Comparative Evaluation of the Bioreactivity and Mutagenic Spectra of Acrolein-Derived α-HOPdG and γ-HOPdG Regioisomeric Deoxyguanosine Adducts

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · SEPTEMBER 2003

Impact Factor: 3.53 · DOI: 10.1021/tx034066u · Source: PubMed

6 AUTHORS, INCLUDING:

Irina G Minko
Oregon Health and Science University
34 PUBLICATIONS **1,217** CITATIONS
SEE PROFILE

R. Stephen Lloyd
Oregon Health and Science University
224 PUBLICATIONS **6,848** CITATIONS
SEE PROFILE

Comparative Evaluation of the Bioreactivity and Mutagenic Spectra of Acrolein-Derived α-HOPdG and γ-HOPdG Regioisomeric Deoxyguanosine Adducts

Ana M. Sanchez,[†,‡] Irina G. Minko,[†,‡] Andrew J. Kurtz,[†,‡] Manorama Kanuri,[†,‡] Masaaki Moriya,[§] and R. Stephen Lloyd*[,†]

Sealy Center For Molecular Science and the Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77555-1071, and Laboratory of Chemical Biology, Department of Pharmacological Sciences, State University of New York, Stony Brook, New York 11794-8651

Received April 2, 2003

Acrolein is a bifunctional electrophile, present as an ubiquitous environmental pollutant and an endogenous cellular product of lipid peroxidation. Reaction of acrolein with deoxyguanosine produces two regioisomeric DNA adducts, specifically γ-hydroxypropanodeoxyguanosine (γ-HOPdG) and α-hydroxypropanodeoxyguanosine (α-HOPdG). While previous investigations have focused on the major γ-HOPdG adduct, little is known about the properties of the minor α-HOPdG adduct. Therefore, this comparative investigation has assessed the following: the ability of each adduct to undergo secondary chemical reactions with biomolecules to form various cross-linked species, in vitro translesion DNA synthesis, and mutagenic properties, following replication in mammalian cells. In contrast to γ-HOPdG, which is capable of forming DNA−DNA, DNA−peptide, and DNA−protein cross-links, α-HOPdG did not form any of these cross-linked species. These results can be attributed to the inability of the α-HOPdG adduct to undergo ring opening, whereas the γ-HOPdG adduct forms the ring open, acyclic N^2 oxopropyl in duplex DNA, which readily reacts with nucleophilic functions. Consistent with this interpretation, when polymerase η replication bypass of DNA containing α-HOPdG was assayed, this lesion posed a stronger block to replication than the γ-HOPdG adduct, closely resembling the results for polymerase η bypass of propanodeoxyguanosine in which the exocyclic adduct remains permanently ring-closed. Cellular replication and mutagenesis assays in COS-7 cells using single-stranded DNA containing a site specific α-HOPdG revealed that this adduct was significantly mutagenic, yielding a nearly identical frequency and spectrum of mutations as compared with the γ-HOPdG adduct.

Introduction

Acrolein is a genotoxic and mutagenic agent widely distributed in nature. It is produced by incomplete combustion of organic materials, including woods, food, tobacco, and fuels (*1*, *2*). Acrolein is also formed endogenously within cells, by peroxidation of membrane lipids (*3*, *4*), and as a metabolic product of both various drugs and chemical carcinogens (e.g., a secondary metabolite of cyclophosphamide) (*5*).

Acrolein is an α,β-unsaturated aldehyde that reacts with deoxyguanosine to form adducts through a pair of regioisomeric Michael additions with initial bond formation occurring either at the 1 or N^2 positions, followed by ring closure to form 3-carbon exocyclic hydroxy-isomeric adducts: the major, γ-HOPdG,[1] and minor, α-HOPdG (Figure 1A) (*6*). These exocyclic adducts have the potential to disrupt base pairing within duplex DNA and may play an important role in mutagenesis and possibly carcinogenesis. Additionally, γ-HOPdG has been detected as a prevalent lesion in a variety of rodent and human tissues, including colon, lung, brain, prostate, kidney, mammary gland, liver, and leukocytes, even without exogenous acrolein treatment (*7*, *8*).

Prior to the development of synthetic strategies to produce site- and stereospecific α- and γ-HOPdG-adducted DNAs, mutagenesis studies were carried out using PdG as a model compound (Figure 1B) (*9*−*12*). Replication assays using PdG determined that it constituted a major block to DNA synthesis, although reports on the mutagenic potential of this lesion varied greatly when the adduct was replicated in *Escherichia coli*. In particular, Moriya observed 100% G to T transversions using a ss pMS2 vector (*11*); however, Burcham and Marnett reported 2% G mutation to T and A when using a M13MB102 vector (*10*). This difference in mutation rates likely reflects the efficient repair of PdG in ds DNA. PdG was also shown to be mutagenic in COS-7 cells, with 8% G mutations to T and A (*11*).

More recent developments have facilitated the synthesis of site specifically modified DNAs containing γ- and α-HOPdG lesions (*13*−*16*). This breakthrough has al-

* To whom correspondence should be addressed. Tel: (409)772-2179. Fax: (409)772-1790. E-mail: rslloyd@utmb.edu.
† University of Texas Medical Branch.
‡ Authors made equal contributions to this investigation.
§ State University of New York.

[1] Abbreviations: γ-HOPdG, γ-hydroxypropanodeoxyguanosine; α-HOPdG, α-hydroxypropanodeoxyguanosine; PdG, propanodeoxyguanosine; T4-pdg, T4 pyrimidine dimer glycosylase/apurinic-apyrimidinic site lyase; pol, polymerase; ds, double-stranded; ss, single-stranded.

10.1021/tx034066u CCC: $25.00
Published on Web 07/29/2003

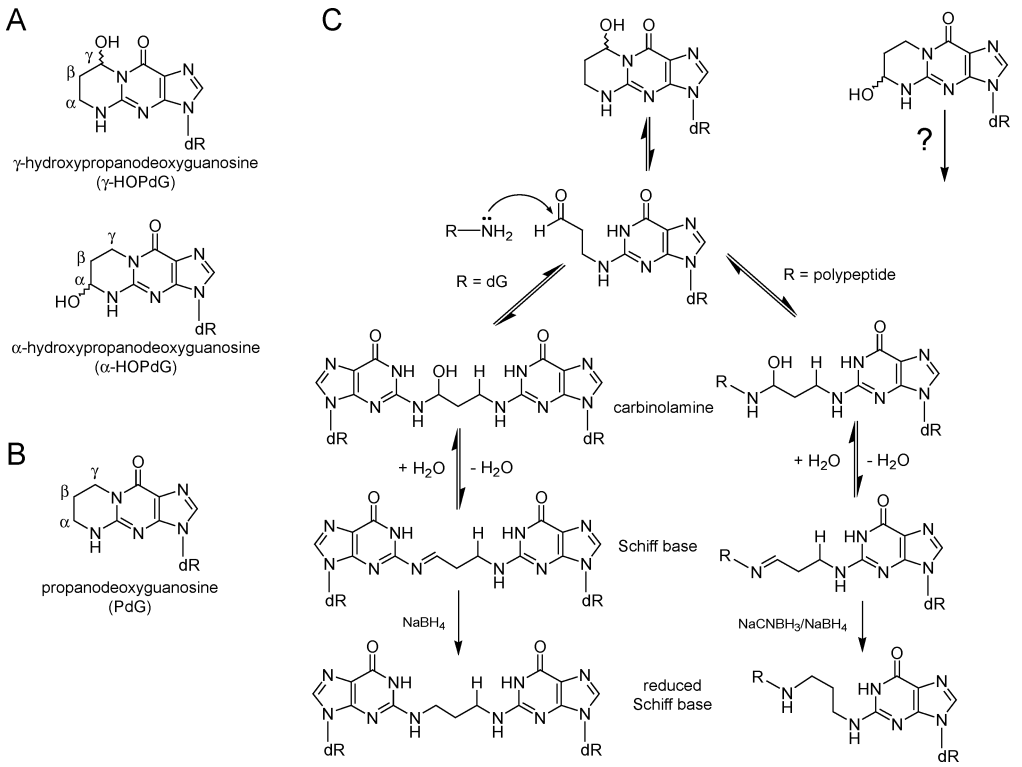

Figure 1. Structures of lesions under investigation. (A) Regioisomers of HOPdG and (B) PdG. (C) The γ-HOPdG nucleoside primarily exists in a closed form in solution, but the equilibrium shifts to the opened structure in duplex DNA. The left and right branches show the pathways leading to DNA−DNA and DNA−protein/peptide cross-links, respectively.

lowed the first structural analysis of γ-HOPdG (*17*), showing that the nucleoside exists in a ring-closed form in solution but undergoes rearrangement to a ring-opened structure in duplex DNA (Figure 1C). Mutagenesis studies using duplex DNAs, revealed that γ-HOPdG was marginally miscoding, inducing ≤1% G to T and G to A base substitutions in HeLa and xeroderma pigmentosum complementation group A and variant cells (*18*), whereas replication of the α-HOPdG adduct in xeroderma pigmentosum A cells induced ∼10% base substitutions, primarily G to T transversions (*19*). In contrast to the mutagenic analyses of the γ-HOPdG adduct in ds DNA, bypass replication analyses using a ss shuttle vector revealed that the γ-HOPdG adduct was significantly mutagenic in COS-7 cells, with the overall mutagenic frequency being 7.4% (*20*). In *E. coli*, replication of γ-HOPdG was not found to be mutagenic, whether ss or ds vectors were used (*20*−*22*).

To identify an activity responsible for bypass of HOPdG adducts, replication studies have been performed in vitro using Klenow exo$^-$ and Klenow exo$^+$ fragments of *E. coli* pol I, the two major eukaryotic DNA polymerases, pol δ and pol ϵ, and a number of yeast and human DNA polymerases proficient in translesion synthesis (*19*−*24*). For all mammalian enzymes tested, γ- and α-HOPdG represented a significant block to both nucleotide insertion opposite the lesion and extension beyond the adducted base when paired with dC (*20*, *23*, *24*). Replication was mutagenic in each case; thus, at present, it cannot be concluded which pol carries out error free bypass of these lesions in humans. However, the collective data suggested involvement of human pol η in the error prone bypass of both γ- and α-HOPdG (*23*, *24*).

As mentioned above, in duplex DNA, the γ-HOPdG lesion exists primarily in a ring-opened form (*17*, *25*). This structural observation suggested that the ring-opened aldehydic group is capable of undergoing further reactions with primary amines in close proximity. Consistent with this prediction, oligodeoxynucleotides containing the γ-HOPdG lesion in a Cp***G** sequence context have been shown to form a reversible DNA−DNA cross-link (Figure 1C) (*26*, *27*). This cross-link occurred between the free aldehyde in the ring-opened form of the adduct (Figure 1C) and the exocyclic amino group of the deoxyguanosine in the opposing strand. It was concluded that there is a mixture of cross-linked species including imine and carbinolamine structures (*27*), and evidence for the interchain carbinolamine intermediate has been obtained by NMR analyses (*25*). This is of major biological importance because interstrand DNA−DNA cross-links constitute one of the most serious lesions in DNA, since they present an absolute block to replication. However, no evidence of cross-link formation was found when the same assays were done in ***G**pC sequence context (*27*). Additionally, ongoing studies in our laboratory have demonstrated that cross-link formation is sequence context-dependent and that γ-HOPdG can form intra- and interstrand cross-links with the exocyclic primary amines of C and A. Further evidence for the secondary reactivity of the ring-opened γ-HOPdG lesion has recently been obtained in which the adduct has been shown to form Schiff base-mediated cross-linked species with oligopeptides (Figure 1C) (*28*).

The goal of this investigation was to conduct a comparative evaluation of the bioreactivity of DNAs containing acrolein-derived γ- and α-HOPdG lesions through their ability both to form in vitro DNA−DNA, DNA−protein, and DNA−peptide cross-links and to modulate DNA synthesis by yeast pol η. Furthermore, the mutagenesis of each lesion was tested in COS-7 cells utilizing a site specifically modified ss pMS2 vector.

Materials and Methods

Materials and Enzymes. The peptide Lys-Trp-Lys-Lys was prepared by the Protein Chemistry Laboratory (A. Kurosky, Director) (NIEHS Center in Environmental Toxicology, University of Texas Medical Branch (UTMB), Galveston, TX). Following initial synthesis, the peptide was analyzed by MS, purified by preparative HPLC, and resuspended in a solution of 20:80 acetonitrile/water. The concentration of Lys-Trp-Lys-Lys in solution was determined by monitoring tryptophan absorbance at 280 nm using a Shimadzu BioSpec-1601 spectrophotometer. The concentration was calculated using 5500 M^{-1} cm^{-1} as the tryptophan molar extinction coefficient (*29*).

Saccharomyces cerevisiae pol η was a generous gift from Dr. L. Prakash and Dr. S. Prakash, UTMB, and was purified as described by Johnson et al. (*30*). Bacteriophage T4-pdg was purified according to a previously published procedure (*31*).

T4 DNA ligase, T4 polynucleotide kinase, uracil DNA glycosylase, and *Eco*RV were obtained from New England BioLabs (Beverly, MA). S1 nuclease was purchased from Life Technologies, Inc. (Rockville, MD). III-SS histones were purchased from Sigma-Aldrich Co. (St. Louis, MO). [γ^{32}P]-ATP was purchased from NEN Life Science Products Inc. (Boston, MA). Bio-Spin columns were purchased from Bio-Rad (Hercules, CA). Centricon 100 concentrators were obtained from Amicon Inc. (Beverly, MA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, Opti-MEM (reduced serum medium), L-glutamine, antibiotic-antimycotic, and lipofectin reagent for tissue culture were obtained from Life Technologies, Inc.. Trypsin-EDTA and HEPES buffer were purchased from Cellgro (Mediatech, VA). PBS was purchased from Sigma Chemical Co.

Oligodeoxynucleotides. The syntheses of the γ-HOPdG and α-HOPdG oligodeoxynucleotides were carried out as described previously (*14*, *16*), and the adducted deoxynucleosides were individually constructed into 12-mer oligodeoxynucleotides with the sequence 5′-GCTAGC***G**AGTCC-3′, where ***G** denotes the adducted base. Following synthesis, adducted oligodeoxynucleotides were purified by HPLC obtaining a mixture of the two stereoisomers. These oligodeoxynucleotides were a generous gift from Drs. T. M. Harris and C. M. Harris (Vanderbilt University, Nashville, TN).

The complementary strand, with sequence 5′-GGACTCGCTAGC-3′, was purchased from Midland Certified Reagent Co. (Midland, TX). All other oligodeoxynucleotides were synthesized by the Molecular Biology Core Laboratory (Thomas G. Wood, Director) of the NIEHS Center in Environmental Toxicology at UTMB and purified via PAGE.

DNA Vectors and Cells. The shuttle vector, ss pMS2 DNA, was purified as described (*32*). COS-7 cells were purchased from American Type Culture Collection (Rockville, MD). *E. coli* DH10B cells were purchased from Life Technologies Inc.

DNA−DNA Cross-Link Formation with α- and γ-HOPdG Adducts. The modified and unmodified 12-mer oligodeoxynucleotides were 5′-end labeled with T4 polynucleotide DNA kinase using [γ^{32}P]-ATP and purified through a P-6 Bio-Spin Column. The DNA containing the α-HOPdG adduct (10 nM) was annealed to a 5-fold molar excess of the complementary 12-mer (50 nM) and incubated at 4 °C for up to 21 days in a reaction buffer containing 100 mM sodium phosphate (pH 7.0) and 5 mM $MgCl_2$. Additional experiments were carried out varying the total DNA concentration up to 1 μM, with no quantitative differences being observed in the percent of cross-linked species. Two aliquots were withdrawn from the reaction mixture at the indicated times. One of them was subjected to treatment with a reducing agent, $NaBH_4$, for 15 min at 25 °C and frozen at −70 °C. Both the imino intermediate formed during the reaction and any unreacted adehydic substrate were reduced by $NaBH_4$. The second aliquot was directly frozen at −70 °C, without being subjected to treatment with the reducing agent. The substrate (12-mer) and the cross-link product (24-mer) were separated in an 8 M urea-denaturing polyacrylamide gel (15%). The reduced samples were heated at 90 °C prior to loading them onto the gel. The heating step was omitted for the aliquots that did not received $NaBH_4$ treatment. Control experiments using nondenaturing electrophoresis conditions demonstrated that all labeled DNA migrated as duplex DNA (data not shown). The comparative experiments carried out with γ-HOPdG and the unmodified oligodeoxynucleotides were carried out under the same experimental conditions for 7 days at 25 °C.

Trapping of DNA−Protein Complexes. The 12-mer nondamaged or adducted oligodeoxynucleotide duplexes (5 nM) were incubated at 22 °C for 20 h with the T4-pdg protein (2 μM) or the III-SS histone fraction (0.25 μg/μL) in the presence of 100 mM HEPES−KOH buffer (pH 7.6), 20 mM NaCl, 50 mM KCl, and where indicated, with 50 mM $NaCNBH_3$. The $NaCNBH_3$ solution was freshly prepared and added to the reactions immediately after the addition of proteins. Reactions were terminated by the addition of an equal volume of loading buffer containing 125 mM Tris-HCl (pH 6.8), 4% SDS, and 20% glycerol (v/v) and boiled for 5 min. Samples were separated by electrophoresis through 12.5% SDS−PAGE.

Trapping of Covalent DNA−Peptide Complexes. For trapping reactions, adducted oligodeoxynucleotide duplexes (75 nM) were incubated with peptide in 50 mM HEPES (pH 7.0) and 100 mM NaCl at 4 °C. The concentrations of Lys-Trp-Lys-Lys and $NaCNBH_3$ in trapping reactions were 1.0 and 50 mM, respectively, and reactions were quenched by the addition of 100 mM $NaBH_4$. Aqueous solutions of both $NaCNBH_3$ and $NaBH_4$ were prepared fresh on the day of use. Each reaction mixture was subsequently diluted 5-fold by the addition of 1.25× loading buffer (59% v/v formamide, 12.5 mM EDTA, 0.012% w/v bromophenol blue, 0.012% xylene cyanol) and heated at 90 °C for 3 min. The products of each reaction were separated on a 15% denaturing polyacrylamide gel (8.3 M urea) in sequencing buffer (134 mM Tris base, 44 mM boric acid, 10 mM EDTA) for 5 h at 1500 V.

In Vitro DNA Replication Studies. To construct site specifically modified templates for in vitro replication assays, control 12-mer oligodeoxynucleotides and 12-mer oligodeoxynucleotides adducted with either α-HOPdG or γ-HOPdG were ligated using T4 DNA ligase with the 26-mer 3′-flanking oligodeoxynucleotide in the presence of the complement scaffold. The ligation products were purified via denaturing PAGE. Their sequences were identical as follows: 5′-GCTAGC***G**AGTCC-GCGCCAAGCTTGGGCTGCAGCAGGTC-3′, where ***G** is α-HOPdG, γ-HOPdG, or nonadducted dG. As primers for in vitro replication reactions, 21-mer oligodeoxynucleotides were used as follows: 5′-AGCCCAAGCTTGGCGCGGACT-3′, which was complementary to the templates from the position −1 to −21 relative to the adducted base (−1 primer), and 5′-GCCCAAG-CTTGGCGCGGACTC-3′, which overlaps the lesion site in modified templates (0 primer). Primer oligodeoxynucleotides were phosphorylated with T4 polynucleotide kinase using [γ^{32}P]-ATP and purified using P-6 Bio-Spin columns supplied with 10 mM Tris-HCl (pH 7.4). The [γ^{32}P]-labeled primers were mixed with the oligodeoxynucleotide substrates at a molar ratio of 1:2 in the presence of 25 mM Tris-HCl (pH 7.6) and 50 mM NaCl, heated at 90 °C for 2 min, and cooled to room temperature overnight.

Primer extension and single nucleotide incorporation experiments with yeast pol η were carried out as described (*30*). The reaction mixture (10 μL) contained 5 nM primer annealed to a template, 25 mM Tris-HCl (pH 7.5), 10 mM NaCl, 5 mM $MgCl_2$, 10% glycerol, 100 μg/mL of BSA, 5 mM DTT, 100 μM of each of the four dNTPs (primer extension experiments) or 10 μM individually (single nucleotide incorporation experiments), and yeast pol η at the concentrations as indicated in the figure legend. Reactions were incubated at 22 °C and terminated by the addition of four times excess of stop solution consisting of 95% (v/v) formamide, 20 mM EDTA, 0.02% (w/v) xylene cyanol, and 0.02% (w/v) bromphenol blue. Reaction products were resolved through a 15% denaturing PAGE.

Construction of Circular ss pMS2 DNA Modified with α- and γ-HOPdG. The ss pMS2 shuttle vector (29 pmols, 50

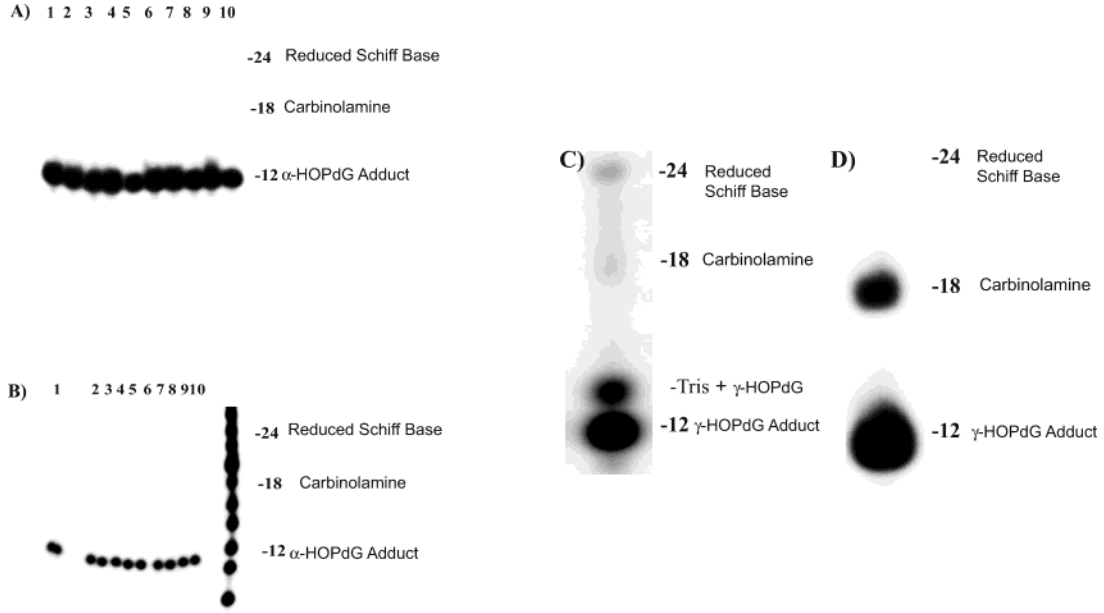

Figure 2. Interstrand DNA−DNA cross-link formation with α-HOPdG and γ-HOPdG. (A) Kinetic analyses of DNA−DNA cross-link formation in which a [^{32}P]-labeled α-HOPdG 12-mer was annealed with a 5-fold molar excess of the complementary 12-mer and incubated for up to 21 days at 4 °C. Following quenching with 25 mM $NaBH_4$, to assay for the covalent reduced Schiff base product, samples were heated to 90 °C and analyzed via PAGE. Lane 1, control α-HOPdG 12-mer; lanes 2−10, 20 min, 1, 3, 6, 9, 12, 15, 18, and 21 days, respectively. (B) Aliquots of the reaction mixture described above were frozen at −70 °C until analyzed using a 15% polyacrylamide gel (8 M urea) in order to assay for the formation of a carbinolamine species. Lane 1, control α-HOPdG 12-mer; lanes 2−10, 20 min, 1, 3, 6, 9, 12, 15, 18, and 21 days, respectively. (C) Duplex DNA containing γ-HOPdG was incubated for 7 days at 25 °C and quenched with $NaBH_4$. The data show the formation of a covalently linked reduced Schiff base product. The reduced Tris-adduct Schiff base is indicated. (D) γ-HOPdG lesion was incubated for 7 days at 25 °C, without $NaBH_4$ treatment, demonstrating the carbinolamine cross-linked product.

μg) was annealed to a 58-mer scaffold DNA [3′-CUUAAGCUC-GAGCCAUGGUCGCUACGAUCGCUCAGGUAGCGAACGUC-CCCGGGAGCUC-5′] (145 pmols/2.34 μg) and digested with *Eco*RV to generate a partially duplex DNA containing a 12 nucleotide gap. Adducted and nonadducted 12-mer oligodeoxynucleotides were annealed and ligated into this gap as described previously (*20*). Ligation products were visualized by ethidium bromide staining following electrophoresis through a 1.4% agarose gel, and the correct sizes were verified by comparison to standard circular and linearized ss pMS2 DNA. pMS2 DNAs were subsequently diluted to 600 μL and purified from non-ligated 12-mer by centrifugation through Centricon-100 tubes. This step was repeated three times, after which DNA was recovered by ethanol precipitation and the DNA concentration determined by measuring absorbance at 260 nm. To prevent the scaffold DNA from being used as a primer for (−) strand replication, ligated DNAs were incubated with uracil DNA glycosylase for 1 h to remove uracil bases and generate abasic sites.

Mutagenesis Experiments. Transfection of modified ss pMS2 into COS-7, isolation of DNA, amplification in *E. coli* DH10B cells, and differential hybridization analyses were carried out as described previously (*20*). Progeny plasmid DNA was hybridized with [γ^{32}P]-labeled 18-mer oligodeoxynucleotide probes of sequence 5′-GATGCTAGC***N**AGTCCATC-3′, where ***N** denotes A, T, G, or C. Whatman 541 filters containing hybridized colonies were exposed to X-OMAT AR film overnight, and autoradiographs were developed to identify mutation frequency and types of mutations. Representative colonies were subjected to dideoxy sequencing (*33*) to confirm the presence of the mutations. A 20-mer primer, approximately 100 nucleotides downstream of the adduct (5′-CCATCTTGTTCAATCAT GCG-3′), was used to sequence the region containing the 12-mer oligodeoxynucleotide in progeny plasmid DNA.

Illustrations. Results were visualized by phosphorimager analysis and autoradiography of wet gels. Product bands were quantitated using ImageQuant (5.0) software.

Results

DNA−DNA Interstrand Cross-Linking. Prior investigations have demonstrated that duplex DNA containing a γ-HOPdG lesion can form interstrand DNA cross-links (*26*, *27*), as well as DNA−peptide cross-links (*28*). To determine if the α-HOPdG lesion could undergo further chemical reactions to form interstrand cross-linked species, the following strategy was adopted in which the initial step was hypothesized to involve the opening of the exocyclic ring of the adduct, which potentially could react with an exocyclic amino group of a guanine in the complementary strand. Such a reaction would proceed through a carbinolamine intermediate, which by dehydration, yields a Schiff base (imine) (Figure 1C). To assay for such a possibility, as described previously in the Materials and Methods, reactions were carried out using control dG and α-HOPdG- and γ-HOPdG-modified 12-mer DNAs (5′-GCTAGC***G**AGTCC-3′ where ***G** = G, α-HOPdG, or γ-HOPdG, respectively). To assay for the covalent, reduced Schiff base cross-link (Figure 2A), aliquots were subjected to a secondary treatment with $NaBH_4$. In the absence of a reducing agent, the assay was designed to detect the carbinolamine cross-link by omitting the heating step. In the case of the α-HOPdG-containing duplex DNA, neither the reduced Schiff base (Figure 2A) nor the carbinolamine (Figure 2B) cross-linked species could be detected following gel electrophoresis. When DNAs containing γ-HOPdG in a Cp***G** sequence context were used to monitor cross-link formation, time points were taken at 0, 1, 3, 5, and 7 days. These data showed that the formation of the Schiff base intermediate was slowly established during the first 3 days (data not shown); Figure 2C illustrates the seventh day of reaction.

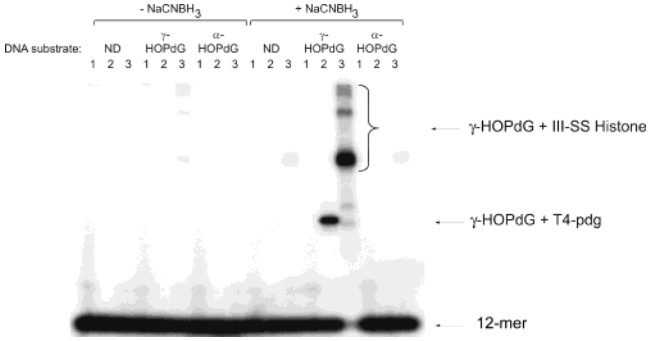

Figure 3. DNA−protein cross-linking at α- and γ-HOPdG adducts. Control (ND) or γ-HOPdG- or α-HOPdG-adducted oligodeoxynucleotides were annealed to the complement 12-mer. DNA duplexes (5 nM) were incubated at 22 °C for 20 h in the presence of 100 mM HEPES−KOH (pH 7.6), 20 mM NaCl, 50 mM KCl without (lane 1), or with addition of proteins: 2 μM T4-pdg (lane 2) or 0.25 μg/μL of the III−SS histones (lane 3). $NaCNBH_3$ (50 mM) was added to reactions as indicated. Reactions were terminated by boiling for 5 min in the presence of 2% SDS. The products were resolved through a 12.5% SDS−PAGE.

The DNA species migrating slightly more slowly than the 12-mer control has been demonstrated by MS to be a reduced Tris:adduct Schiff base (data not shown). In contrast, when γ-HOPdG DNA aliquots were not exposed to $NaBH_4$ or heating, a major cross-linked species (carbinolamine) was detected (Figure 2D) after the same period of time. The assignment of the slower migrating species (Figure 2D) as a carbinolamine cross-link was based on its thermal instability and inability to be reduced by $NaBH_4$. A carbinolamine cross-link was previously observed by NMR (*25*). Additionally, the kinetics of the appearance of this cross-linked species (data not shown) was in good agreement with a prior report using nuclease digestion and HPLC analyzes (*26*, *27*). We attribute the difference in the percentages of product observed to the differences in the experimental conditions and the analytical methodology used in these two investigations. Identical reaction conditions and analyses using nonadducted G in place of γ-HOPdG yielded no cross-linked species (data not shown).

DNA−Protein Cross-Linking at the α- and γ-HOPdG Adducts. The catalytic mechanism of the bifunctional glycosylase/apurinic/apyrimidinic lyase enzyme, T4-pdg, involves the formation of an imino (Schiff base) complex between the α-amino group of the protein and a reactive aldehyde at an abasic site (*34*). Because γ-HOPdG forms an aldehydic, ring-opened structure, in analogy with the ring-opened abasic site, it may also be subject to nucleophilic attack and, therefore, have the potential to form Schiff base-mediated complexes with proteins. To test this possibility, an experimental strategy was designed that allowed for the stabilization of the imino complexes by $NaCNBH_3$. Incubation of the 12-mer γ-HOPdG-containing duplex DNA with T4-pdg enzyme resulted in formation of a product that was detected only when $NaCNBH_3$ was added to the reaction (Figure 3). Accumulation of this product was time-dependent and was not observed with the prereduced γ-HOPdG-adducted oligodeoxynucleotide duplexes (data not shown). Formation of γ-HOPdG−DNA−protein complexes under reducing conditions was also detected in reactions with III-SS histone fraction, enriched in histone I (Figure 3), whole histone fractions, and nuclear extracts (data not shown). Therefore, these data indicated that γ-HOPdG was capable of forming imino complexes with a variety of proteins and that the chemical bond generated involved the aldehydic group of the adduct. Because the currently available data on the structure of α-HOPdG were not sufficient to conclude whether this adduct undergoes ring opening like γ-HOPdG or is always in its ring-closed form and because formation of the Schiff base complexes ultimately requires an aldehydic moiety, the ability of α-HOPdG to form DNA−protein complexes in the same manner as γ-HOPdG was tested. Formation of cross-linked species would be indicative of the ring-opened conformation of the adduct. As shown in Figure 3, no product was accumulated when the α-HOPdG-modified duplexes were incubated with the T4-pdg enzyme or the III-SS histones. These results are consistent with the proposal that the α-HOPdG adduct remains predominantly ring-closed within the duplex DNA.

DNA−Peptide Cross-Linking at α- and γ-HOPdG Adducts. The strategies described above were unsuccessful in generating data that would suggest that the α-HOPdG exists in equilibrium as a species that could form DNA or protein cross-links. To minimize the possibility that steric hindrances prevented the formation of such species for α-HOPdG, small basic oligopeptides were incubated with α- and γ-HOPdG-adducted DNAs to probe for transient ring opening. To test this possibility and serve as a positive control, the 12-mer oligodeoxynucleotide substrate containing the centrally located γ-HOPdG adduct was annealed to its complement and reacted with the peptide Lys-Trp-Lys-Lys in the presence of $NaCNBH_3$ (*28*). To examine the kinetics of cross-link formation, reaction mixtures were quenched at the designated time points by the addition of $NaBH_4$ to facilitate the rapid reduction of aldehydic substrate. Although modified relative to DNA−protein cross-linking reactions, the conditions for DNA−peptide reactions were optimized to achieve efficient complex formation. As shown in Figure 4A, a radioactively labeled complex was observed that migrated more slowly than unreacted labeled DNA through a denaturing polyacylamide gel. The kinetics of complex formation were evaluated by monitoring the accumulation of this major DNA−peptide product band, and at the end of a 1 h time course, 87% of the starting γ-HOPdG substrate was reacted to form a cross-link. When reducing agent was omitted from the reaction, the amount of shifted complex that was observed was dramatically diminished, a result consistent with the necessity of stabilizing the Schiff base complex by reduction prior to denaturing PAGE analysis. To evaluate the relative ring-opened/ring-closed equilibria for the α-HOPdG adduct, an analogous substrate containing the α-HOPdG adduct was reacted with Lys-Trp-Lys-Lys under identical trapping conditions. In contrast to the results obtained for the γ-HOPdG adduct, no accumulation of a DNA−peptide cross-link was observed for the α-HOPdG adduct, either in the presence or in the absence of reducing agent (Figure 4B). These data would suggest that the α-HOPdG adduct favors the ring-closed tautomer in duplex DNA, whereas the γ-HOPdG adduct favors the ring-opened tautomer, consistent with previous structural studies.

In Vitro Lesion Bypass with Yeast DNA pol η. Because all experimental data were consistent with an interpretation that the α-HOPdG exists in a ring-closed form, it was possible to further indirectly probe its structure by measuring the relative efficiency of replica-

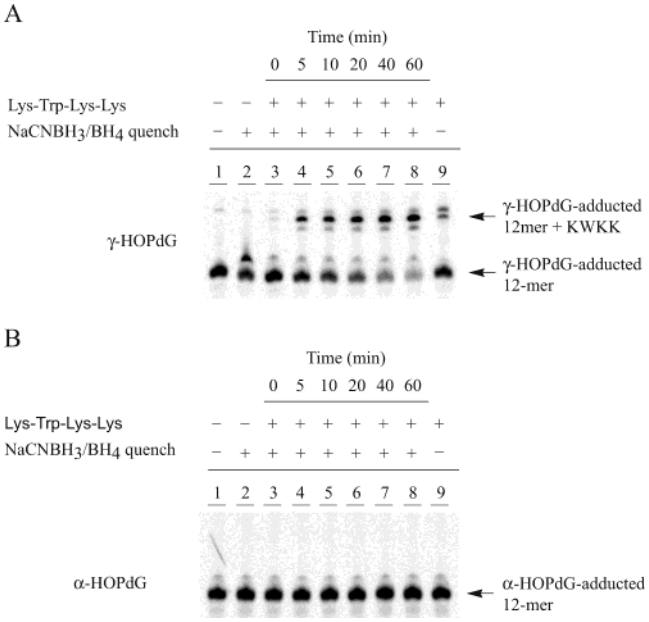

Figure 4. DNA−peptide cross-linking at α- and γ-HOPdG adducts. (A) For trapping reactions, γ-HOPdG-adducted oligodeoxynucleotide duplexes (75 nM) were incubated with 1.5 mM Lys-Trp-Lys-Lys (lanes 3−9) in the absence (lanes 1, 9) or the presence (lanes 2−8) of 50 mM $NaCNBH_3$. Reactions were carried out in 50 mM HEPES (pH 7.0) and 100 mM NaCl at 4 °C and were incubated for 0, 5, 10, 20, 40, or 60 min as shown. Control reactions were incubated for 60 min, in which peptide (lanes 1−2) or reducing agent (lane 9) was omitted. Labels indicate the positions of the substrate 12-mer DNAs and the major reduced Schiff base complexes (12-mer + peptide) following denaturing PAGE analysis. (B) Trapping reactions were carried out for the end-labeled α-HOPdG-adducted DNA exactly as described above. The label indicates the position of the substrate 12-mer DNA.

tion bypass. Both γ- and α-HOPdG adducts and the PdG adduct (a structural analogue to the ring-closed form of γ-HOPdG) represent a significant block to in vitro replication (*19*−*24*, *35*, *36*); however, replicative blockage caused by γ-HOPdG appears to be considerably weaker than that caused by PdG (*19*, *21*, *23*). In the case of yeast pol *η*, the efficiency of DNA synthesis past PdG relative to γ-HOPdG was reduced ∼8-fold at the step of nucleotide incorporation opposite the lesion and ∼900-fold at the extension step (*23*). Importantly, replication across the reduced γ-HOPdG adduct, which mimics the ring-opened form of γ-HOPdG, was much more efficient than replication across γ-HOPdG. These data were consistent with a previously proposed model of bypass of γ-HOPdG (*17*), which suggested that structural rearrangement of the adduct from the ring-closed to the ring-opened form is important for both nucleotide incorporation opposite the lesion and subsequent extension from the resulting base pair. Comparative in vitro replication studies using yeast pol *η* were performed on α-HOPdG- and γ-HOPdG-adducted oligodeoxynucleotides as templates. First, −1 and 0 primers were annealed to the template DNAs and extended by yeast pol *η* in the presence of all four dNTPs (Figure 5A). Although formation of the full-length products could be observed on each of the six primer/template combinations, the efficiencies of replication differed notably among the various templates. In reactions with −1 primers, replicative blockage was observed on both adducted templates and was much stronger on the α-HOPdG-modified substrate (lanes 11−15) in comparison with the γ-HOPdG-modified substrate (lanes 6−10). This difference between the adducted substrates was even more striking in reactions with 0 primers: whereas extension from α-HOPdG paired with dC was strongly inhibited (lanes 26−30), no significant effect on replication was observed when γ-HOPdG was primed with dC (lanes 21−25). Thus, translesional DNA synthesis past α-HOPdG by yeast pol *η* resembles bypass of the ring-closed PdG adduct but not the γ-HOPdG adduct. These data support the hypothesis that the inability of α-HOPdG to participate in the canonical Watson−Crick base pairing significantly contributes to the replicative blockage caused by this adduct, affecting both incorporation and extension steps of DNA synthesis. A similar observation has been reported recently for Klenow exo$^-$ extension of a primer in which dC was positioned opposite either PdG or α-HOPdG. DNA synthesis was relatively efficient on a γ-HOPdG-containing substrate, but it was greatly inhibited on PdG- and α-HOPdG-containing substrates (*19*).

Yeast pol *η* was the first pol identified that synthesizes accurately past γ-HOPdG and PdG adducts, preferentially inserting a C opposite these lesions (*23*). To examine the specificity of nucleotide incorporation opposite α-HOPdG, single nucleotide incorporation experiments were carried out using a −1 primer (Figure 5 B). On the α-HOPdG-adducted template (lanes 12−16), primers were most efficiently extended by yeast pol *η* in the presence of C, but some extension was also observed in the presence of each of the other dNTPs. Ratios of the correct vs incorrect nucleotide incorporation were almost identical for α- and γ-HOPdG adducts (lanes 7−11). Thus, similarly to γ-HOPdG and PdG, α-HOPdG does not appear to be miscoding for yeast pol *η*.

Mutagenicity of α-HOPdG Modified ss Vectors in Simian Kidney Cells. Prior investigations have established that replication in mammalian cells of ss vs ds vectors containing site specific γ-HOPdG adducts yielded significantly different mutagenic frequencies, ∼7 and 1%, respectively (*18*, *20*). Replication of duplex DNAs containing α-HOPdG generated ∼10−12% mutations per replication (*19*). In this investigation, the ss shuttle vector, pMS2, was used to establish mutation frequencies and spectra for α- and γ-HOPdG (Figure 6). To assess the mutagenic potential of these adducts, 480 colonies were picked and individually grown in 96 well plates for analysis, using differential hybridization strategy to reveal the kind of mutations that may have occurred at the site of the adducted guanine. Using differential DNA hybridization techniques, followed by direct sequence analyses, 5.6 and 2.6% of the transformants had targeted G to T and G to C transversions, respectively, while no G to A transitions were detected. No data were obtained that suggested deletion mutagenesis was operative. Differential hybridization of control, nonadducted ss pMS2 (dG) revealed no mutations. To efficiently increase the total number of *E. coli* transformants that could readily be assayed, instead of individually hand picking growing and analyzing colonies, direct colony lifts were also performed on transformants and probed with site specific A, T, G, or C probes. Because all of the transformed colonies were lifted for mutational analysis by differential hybridization strategy, sequencing for these DNAs was not possible, but mutation frequencies were confirmed. The percentages obtained were in good agreement with data shown in Figure 6.

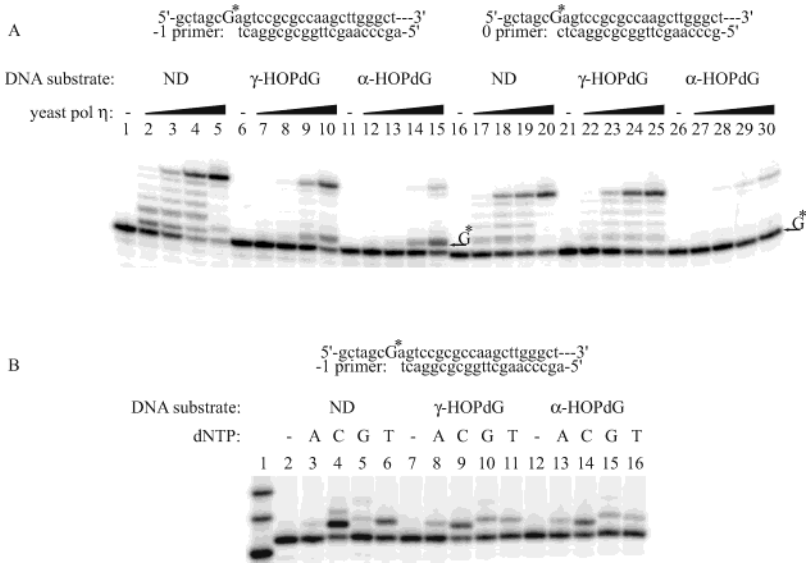

Figure 5. Primer extension (A) and single nucleotide incorporation (B) catalyzed by *S. cerevisiae* pol *η* on the γ-HOPdG- and α-HOPdG-adducted templates. The 21-mer primers were annealed to the nondamaged (ND), γ-HOPdG-adducted, or α-HOPdG-adducted 38-mer templates. The DNA substrates (5 nM) were incubated at 22 °C in the presence of all four dNTPs (100 μM) and increasing concentrations (3−200 pM) of *S. cerevisiae* pol *η* for 20 min (A) or in the presence of one of the four dNTPs (10 μM) (− = no nucleotide added, A = dATP, C = dCTP, G = dGTP, T = dTTP) and *S. cerevisiae* pol *η* (1 nM) for 10 or 40 min using ND or adducted templates, respectively (B). G* indicates the position of the modified G on the template.

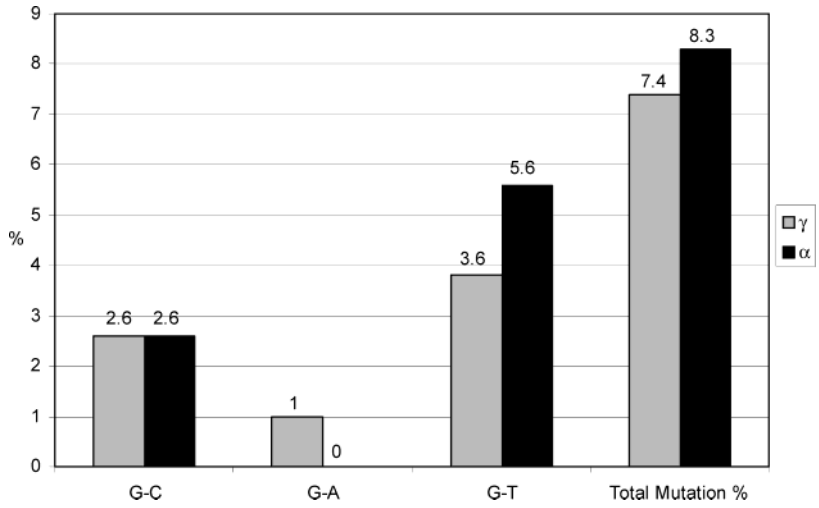

Figure 6. Percentages of mutations detected in COS-7 cells when replicated past γ-HOPdG and α-HOPdG adducts.

Discussion

Prior investigations have demonstrated that the primary DNA adduct that is formed in the reaction of acrolein with DNA is γ-HOPdG (*6*). Although commonly depicted in its ring-closed form, NMR solution structures have revealed that within duplex DNA, γ-HOPdG exists primarily in the ring-opened form (*17*, *25*). In addition, within a specific sequence context (Cp***G**), the γ-HOPdG adduct exists in equilibrium not only with the ring-opened tautomer but also with imino and carbinolamine interstrand cross-links (*25*). It was determined by NMR[2] that the equilibrium distribution of the cross-linked species was ∼15% carbinolamine cross-link and ∼3% Schiff base cross-link. These observations are in strong agreement with the data presented here for γ-HOPdG, showing that the major cross-linked product observed corresponded to a carbinolamine cross-link based on its thermal instability and inability to be reduced by $NaBH_4$ (Figure 2D).

The existence of these DNA−DNA cross-links has been verified (*26*, *27*), thus clearly establishing the existence of a reactive, ring-opened intermediate adduct structure that is capable of participating in secondary chemical reactions. Additional data confirming the secondary reactivity of the γ-HOPdG adduct have been shown through the rapid accumulation of DNA−peptide cross-links when reactions were carried out with a mild reducing agent (*28*). However, no structural studies are currently available that would shed insight on the capacity of the α-HOPdG adduct to also undergo transient ring opening. In summary, this investigation sought to obtain indirect evidence for the existence of such a reactive intermediate, initially by assaying for the formation of DNA−DNA, DNA−protein, and DNA−peptide cross-linked species. Although cross-link formation was readily evident for reactions initiated with the γ-HOPdG lesion, no data were obtained that even hinted at the

[2] M. Stone, personal communication.

formation of these various cross-linked species with the α-HOPdG-containing DNAs (Figures 2−4).

If one considers only the inability to detect DNA−DNA cross-linked species using DNAs containing the site specific α-HOPdG in a Cp***G** sequence context, these data suggested either that this lesion exists almost exclusively in a ring-closed form or that the exact DNA geometry required to achieve a cross-linked intermediate was not possible in the sequence context examined. Although there were no data suggestive of ring opening for the α-HOPdG lesion, due to the limited extent of formation of stable DNA−DNA cross-linked species with the γ-HOPdG containing DNAs, and the concerns over inappropriate geometry to form these cross-linked species, investigations were continued to assay for DNA−protein and DNA−peptide cross-linked species. Even though it was possible to readily observe the accumulation of covalent complexes corresponding to protein and peptide cross-links with the γ-HOPdG adduct using borohydride trapping methodologies (*28*) (Figures 3 and 4), no data were obtained that suggested any formation of reducible Schiff base intermediates using the α-HOPdG-containing DNAs. Because ring opening is a requisite step in the cross-linking reaction, the inability of α-HOPdG to form a reduced Schiff base in the presence of an excess of protein or peptides indicates that the adduct is predominantly in a ring-closed structure. In support of this interpretation, it was shown previously that a ss oligodeoxynucleotide containing the γ-HOPdG adduct exhibits significantly slower kinetics of DNA−peptide cross-link formation when compared to a duplex substrate (*28*). Such a result is consistent with the structural observation that duplex DNA catalyzes ring opening for the γ-HOPdG adduct (*17*) and further supports a mandatory ring opening in cross-link formation. Collectively, the results of DNA−DNA, DNA−protein, and DNA−peptide cross-linking are consistent with the interpretation that the equilibrium between the ring-opened and the ring-closed tautomers of α-HOPdG lies toward the ring-closed configuration.

A mechanism for accelerating ring opening has been previously proposed for malondialdehyde-adducted DNA (*37*); those data demonstrated that the ring opening of the adduct was greatly enhanced only when cytosine was opposite the lesion, not when thymine, adenine, or guanine were present in the complementary position. It was proposed that the exocyclic amino group of cytosine forms a transient Schiff base with the malondialdehyde-modified base. Addition of water to the Schiff base would regenerate the catalytic cytosine and the ring-opened base (*37*). Similarly, it could be proposed that a cytosine in the complementary strand opposite the γ-HOPdG catalyzes its ring opening, and once-ring opened, correct base pairing further stabilizes the ring-opened structure. In contrast, the OH group of the α-HOPdG adduct may not be close enough to the exocyclic amino group to promote Schiff base formation; thus, ring opening is not favored for this lesion. These data also raise the possibility that within the cellular context of the nucleus, the γ-HOPdG adduct never exists in the ring-closed form due to stabilization of the ring-opened species.

The preliminary conclusion that the α-HOPdG lesion exists almost exclusively in the ring-closed, nonreactive state was further supported by in vitro replication data (Figure 5). Consistent with this hypothesis, replication of DNA containing an α-HOPdG adduct was qualitatively similar to the replication of the permanently ring-closed PdG adduct, while replication of the γ-HOPdG was qualitatively similar to data obtained following replication of DNA containing the reduced, permanently ring-opened γ-HOPdG lesion (*23*).

To further substantiate the assertion that there are fundamental differences in the equilibrium structures of the α- vs γ-HOPdG adducts, cellular replication studies have demonstrated a major difference in the relative mutagenicity of these two lesions in fully duplex DNA (*18, 19*). In contrast to this current investigation in which replication of ss DNAs containing the α- and γ-HOPdG lesions revealed about equally mutagenic frequencies and spectra, prior investigations with ds DNA containing α- vs γ-HOPdG revealed a >30-fold difference in mutagenic frequencies (*18, 19*). Thus, the question is raised concerning the chemical and the molecular basis for the different results and conclusions that were drawn concerning the same lesions—in other words, why should the use of ss vs ds DNAs be so critical in the outcome of mutagenic assays? The answer is likely to be relatively straightforward and reflects the dynamic equilibrium and fidelity of replication bypass between the ring-opened and -closed forms of these lesions in ss vs ds DNAs. Within ss DNA, the γ-HOPdG lesion primarily exists in the ring-closed form (*17*), so that the ability of the N1 to participate in hydrogen bonding is blocked by modification. In contrast, within ds DNA, the ring-opened adduct structure enables the formation of a canonical Watson−Crick base pair (*17*), suggesting a lower miscoding potential of this latter form of the lesion. The assumption that the ring-opened conformation is less mutagenic was supported recently by in vitro replication studies using human pol *η*: whereas incorporation of a correct nucleotide (C) predominated opposite the reduced, permanently ring-opened γ-HOPdG adduct, misinsertions were the only events observed opposite PdG, which represents the ring-closed analogue of γ-HOPdG (*23*). Thus, for cellular replication studies using ss vectors, the structural equilibrium of the γ-HOPdG lies strongly toward the ring-closed, more miscoding form, and the rate of ring opening is slow relative to the rate of polymerization past the lesion site. Replication of duplex DNA, where the lesion is primarily preopened, results in predominantly error free bypass, suggesting that the rate of polymerization is faster than the rate of ring closure at the replication fork. By comparison, the data presented here indicate that in either ss or ds DNA, the α-HOPdG adduct exists as the ring-closed structure, giving comparable mutagenic frequencies and spectra in both systems. Therefore, using ss shuttle vectors, the fundamental structural differences between α- vs γ-HOPdG are made evident by converting the routinely ring-opened γ-HOPdG to a ring-closed configuration. These analyses suggest that for lesions whose basic equilibrium structures can be modulated by the presence of the complementary strand, analyses using ds vectors are preferable, even though they open the possibilities for repair and recombination processes to modulate the mutagenic capacity.

Finally, in the experiments presented above, we have utilized a protein- and peptide-trapping assay as a means to probe the relative ability of the α-HOPdG and γ-HOPdG adducts to undergo ring opening. The results of this in vitro assay not only provide insight into the ring structure of each adduct but also suggest the possibility that the γ-HOPdG adduct can react to form DNA−protein

cross-links as secondary adducts within the cell. It has been well-documented that acrolein produces DNA−protein cross-links (*38*). In addition, the γ-HOPdG adduct has been detected in human tissues as a biomarker of smoking-induced acrolein exposure (*39*) and also in human and rodent tissues as a background DNA lesion (*7*, *8*). Thus, it remains plausible that the γ-HOPdG adduct represents an intermediate along a pathway of formation for both DNA−DNA and DNA−protein cross-links in vivo. In this study, we concluded that amines can react with the γ-HOPdG adduct to form cross-links via Schiff base linkage because the isolated DNA−protein and DNA−peptide complexes were stabilized in the presence of a reducing agent. The potential for such a linkage suggests that lysine-rich histone proteins, which remain closely associated with DNA, may participate in cross-linking reactions with this adduct. In any case, the ability of γ-HOPdG to form secondary adducts confounds the mutagenic and cytotoxic potential for this lesion, since both interstrand DNA−DNA and DNA−protein cross-links are expected to pose significant (if not complete) blocks to replication and transcription. The mechanisms by which these secondary adducts might be repaired is as yet unclear, although nucleotide excision repair (NER) has been implicated in the repair of DNA−protein cross-links in both human cells (*40*) and *E. coli* (*41*). For DNA−DNA cross-links, the repair pathway may involve a subset of the NER proteins, which function in the recombinatorial repair of these lesions (*42*).

Acknowledgment. This work has been supported by Grant PO1 ES05355 (M. Stone, PI, R.S.L., PI Project 2), P30 ES06766 (R.S.L., PI). R.S.L. is the recipient of the Mary Gibbs Jones Distinguished Chair in Environmental Toxicology from the Houston Endowment. This work was also supported in part by NIEHS Award T32 ES07254-10 (to A.J.K.). We are grateful to Drs. M. L. Dodson, T. M. Harris, and M. P. Stone for helpful discussions. We acknowledge Drs. L. Prakash, S. Prakash, and M. T. Washington for the generous gift of yeast pol η. We acknowledge Ritchie Jabil for purification of T4-pdg. We also acknowledge The Molecular Biology Core Laboratory of the NIEHS Toxicology Center (University of Texas Medical Branch, Galveston, TX) for oligodeoxynucleotide synthesis and the Protein Chemistry Laboratory (University of Texas Medical Branch, Galveston, TX) for the synthesis of the oligopeptides. The secretarial support of Rosemary Martinez is gratefully acknowledged.

References

(1) Trietman, R. D., Burgess, W. A., and Gold, A. (1980) Air contaminants encountered by firefighters. *Am. Ind. Hyg. Assoc. J.* **41**, 796−802.

(2) Graedel, T. E. (1979) *Chemical Compounds in the Atmosphere*, Academic Press, New York.

(3) Esterbauer, H., Schaur, R. J., and Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biol. Med.* **11**, 81−128.

(4) Uchida, K., Kanematsu, M., Morimitsu, Y., Osawa, T., Noguchi, N., and Niki, E. (1998) Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low-density lipoproteins. *J. Biol. Chem.* **273**, 16058−16066.

(5) Brock, N., Stekar, J., Pohl, J., Niemeyer, U., and Scheffler, G. (1979) Acrolein, the causative factor of urotoxic side-effects of cyclophosphamide, ifosfamide, trofosfamide and sufosfamide. *Arzneimittelforschung* **29**, 659−661.

(6) Chung, F. L., Young, R., and Hecht, S. S. (1984) Formation of cyclic 1, N^2-propanodeoxyguanosine adducts in DNA upon reaction with acrolein or crotonaldehyde. *Cancer Res.* **44**, 990−995.

(7) Nath, R. G., and Chung, F. L. (1994) Detection of exocyclic 1, N^2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7491−7495.

(8) Nath, R. G., Ocando, J. E., and Chung, F. L. (1996) Detection of 1, N^2-propanodeoxyguanosine adducts as potential endogenous DNA lesions in rodent and human tissues. *Cancer Res.* **56**, 452−456.

(9) Benamira, M., Singh, U., and Marnett, L. J. (1992) Site-specific frameshift mutagenesis by a propanodeoxyguanosine adduct positioned in the $(CpG)_4$ hot-spot of *Salmonella typhimurium* hisD3052 carried on an M13 vector. *J. Biol. Chem.* **267**, 22392−22400.

(10) Burcham, P. C., and Marnett, L. J. (1994) Site-specific mutagenesis by a propanodeoxyguanosine adduct carried on an M13 genome. *J. Biol. Chem.* **269**, 28844−28850.

(11) Moriya, M., Zhang, W., Johnson, F., and Grollman, A. P. (1994) Mutagenic potency of exocyclic DNA adducts: marked differences between *Escherichia coli* and simian kidney cells. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11899−11903.

(12) Johnson, K. A., Fink, S. P., and Marnett, L. J. (1997) Repair of propanodeoxyguanosine by nucleotide excision repair in vivo and in vitro. *J. Biol. Chem.* **272**, 11434−11438.

(13) Khullar, S., Varaprasad, C. V., and Johnson, F. (1999) Postsynthetic generation of a major acrolein adduct of 2′-deoxyguanosine in oligomeric DNA. *J. Med. Chem.* **42**, 947−950.

(14) Nechev, L. V., Harris, C. M., and Harris, T. M. (2000) Synthesis of nucleosides and oligonucleotides containing adducts of acrolein and vinyl chloride. *Chem. Res. Toxicol.* **13**, 421−429.

(15) Huang, Y., and Johnson, F. (2002) Regioisomeric synthesis and characteristics of the alpha-hydroxy-1,N(2)-propanodeoxyguanosine. *Chem. Res. Toxicol.* **15**, 236−239.

(16) Nechev, L. V., Kozekov, I. D., Brock, A. K., Rizzo, C. J., and Harris, T. M. (2002) DNA adducts of acrolein: site-specific synthesis of an oligodeoxynucleotide containing 6-hydroxy-5,6,7,8-tetrahydropyrimido[1,2-a]purin-10(3H)-one, an acrolein adduct of guanine. *Chem. Res. Toxicol.* **15**, 607−613.

(17) de los Santos, C., Zaliznyak, T., and Johnson, F. (2001) NMR characterization of a DNA duplex containing the major acrolein-derived deoxyguanosine adduct γ-OH-1,N^2-propano-2′-deoxyguanosine. *J. Biol. Chem.* **276**, 9077−9082.

(18) Yang, I. Y., Johnson, F., Grollman, A. P., and Moriya, M. (2002) Genotoxic mechanism for the major acrolein-derived deoxyguanosine adduct in human cells. *Chem. Res. Toxicol.* **15**, 160−164.

(19) Yang, I. Y., Chan, G., Miller, H., Huang, Y., Torres, M. C., Johnson, F., and Moriya, M. (2002) Mutagenesis by acrolein-derived propanodeoxyguanosine adducts in human cells. *Biochemistry* **41**, 13826−13832.

(20) Kanuri, M., Minko, I. G., Nechev, L. V., Harris, T. M., Harris, C. M., and Lloyd, R. S. (2002) Error prone translesion synthesis past gamma-hydroxypropano deoxyguanosine, the primary acrolein-derived adduct in mammalian cells. *J. Biol. Chem.* **277**, 18257−18265.

(21) Yang, I. Y., Hossain, M., Miller, H., Khullar, S., Johnson, F., Grollman, A., and Moriya, M. (2001) Responses to the major acrolein-derived deoxyguanosine adduct in *Escherichia coli*. *J. Biol. Chem.* **276**, 9071−9076.

(22) VanderVeen, L. A., Hashim, M. F., Nechev, L. V., Harris, T. M., Harris, C. M., and Marnett, L. J. (2001) Evaluation of the mutagenic potential of the principal DNA adduct of acrolein. *J. Biol. Chem.* **276**, 9066−9070.

(23) Minko, I. G., Washington, M. T., Kanuri, M., Prakash, L., Prakash, S., and Lloyd, R. S. (2003) Translesion synthesis past acrolein-derived DNA adduct, gamma-hydroxypropanodeoxyguanosine, by yeast and human DNA polymerase eta. *J. Biol. Chem.* **278**, 784−790.

(24) Yang, I. Y., Miller, H., Wang, Z., Frank, E. G., Ohmori, H., Hanaoka, F., and Moriya, M. (2003) Mammalian translesion DNA synthesis across an acrolein-derived deoxyguanosine adduct: Participation of pol eta in error-prone synthesis in human cells. *J. Biol. Chem.* **12**, 12.

(25) Kim, H. Y., Voehler, M., Harris, T. M., and Stone, M. P. (2002) Detection of an interchain carbinolamine cross-link formed in a CpG sequence by the acrolein DNA adduct γ-OH-1,N(2)-propano-2′-deoxyguanosine. *J. Am. Chem. Soc.* **124**, 9324−9325.

(26) Kozekov, I. D., Nechev, L. V., Sanchez, A., Harris, C. M., Lloyd, R. S., and Harris, T. M. (2001) Interchain cross-linking of DNA mediated by the principal adduct of acrolein. *Chem. Res. Toxicol.* **14**, 1482−1485.

(27) Kozekov, I. D., Nechev, L. V., Moseley, M. S., Harris, C. M., Rizzo, C. J., Stone, M. P., and Harris, T. M. (2003) DNA interchain cross-links formed by acrolein and crotonaldehyde. *J. Am. Chem. Soc.* **125**, 50−61.

(28) Kurtz, A. J., and Lloyd, R. S. (2003) 1,N^2-Deoxyguanosine adducts of acrolein, crotonaldehyde, and *trans*-4-hydroxynonenal cross-link to peptides via Schiff base linkage. *J. Biol. Chem.* **278**, 5970−5976.

(29) Pace, C. N., Vajdos, F., Fee, L., Grimsley, G., and Gray, T. (1995) How to measure and predict the molar absorption coefficient of a protein. *Protein Sci.* **4**, 2411−2423.

(30) Johnson, R. E., Prakash, S., and Prakash, L. (1999) Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Pol eta. *Science* **283**, 1001−1004.

(31) Jaruga, P., Jabil, R., McCullough, A. K., Rodriguez, H., Dizdaroglu, M., and Lloyd, R. S. (2002) *Chlorella virus* pyrimidine dimer glycosylase excises ultraviolet radiation- and hydroxyl radical-induced products 4,6-diamino-5-formamidopyrimidine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine from DNA. *Photochem. Photobiol.* **75**, 85−91.

(32) Pandya, G. A., and Moriya, M. (1996) 1,N^6-ethenodeoxyadenosine, a DNA adduct highly mutagenic in mammalian cells. *Biochemistry* **35**, 11487−11492.

(33) Sanger, F., Nicklen, S., and Coulson, A. R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463−5467.

(34) Schrock, R. D., 3rd, and Lloyd, R. S. (1991) Reductive methylation of the amino terminus of endonuclease V eradicates catalytic activities. Evidence for an essential role of the amino terminus in the chemical mechanisms of catalysis. *J. Biol. Chem.* **266**, 17631−17639.

(35) Hashim, M. F., and Marnett, L. J. (1996) Sequence-dependent induction of base pair substitutions and frameshifts by propanodeoxyguanosine during in vitro DNA replication. *J. Biol. Chem.* **271**, 9160−9165.

(36) Hashim, M. F., Schnetz-Boutaud, N., and Marnett, L. J. (1997) Replication of template-primers containing propanodeoxyguanosine by DNA polymerase beta. Induction of base pair substitution and frameshift mutations by template slippage and deoxynucleoside triphosphate stabilization. *J. Biol. Chem.* **272**, 20205−20212.

(37) Mao, H., Schnetz-Boutaud, N. C., Weisenseel, J. P., Marnett, L. J., and Stone, M. P. (1999) Duplex DNA catalyzes the chemical rearrangement of a malondialdehyde deoxyguanosine adduct. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6615−6620.

(38) Kuykendall, J. R., and Bogdanffy, M. S. (1992) Efficiency of DNA-histone cross-linking induced by saturated and unsaturated aldehydes *in vitro*. *Mutat. Res.* **283**, 131−136.

(39) Nath, R. G., Ocando, J. E., Guttenplan, J. B., and Chung, F. L. (1998) 1,N^2-propanodeoxyguanosine adducts: potential new biomarkers of smoking-induced DNA damage in human oral tissue. *Cancer Res.* **58**, 581−584.

(40) Fornace, A. J., Jr., and Seres, D. S. (1982) Repair of trans-Pt(II) diamminedichloride DNA-protein cross-links in normal and excision-deficient human cells. *Mutat. Res.* **94**, 277−284.

(41) Minko, I. G., Zou, Y., and Lloyd, R. S. (2002) Incision of DNA-protein cross-links by UvrABC nuclease suggests a potential repair pathway involving nucleotide excision repair. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 1905−1909.

(42) Zhang, N., Zhang, X., Peterson, C., Li, L., and Legerski, R. (2000) Differential processing of UV mimetic and interstrand cross-link damage by XPF cell extracts. *Nucleic Acids Res.* **28**, 4800−4804.

TX034066U