nM Klenow fragment. The reaction was

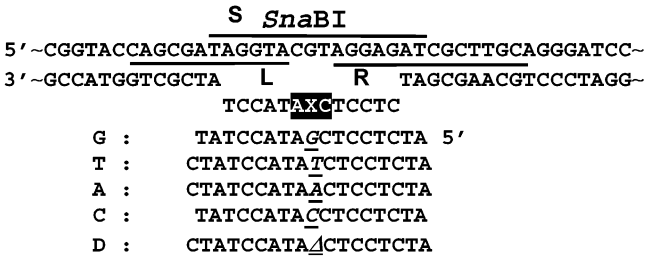

FIGURE 4: Nucleotide sequence surrounding a DNA adduct and probes used for oligonucleotide hybridization. L and R probes (underlined) were used to identify progeny containing the 13-mer insert. Probe S (overscored) hybridizes to the unmodified complementary strand. Probes G, T, A, C, and D were used to determine the base at the site of the DNA adduct (X). Three mismatches are highlighted. The unique *Sna*BI restriction site is located opposite a DNA adduct.

conducted at room temperature for 30 min and stopped by addition of 10 μL of formamide dye mixture (90% formamide, 0.1% xylene cyanol, 0.1% bromophenol blue, 20 mM EDTA). Samples were heated at 95 °C for 5 min, and aliquots (2.5 μL) were subjected to electrophoresis in denaturing 20% polyacrylamide gel. The intensity of radioactive bands was quantified by a PhosphorImager and ImageQuant software (Molecular Dynamics).

RESULTS

To determine the genotoxic properties of the two naturally occurring PdG adducts derived from acrolein, we synthesized oligonucleotides containing α-OH-PdG, γ-OH-PdG, and the model adduct PdG. These modified oligonucleotides were used to conduct in vitro DNA repair and primer extension studies and in vivo genotoxic studies in human cells.

Base Excision Repair of α- *and* γ*-OH-PdG and PdG.* To examine the removal of α- and γ-OH-PdG by base excision repair, a radiolabeled duplex 25-mer containing a site-specifically placed adduct was treated with a HeLa cell extract (Figure 5). When a DNA adduct is excised by DNA glycosylase, a radioactive 12-mer is generated following treatment of the reaction product with NaOH, which cleaves the modified 25-mer at an abasic site generated by DNA glycosylase. Incubation of duplex 25-mer containing α-OH-PdG, γ-OH-PdG, or PdG with the extract failed to generate 12-mer (Figure 5, lane 2). Similarly, DNA glycosylase activity was not detected when the 25-mer containing α-OH-PdG was paired with a complementary strand containing the same three mismatches as in the plasmid construct (lane 3). When duplex 25-mer containing ϵdA was used as a substrate, the treatment generated a radioactive band comigrating with standard 12-mer (5′ ^{32}P-GACCTGCTCCTC) (lane M), reflecting excision of ϵdA by a DNA glycosylase. These results suggest that base excision repair does not efficiently remove α-OH-PdG, γ-OH-PdG, or PdG from DNA in human cells.

Lanes 1 and 2 for γ-OH-PdG show a band that migrates much more slowly than the 25-mer. This band may represent an interstrand cross-linked product since DNA containing γ-OH-PdG in a 5′-CpG sequence context has been shown to form an interstrand cross-link with dG in the complementary strand (*31*).

Blocking of DNA Synthesis in Human Cells. PdG and γ-OH-PdG are shown to be substrates for nucleotide excision repair (*11*, *16*). As noted above, the three adducts are not removed by base excision repair. Therefore, it is expected that these adducts are not removed in XPA cells and that the ratio of progeny derived from each strand should represent the relative degree of DNA synthesis block by any given adduct. In our construct, a DNA adduct was embedded in a mismatched region, serving as a strand-specific tag. When unmodified (dG), mismatch-containing plasmid was replicated in XPA cells, the ratio of progeny plasmid derived from each strand, as detected by the S and G probes, respectively, was approximately 50:50 (Figure 6), indicating equal replication of the two strands. When modified, mismatch-containing plasmid was used, the ratio favored progeny derived from the unmodified strand, indicating that DNA synthesis is blocked by the adduct. Fractions (average of two experiments) of progeny derived from the modified strand were 52%, 40%, 9%, and 11% for dG, γ-OH-PdG, α-OH-PdG, and PdG, respectively, demonstrating that γ-OH-PdG is much less blocking than α-OH-PdG and PdG. On the basis of these results, translesion synthesis efficiencies

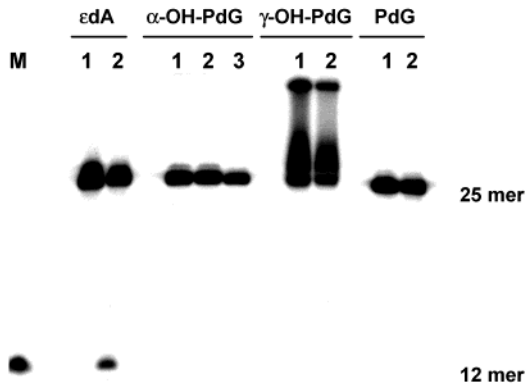

FIGURE 5: Base excision of α-OH-PdG, γ-OH-PdG, PdG and ϵdA with HeLa cell extract. A 5′-^{32}P-labeled modified 25-mer was annealed to the complementary 25-mer and then incubated with HeLa whole cell extract as described in the text. Oligonucleotides were precipitated with ethanol following proteinase K treatment and phenol extraction, treated with 10 mM NaOH−95% formamide at 90 °C for 3 min, and subjected to electrophoresis in denaturing 20% polyacrylamide gel. Radioactive bands were detected by a PhosphorImager. Lanes: M, 12-mer marker; 1, duplex 25-mer without cell extract treatment; 2, duplex 25-mer with cell extract treatment; 3, same as lane 2 but the duplex contained three base mismatches as shown in Figure 3.

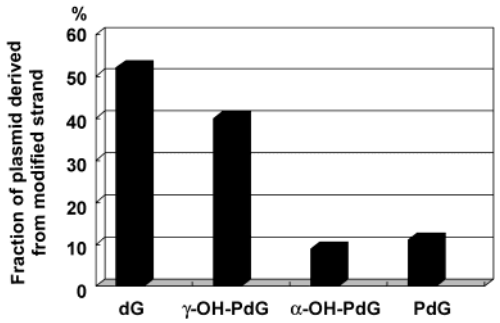

FIGURE 6: Translesion synthesis efficiency in human XPA cells. Progeny plasmid was recovered from transfected cells, digested with *Dpn*I, and used to transform *E. coli*. *E. coli* transformants were hybridized with probes shown in Figure 4 to determine the strand from which progeny was generated. The results are averages of two independent transfection experiments. The ordinate (*Y*-axis) shows the percent of progeny derived from the modified strand.

calculated for the γ-OH-PdG, α-OH-PdG, and PdG adducts are 77%, 17%, and 21%, respectively.

Miscoding Events in Human Cells. Fidelity of translesion synthesis was determined in XPA cells. Recovered plasmid was digested with *Dpn*I and *Sna*BI prior to transformation of *E. coli.* Transformants were selected on blasticidin S and ampicillin-containing plates and hybridized to G, T, A, C, and D probes to establish which base had been incorporated opposite the adduct. γ-OH-PdG coded exclusively for dG in XPA cells: the miscoding frequency was <0.36% (Table 1), consistent with previous results (*11*−*13*). The model adduct, PdG, was associated with a miscoding frequency of 4.6% (average of three experiments) with PdG → T being the dominant miscoding event. α-OH-PdG miscoded at frequencies of 10.4% and 12.5% in two independent experiments with α-OH-PdG → T being predominant. The miscoding frequency of α-OH-PdG was approximately 2-fold higher than for PdG. G → A and G → C base substitutions were also observed for both adducts.

Primer Extension Studies. Having shown that α-OH-PdG and PdG are more blocking and miscoding than γ-OH-PdG, the primer extension approach was employed to further characterize the blocking effects of these adducts. Since incorporation of dCMP is the major translesional synthesis event in human cells for all adducts, this study focused on extension from a dCMP terminus. We first compared primer extension from a 3′ dC terminus (primer 1) located opposite a DNA adduct (Figure 7A). This experiment, using varying amounts of Klenow enzyme, showed that primer extension was most efficient on the γ-OH-PdG-containing 28-mer template; fully extended primer accounted for 57%, 8%, and 5% with the γ-OH-PdG, α-OH-PdG, and PdG templates, respectively, when 33.8 nM enzyme was used. A 29-mer product also was produced by the terminal deoxynucleotidyl transferase activity of Klenow enzyme.

We also compared efficiencies of extension from each of the four dNMP termini using 2.11 nM enzyme (Figure 7B). This experiment showed that the primer with dC at the 3′ terminus was more readily extended on the 28-mer template containing γ-OH-PdG while extension from the comparable dA, dG, and dT termini was inefficient. For the α-OH-PdG-containing template, extension from the dT terminus was more efficient than from dA and dC termini. The PdG-containing template showed a similar tendency with α-OH-PdG with a greatly reduced efficiency of extension. When primer 2 was used, extension from the dG terminus was relatively efficient for all modified templates (Figure 7C). Primer extension on the γ-OH-PdG-containing template was most efficient with full-length products observed at the lowest concentration (0.13 nM) of enzyme. When the α-OH-PdG and PdG templates were used, full-length products were obtained with 2.11 nM enzyme. Overall, these studies indicate that α-OH-PdG and PdG are much more inhibitory than γ-OH-PdG to extension from the 3′ dC terminus located opposite the adduct.

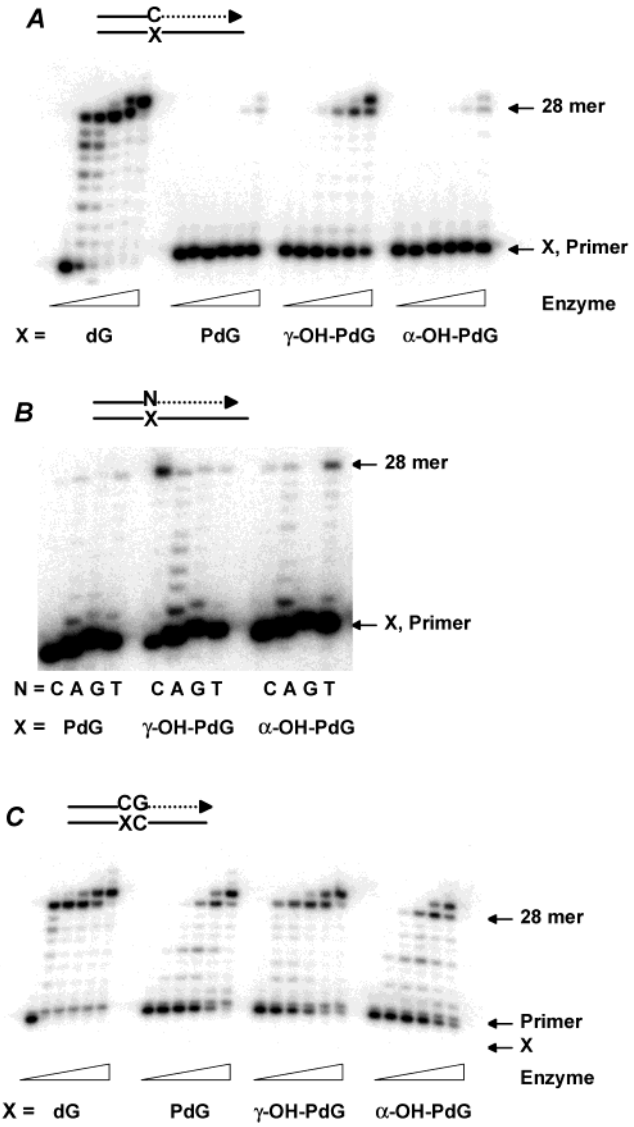

FIGURE 7: In vitro primer extension catalyzed by exonuclease-deficient Klenow fragment. 28-mer templates were primed with primer 1 (A and B) or primer 2 (C). X represents the position of a DNA adduct. Enzyme concentrations were 0, 0.13, 0.53, 2.11, 8.45, and 33.8 nM (from left to right) for each adduct in (A) and (C). The enzyme concentration was 2.11 nM for all lanes in (B). The annealing reaction was conducted by slow cooling following heating at 70 °C for 5 min and then kept at 37 °C for 3 h. The extension reaction was performed for 30 min at room temperature in a volume of 10 μL. A portion was subjected to electrophoresis in denaturing 20% polyacrylamide gel.

DISCUSSION

In this study, we have compared the genotoxicity of the authentic acrolein-derived major and minor dG adducts with that of the model adduct, PdG. In human cells, the minor adduct, α-OH-PdG, and PdG were much more blocking and miscoding than the major adduct, γ-OH-PdG. Miscoding events were not detected with γ-OH-PdG in XPA cells (Table 1), consistent with previous results (*11*−*13*). α-OH-PdG miscodes more frequently than PdG, inducing base substitutions with G → T transversions being predominant. In a recent study with plasmid modified randomly with acrolein, G·C → T·A transversions were most commonly observed followed by G·C → A·T transitions (*7*). The results of the present study suggest that the minor α-OH-PdG adduct contributes significantly to the mutagenesis by acrolein. Acrolein also forms intra- and interstrand dG-dG cross-links (*7*, *31*); such lesions may also contribute to the mutagenicity.

The exocyclic ring of γ-OH-PdG opens when paired to dC, enabling this adduct to form a conventional Watson−Crick base pair (*21*). The acrolein-derived moiety, linked

through the N^2 position of dG, lies in the minor groove (*21*). This structure may explain the efficient (Figure 6) and accurate (Table 1) translesion DNA synthesis observed for γ-OH-PdG as well as the relatively facile extension from a dC terminus in primer extension in vitro (Figure 7). In the case of the malondialdehyde-derived exocyclic propeno-dG adduct, ring opening is thought to be catalyzed by dC (*32*). Since γ-OH-PdG paired to dG in our mismatched plasmid construct (Figure 3), the ring opening may occur when a DNA polymerase catalyzes the insertion of dCMP opposite the adduct, followed by the formation of a stable Watson−Crick pair. This mechanism may not operate for α-OH-PdG since, when the exocyclic ring of α-OH-PdG opens, the acrolein-derived moiety is linked through the 1 position of dG, preventing the Watson−Crick pairing to dC. Thus, the structural difference between the two adducts may result in different blocking and miscoding properties. As shown in this study, the biological effects of α-OH-PdG are very similar to those of PdG. Therefore, the exocyclic ring of this adduct may remain closed in DNA. A structural study to test this possibility is in progress. However, the mechanism proposed does not explain the high-fidelity translesion synthesis across α-OH-PdG and PdG in human cells. Although miscoding frequencies for both adducts are much higher than for γ-OH-PdG, 88−95% of translesion synthesis is still accurate. The accurate synthesis cannot be mediated by Watson−Crick base pairing and may reflect involvement of the recently discovered translesion-specialized DNA polymerases.

Our in vitro primer extension studies show that extension from the 3′ dC terminus opposite γ-OH-PdG is much more efficient than extension from the same terminus opposite α-OH-PdG or PdG (Figure 7A). This result may be explained by the exocyclic ring-opening mechanism as discussed above. However, efficient and preferential incorporation of dCMP opposite γ-OH-PdG was not observed when mammalian DNA polymerase δ/proliferating cell nuclear antigen or 3′→5′ exonuclease-deficient Klenow fragment was used (*11*, *12*, *33*). Preferential dCMP incorporation may be catalyzed by translesion-specialized DNA polymerases or conducted by DNA polymerase δ in the presence of other accessory factors such as replication protein A and replication factor C. The roles for those DNA polymerases are currently under investigation.

Kanuri et al. reported that γ-OH-PdG miscodes at a frequency of ∼7.5% in simian kidney COS cells (*33*). This frequency is much higher than those recorded in our laboratory (this study and ref *13*). The reason for this apparent discrepancy is presently unclear. However, we observed a similar phenomenon in experiments with ϵdA (*26*, *29*), where the mutation frequency was much higher when this adduct was incorporated into single-stranded DNA and replicated in COS cells than when inserted in double-stranded DNA and replicated in human cells. Differences in DNA repair do not account for the discrepancy since, in both experiments with single- and double-stranded DNA, plasmid derived from translesion synthesis was analyzed to determine fidelity. It might reflect differences in vector (single strand vs double strand), sequence context, and/or host cells used.

Chung et al. (*1*, *8*, *10*) detected acrolein-derived dG adducts in DNA isolated from experimental animals and human tissues at levels ranging between 0.01 and 0.753 μmol/mol of guanines. They also found that oxidative stress, enhanced levels of lipid peroxidation, decreased levels of glutathione, and smoking markedly increase tissue levels of propano-dG adducts (*1*). Although the α-OH-PdG adduct constitutes a minor fraction of the total endogenous DNA damage, it is possible that this adduct contributes to the mutagenic burden at a significant level in human cells under conditions of stress.

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