The Hydantoin Lesions Formed from Oxidation of 7,8-Dihydro-8-oxoguanine Are Potent Sources of Replication Errors in Vivo†

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ABSTRACT: Single-stranded DNA genomes have been constructed that site-specifically contain the 7,8-dihydro-8-oxo-2′-deoxyguanine (8-oxoG) oxidation products guanidinohydantoin (Gh) and the two stable stereoisomers of spiroiminodihydantoin (Sp1 and Sp2). The circular viral genomes were transfected into wild-type AB1157 *Escherichia coli*, and the efficiency of lesion bypass by DNA polymerase(s) was assessed. Viral progeny were analyzed for mutation frequency and type using the recently developed restriction endonuclease and postlabeling (REAP) assay. Gh was bypassed nearly as efficiently as the parent 8-oxoG but was highly mutagenic, causing almost exclusive G → C transversions. The stereoisomers Sp1 and Sp2 were, in comparison, much stronger blocks to DNA polymerase extension and caused a mixture of G → T and G → C transversions. The ratio of G → T to G → C mutations for each Sp lesion was dependent on the stereochemical configuration of the base. All observed mutation frequencies were at least an order of magnitude higher than those caused by 8-oxoG. Were these lesions to be formed in vivo, our data show that they are absolutely miscoding and may be refractory to repair after translesion synthesis.

Genotoxicity due to degradation of DNA by oxygen radicals is associated with aging and carcinogenesis (*1*). An understanding of the processes of spontaneous, oxidant-induced and ionizing radiation-induced mutagenesis requires knowledge of the exact types and biological significance of oxidative lesions formed in DNA. Both endogenous and exogenous agents have been implicated in the in vivo oxidation of DNA (*2*). The observation that oxidative damage to DNA naturally occurs in vivo was a major discovery because it demonstrated that DNA damage could occur endogenously as a consequence of normal metabolism (*3*−*5*). Currently, over 50 oxidative DNA lesions have been characterized (*6*). The 7,8-dihydro-8-oxo-2′-deoxyguanine (8-oxoG,[1] Figure 1) lesion has been studied extensively because it is a major oxidative DNA lesion (*7*) and can be detected by a variety of assays (*8*). 8-OxoG is mutagenic because of its propensity to mispair with A during replication by DNA polymerases. Incorporation of A opposite 8-oxoG leads to a predominant G → T transversion mutation (*9*−*11*), which is the second most common somatic mutation in human carcinomas (*12*) and is especially frequent in the mutational spectrum of the p53 tumor suppressor gene (*13*).

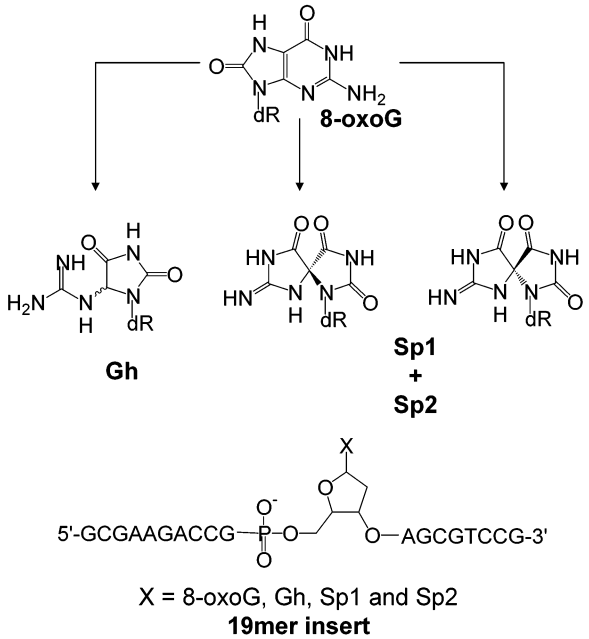

FIGURE 1: Structures of 8-oxoG, Gh, Sp1, Sp2 and the oligonucleotide sequence used for genome construction.

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[1] Abbreviations: AP, abasic; Gh, guanidinohydantoin; HPLC, high-pressure liquid chromatography; Iz, imidazalone; MALDI-TOF, matrix-assisted laser desorption ionization−time of flight; Oa, oxaluric acid; $ONOO^-$, peroxynitrite; 8-oxoG, 7,8-dihydro-8-oxo-2′-deoxyguanine; Oz, oxazalone; PAGE, polyacrylamide gel electrophoresis; Pol I, DNA polymerase I; PNK, polynucleotide kinase; REAP, restriction endonuclease and postlabeling; Sp, spiroiminodihydantoin; ss, single-stranded; THF, tetrahydrofuran; TLC, thin-layer chromatography; MW, molecular weight.

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Several studies have shown that 8-oxoG, which has a lower oxidation potential than any of the four normal DNA bases (*14*), is highly reactive toward oxidizing reagents. This suggests that 8-oxoG may also be sensitive to in vivo DNA oxidation. Peroxynitrite ($ONOO^-$), which is formed endogenously by the reaction of nitric oxide with superoxide, is a potent oxidant that decomposes into radical species capable of oxidizing DNA and other cellular constituents including thiols, lipids, and proteins (*15*−*17*). Oxidation of DNA is known to have mutagenic consequences. For example, $ONOO^-$ treatment of plasmid DNA containing the *supF* gene, followed by transfection into mammalian or bacterial cells, induces mainly G → T and some G → C transversions (*18*). Reaction of $ONOO^-$ with an oligonucleotide containing a portion of the *supF* gene followed by incubation with hot piperidine, which cleaves DNA preferentially at oxidized 8-oxoG nucleotides, causes strand scission at sites coinciding with the mutational "hot spots" observed after replication in bacterial cells (*19*). Since 8-oxoG is not a known source of G → C transversions and 8-oxoG in DNA is refractory to cleavage by piperidine (*20*, *21*), these experiments suggest that additional DNA lesions are forming and that these lesions may contribute to $ONOO^-$ mutagenicity.

Peroxynitrite is known to react preferentially with 8-oxoG in DNA to form several mutagenic 2′-deoxy-β-D-*erythro*-pentofuranosyl products, including cyanuric acid, oxaluric acid, and oxazalone (*22*), which were recently reported to cause predominantly G → T transversion mutations in vitro using purified DNA polymerases (*23*−*25*) and in vivo by transfection of site-specifically adducted bacteriophage DNA into *Escherichia coli* (*26*). Determination of the in vivo mutagenic consequences of such lesions typically has taken the following course: (i) synthesis of the modified oligonucleotide containing the DNA adduct of interest, (ii) insertion of the oligonucleotide into the genome of a virus or plasmid, (iii) introduction of the site-specifically modified genome into a bacterial or mammalian host where replication, and possibly repair, occur, and (iv) enumeration and characterization of mutant progeny (*27*).

Several 8-oxoG oxidation products have yet to be analyzed for their in vivo mutagenic potential. Recently, two major products of 8-oxoG oxidation were identified: guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp) (*28*−*30*), both of which exist as stereoisomers as shown in Figure 1. Although these lesions can be made by oxidation of 8-oxoG with $ONOO^-$ (mimicking the chemistry of the in vivo inflammatory response) (*31*) or by oxidation of guanine with singlet oxygen or other oxidants, a difficult to separate mixture of products results when the reaction is carried out using oligonucleotides (unpublished data). Using a temperature-dependent method of synthesis, Burrows and coworkers developed selective methods that yield oligonucleotides containing Gh and Sp via oxidation of 8-oxoG using the one-electron oxidant Na_2IrCl_6, a compound whose redox potential is specific for 8-oxoG as compared with the four normal DNA bases (*21*, *32*−*34*).

In view of the reported susceptibility of 8-oxoG to oxidation, the mutagenic potential of Gh and Sp was tested in vivo by inserting oligonucleotides containing each well-defined lesion into bacteriophage DNA, followed by transfection into wild-type *E. coli*. This report describes that each lesion was bypassed efficiently enough during replication in vivo to yield progeny phage. The viral progeny was analyzed for mutation frequency and specificity, and it was shown that these oxidation products were much more mutagenic than the parent 8-oxoG.

EXPERIMENTAL PROCEDURES

Oligonucleotides. The DNA base lesion 8-oxoG and tetrahydrofuran abasic (AP) site control 19mer oligonucleotides were made using phosphoramidite solid-phase methods and purified as described (*26*). Oligodeoxynucleotides containing Gh or Sp were prepared by reacting 12 μM 5′-GCGAAGACCGXAGCGTCCG-3′ (X = 8-oxoG) with the oxidizing agent Na_2IrC1_6 (175 μM final concentration) in 100 μL of 10 mM sodium phosphate and 100 mM NaCl for 1 h. Selective formation of Gh was accomplished by incubation of the 8-oxoG-containing oligonucleotide at pH 7.0 and 4 °C, while incubation at pH 6.5 and 60 °C afforded the Sp lesion. The reaction mixture was dialyzed against H_2O using 2000 molecular weight cutoff dialysis tubing for 24 h. The samples were analyzed by negative ion electrospray mass spectrometry (Micromass Quattro II), and their purity was estimated to be ∼95% on the basis of the intensities of related molecular ions. Although both lesions were formed as a mixture of stereoisomers, the Sp-containing oligonucleotides were separable by anion-exchange HPLC. The pure stereoisomer-containing strands are termed Sp1 and Sp2, although their absolute stereochemistry has not been assigned. Purification of the Gh oligodeoxynucleotide was accomplished by HPLC using a Dionex DNA Pac PA-100 4 × 250 mm column and an isocratic buffer system consisting of 50% solvent A (10% acetonitrile and 90% H_2O) and 50% solvent B [10% acetonitrile and 90% 1.5 M ammonium acetate (pH 7)]. The flow rate was 1.0 mL/min, and UV spectra were recorded at 260 nm. Oligodeoxynucleotides containing the Sp isomers were purified in a similar manner, except that an isocratic buffer system consisting of 30% solvent A and 70% solvent B was used. Prior to use, the HPLC-purified oligodeoxynucleotides were dialyzed against H_2O for 72 h, followed by dialysis against 1 mM NaCl for 72 h, to minimize the possibility of polynucleotide kinase inhibition by ammonium cations. The purity of the HPLC-purified oligodeoxynucleotides was determined by HPLC to be >99% using a Dionex DNA Pac PA-100 4 × 250 mm column and a linear gradient of 35% solvent B to 100% solvent B in 30 min. MALDI-TOF mass spectrometry was used to verify the molecular weight (MW) of each strand after purification [Gh, MW = 5868.9 (calculated), 5869.4 (observed); Sp1 and Sp2, MW = 5894.9 (calculated), 5895.6 and 5895.7 (observed), respectively]. The synthetic AP site 19mer control and a 20mer oligonucleotide were used to calibrate the mass spectrometer, which allowed measurement of the MW to within 1 atomic mass unit of the calculated value. The stability of the lesions to the genome construction protocol was verified by MALDI-TOF mass spectrometry. Lesion-containing oligonucleotides were exposed to all reagents and conditions, with the omission of viral DNA and T4 polynucleotide kinase. After the incubations, mass spectrograms of Gh, Sp1, and Sp2 were essentially identical to those of the purified strands prior to genome construction. HPLC chromatograms and mass spectra for oligonucleotides containing each lesion are available as Supporting Information.

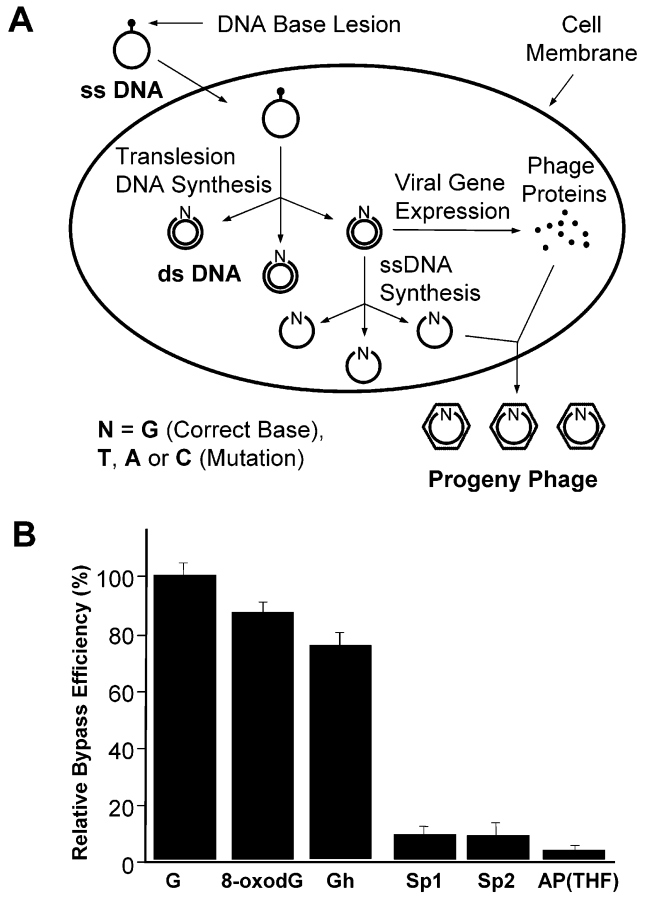

FIGURE 2: (A) Strategy for determination of in vivo translesion DNA polymerase bypass of site-specific lesions in a ss bacteriophage DNA [M13mp7(L2)]. A site-specifically adducted ss viral DNA was transfected into the host cell, followed by translesion DNA synthesis to afford a ds replicative form intermediate. Expression of gene products from the ds intermediate enabled the formation of infective progeny phage. Plating of the transformation mixture onto a lawn of generic bacteria immediately after transfection caused formation of plaques, whose number allowed for the calculation of the efficiency of polymerase bypass for each lesion relative to a guanine control. The base composition (N) at the site that had contained the lesion prior to transfection gave the mutation frequency and type. (B) Relative bypass efficiency data for Gh, Sp1, and Sp2. Controls for DNA polymerase bypass efficiency include G and 8-oxoG (well bypassed) and a synthetic THF AP site (blocking). The error bars represent a 95% confidence interval of the mean based on individual transfections from three independently constructed genomes.

DNA Polymerase Bypass Efficiency. Lesion bypass experiments in wild-type AB1157 *E. coli* were performed as described (*26*, *35*) in triplicate using genomes made from the purified oligodeoxynucleotides 5′-GCGAAGACCGX-AGCGTCCG-3′ (X is the lesion or guanine for the unmodified insert control), as shown in Figure 2. Briefly, 1 pmol of ss M13mp7(L2) was linearized by *Eco*RI digestion of a hairpin contained in the M13 polylinker region that interrupts the *lacZ* gene. Equimolar amounts of two "scaffolds" (oligonucleotides that are complementary to and span the 5′- and 3′-ends of both the vector and the lesion-containing insert) were annealed to the genome. Two scaffolds were used in order to leave a ss gap around the lesion that allowed the subsequent ligation efficiencies to be equal, regardless of the lesion employed. An equimolar amount of the 5′-phosphorylated 19mer insert was added and covalently joined into the genome by incubation with T4 DNA ligase (16 °C, 2 h). The ligation efficiencies were confirmed to be equal by electrophoresis of an aliquot of the ligation mixture on a 1% agarose gel, staining of the gel with ethidium bromide, and image analysis with digital photography (data not shown). AB1157 *E. coli* were made chemically competent with $CaCl_2$ and were immediately plated after transfection using 20 ng of ss viral DNA and $\sim 10^8$ cells as described (*26*). For each successful initial round of viral genome replication within each cell, viral progeny leaves a transfected AB1157 cell and infect nearby NR9050 *E. coli* (containing the F′ episome required for infection). The resulting localized infection slows the growth of the NR9050 cells, which is visible as a transparent spot (plaque) surrounded by a dense lawn of uninfected cells. A control for the presence of viable genomes that lacked an insert was made by constructing a genome containing a nonphosphorylated AP-containing 19mer insert. The number of plaques arising from this control was subtracted from the number of plaques derived from genomes containing 5′-phosphorylated inserts; the latter inserts contained the lesion of interest. The number of plaques formed by each lesion relative to that of the guanine control genome is indicated as "relative bypass efficiency", with the corrected guanine reference averaging 217 plaques per plate, as shown in Supporting Information.

Mutation Frequency and Type. Mutational analysis was performed using the recently developed *r*estriction *e*ndonuclease *a*nd *p*ostlabeling (REAP) assay with triplicate genome constructions and transfections for each lesion (*26*, *35*−*37*). Briefly, 100 μL of cells was transformed by electroporation (2.5 kV, 129 ohms, 2 mm gap cuvette) of 50 ng (1 ng/μL) of the constructed genome, after which they were added to 10 mL of LB media and incubated on a roller drum for 6 h at 37 °C. This amplified the $\sim 10^4$ initial events to $\sim 10^9$ progeny. The cells were pelleted, and the supernatant was decanted into 15 mL polypropylene tubes and stored at 4 °C. To eliminate PCR artifacts due the presence of nontransfected DNA, 50 μL of the phage suspension was added to 9 mL of LB and 50 μL of saturated SCS110 *E. coli*. The cells were grown an additional 4 h producing $\sim 3 \times 10^{11}$ phage, thus reducing the amount of nontransfected DNA template to less than 0.1% with respect to the template from progeny phage. We believe this subsequent infection will produce no change in the progeny population, since the genome was modified with the lesion in a nonessential gene, and reconstruction experiments in which progeny phage containing different mixtures of G and A at the lesion site retained their initial mixture composition after subsequent infection (*36*). Single-stranded viral template DNA was prepared using 700 μL of phage supernatant (QIAPrep spin M13 kit, Qiagen), from which 15% was used for each PCR reaction. As shown in Figure 3, the region that had contained the lesion in each progeny phage sample was PCR amplified to yield a 101 bp product whose purity was assayed by agarose gel electrophoresis. The resulting DNA duplex was cleaved with *Bbs*I (a type IIs restriction endonuclease that cleaves a fixed numbers of bases away from its binding site) at the position that had originally contained the lesion in the template vector, thus affording a 55mer whose newly formed 5′-end contained the lesion site. The 55mer was dephosphorylated with shrimp alkaline phosphatase, which allowed the lesion site at the 5′-overhang to be radiolabeled using PNK

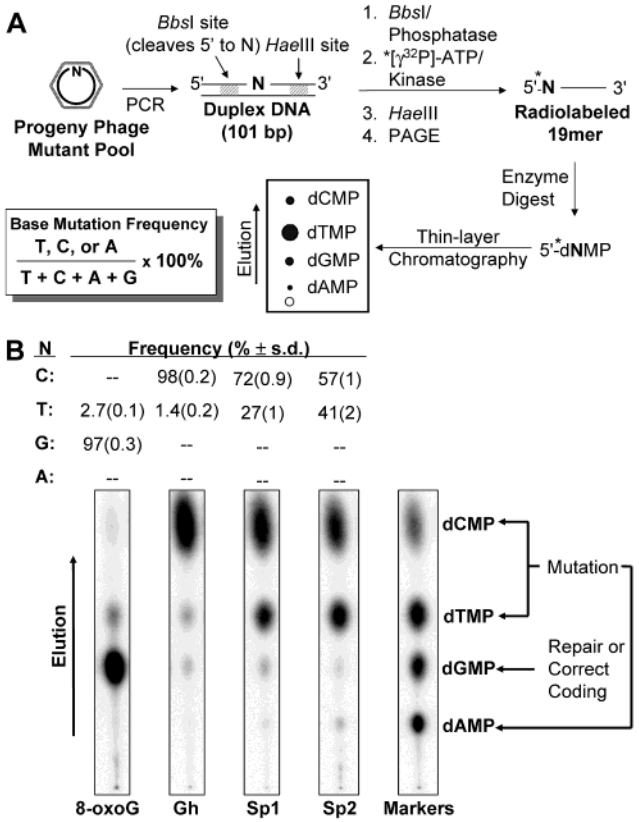

FIGURE 3: (A) Detection of mutations using the REAP assay. A site-specifically adducted genome was transfected into a repair-proficient *E. coli* strain to afford a population of progeny phage. PCR amplification of the region from the progeny phage genome that had originally contained the site-specific adduct was performed for each transformation. DNA was cleaved at the position that had contained the lesion in the parental vector using the restriction endonuclease *Bbs*I. The 5′-ends formed by *Bbs*I digestion were radiolabeled, and treatment with *Hae*III liberated a 19mer, from which the base composition at the 5′-end provided the mutation frequency. The 19mer was digested to 5′-dNMPs. Separation of the radioactive 5′-dNMPs on a TLC plate, followed by PhosphorImager analysis, provided the mutation frequency and type at the lesion site. (B) TLC showing mutation frequency analysis by the REAP assay for Gh, Sp1, and Sp2. Nucleotide insertion frequencies were determined in triplicate experiments, except for Sp2, which was performed in duplicate. The 8-oxoG lesion was included as a control of known mutation frequency and type, and 5′-dNMP markers were derived from digestion of a 13mer oligonucleotide that contained a radiolabeled degenerate 5′-end.

and [γ-^{32}P]ATP. Incubation with the restriction endonuclease *Hae*III yielded a 19mer, allowing for PAGE purification of the radiolabeled fragment of interest. Digestion of the desalted 19mer to 5′-deoxynucleotide monophosphates (5′-dNMPs) was followed by the partitioning of the mixture on a 20 × 20 cm polyethyleneimine TLC plate developed in saturated $(NH_4)_2HPO_4$ (adjusted to pH 6.1 with H_3PO_4). The separated radiolabeled nucleotides were quantified by PhosphorImager analysis, thus providing the fractional base composition of each nucleotide at the lesion site, from which the mutation frequency and type were determined.

RESULTS

The mutagenic potential of DNA lesions is described by two key metrics: (1) the ability of DNA polymerases to undergo translesion DNA synthesis (bypass efficiency) and (2) the resulting mutation frequency and type. Since the viral DNA used in this study was single-stranded, translesion DNA synthesis must have occurred in order for progeny phage to form in the *E. coli* cells. A comparison of the number of plaques (slow-growing colonies of successfully transfected bacteria on a lawn of infectable cells) formed by viral replication of lesion-bearing versus control genomes provided a measure of translesion bypass by DNA polymerases in the cell. The REAP assay was used to determine the identity of the base in the progeny DNA at the position that had contained the lesion, which gave the mutation frequency and type.

Translesion Bypass Efficiency. Since lesions in DNA may block or inhibit replication, the relative number of plaques formed from the immediate plating of the transformation mixture gives a measure of the efficiency of translesion DNA synthesis. A comparison of the number of plaques obtained after transformation of the damaged phage genome to that from an identically constructed vector that did not carry any oxidative damage allowed calculation of the bypass efficiency for each lesion. Transfections were performed in triplicate, and the bypass efficiency relative to the G control was calculated to within a 95% confidence interval of the mean for each lesion. As shown in Figure 2, vector survival for the 8-oxoG control was 87 ± 4% with respect to the G control, which clearly demonstrated that 8-oxoG only weakly inhibited replication. This result agrees, within experimental error, with previously reported values, both in *E. coli* and in mammalian cells (*10*, *11*, *38*−*41*). Synthesis past the secondary oxidative lesion Gh was effective for 75 ± 5% of the vectors, which was nearly the same as for 8-oxoG, and slightly less efficient than the G control. Sp1 and Sp2 were clearly more blocking to DNA polymerase bypass than Gh, with survival percentages of 9 ± 3% and 9 ± 4%, respectively. All of the lesion-containing genomes afforded enough progeny phage for mutation analysis (vide infra). As a control demonstrating DNA replication blockage, lesion survival for a synthetic tetrahydrofuran AP site was 4 ± 2%, which is somewhat higher than that reported for the natural aldehydic AP site placed site-specifically in a nearly identical bacteriophage system (*42*). In contrast, the bypass efficiency measured for the AP site is an order of magnitude lower than that reported for transfection of a plasmid carrying the AP site in a gapped region into cultured human cells (*43*), which may be due to ubiquitous expression of Y-family DNA error-prone bypass polymerases in mammalian cells whose homologues are present at negligible concentrations in uninduced *E. coli* (*44*). Since the 8-oxoG oxidation products are appreciably bypassed (especially the Gh lesion) by polymerases in *E. coli*, they are potential contributors to mutagenesis.

Mutation Frequency and Type. As shown in Figure 3, the mutation frequencies were determined alongside an 8-oxoG control, with radiolabeled 5′-dNMPs as markers for the relative migration of each nucleotide. The mutation frequency of 8-oxoG was modest, providing a 3% G → T transversion. In contrast to the low mutation frequency of 8-oxoG, the mutation frequencies were essentially 100% for Gh, Sp1, and Sp2. The well-bypassed lesion Gh caused 98% G → C and 2% G → T mutations. Interestingly, the same mutation frequencies were observed for the Sp steroisomers, but the mutation types were distributed differently, with G → C being the predominant mutation for both Sp1 and Sp2

stereoisomers. We observed 72% G → C and 27% G → T transversions for Sp1 and 57% G → C and 41% G → T transversions for Sp2.

DISCUSSION

8-OxoG is one of the most common oxidative DNA base lesions due to the low oxidation potential of guanine (*14*). The further oxidation of 8-oxoG after its formation from guanine is reasonable, since the adduct has a lower oxidation potential than the four normal nucleotides. Additionally, base radical cations (the initial products of one-electron oxidation of DNA bases) can migrate over long distances (>55 bp) in duplex DNA to become trapped preferentially at 8-oxoG sites (*45*−*48*), resulting in oxidation of the lesion (*48*). Oxidation of 8-oxoG results in the formation of the hydantoin derivatives described in the present study, as well as a variety of products such as cyanuric acid, oxaluric acid, and a 2,5-diaminoimidazalone, which undergoes slow hydrolysis to form oxazalone (*49*, *50*). The hydantoin products may also be obtained directly from guanine by use of four-electron oxidants such as singlet oxygen or type I photochemistry involving superoxide (*29*, *50*), which implicates them as general products of guanine oxidation in DNA.

We investigated the mutagenic potential of three significant products of peroxynitrite oxidized 8-oxoG in DNA: Gh, Sp1, and Sp2. Convenient synthesis of each defined lesion was enabled by reaction of an 8-oxoG-containing oligonucleotide with the oxidant Na_2IrCl_6 under conditions that favored nearly exclusive formation of either Gh or Sp from 8-oxoG. Each lesion was placed site-specifically into a bacteriophage genome and assayed for bypass efficiency and mutation type following transfection into *E. coli*. A modest mutation frequency (3%) was observed for 8-oxoG, as expected on the basis of literature values (*10*, *11*, *26*, *39*, *40*). The predominant mutation for this lesion was G → T. It is known that 8-oxoG, placed site-specifically in a ss DNA vector, is subject to repair in *E. coli*, since the mutation frequency increases approximately 10-fold in cells deficient in the base-excision repair glycosylases MutM (which catalyzes cleavage of a variety of oxidized guanine lesions from duplex DNA) and MutY (predominantly removes A opposite 8-oxoG) (*10*, *51*). Presumably, the repair occurs after the first round of replication, since both MutM and MutY require duplex substrates (*10*, *52*−*54*). In *E. coli*, it appears that no mechanism exists to restore the original G·C pair from the secondary oxidation products of 8-oxoG after translesion DNA synthesis, since there were negligible amounts of G in the position of the bacteriophage that had contained Gh or Sp, as measured by the REAP assay. Additionally, the lesions Gh, Sp1, and Sp2 are well-bypassed by the *E. coli* DNA polymerase(s). This combination of translesion bypass and miscoding properties apparently contributes to potent mutagenicity of the guanine oxidation products presented in this report. The ∼100% mutation frequencies observed may implicate these lesions collectively as a primary source for the frequently observed G → T and G → C transversions caused by the endogenous oxidation of DNA (*2*, *13*, *18*, *51*, *55*).

It is not known if the sequence context surrounding the lesions influences the mutagenesis of the adducts nor if nontargeted mutations can arise from Gh and Sp. Such a sequence context effect on mutation frequency is observed for 8-oxoG (*56*, *57*). Studies of context effects on Gh and Sp mutagenesis and toxicity would be useful. In this regard, the present work may shed light on the context-dependent mutagenesis induced by methylene blue plus UV light in studies by Loeb and co-workers (*58*). They observed frequent G → C mutations under conditions now known to produce abundant amounts of Sp (*31*).

Significant frequencies of small deletions or insertions would have been detected by the REAP assay, since the *Bbs*I−*Hae*III cleavage products would vary detectably in length. The homogeneity of the resulting 19mer, as assayed by PAGE, indicated that point, rather than frame-shift, mutations predominated in the sequence context evaluated (data not shown).

Lesions derived from 8-oxoG have yet to be detected in cells, but there is indirect evidence that their formation in DNA is relevant in cell-based mutagenesis studies. Juedes and Wogan investigated the mutagenicity of peroxynitrite using the *supF* gene of the pSP189 shuttle vector as a mutation target in bacterial and mammalian cells (*18*). Exposure to $ONOO^-$ caused the mutation frequency to increase 21-fold in the vector when replicated in *E. coli* MBL50 cells and 9-fold upon replication in human Ad293 cells compared to untreated vector. In both systems, mutations occurred at G·C base pairs, predominantly involving G → T transversions (65% when replication was in bacteria and 63% when in human cells). However, G → C transversions were observed but at a lower frequency (28% in MBL50 and 11% in Ad293 cells). In a related study, Termini and co-workers reported that, upon transfection of peroxy radical exposed bacteriophage DNA into *E. coli* (*55*), approximately 88% of the mutant progeny consisted of nearly equal numbers of G → T and G → C transversions. The authors could not detect 8-oxoG by HPLC/electrochemical analysis, suggesting that the G → T transversions were not caused by this base lesion. The work presented here provides at least a partial explanation for the mutation spectrum observed in the *supF* and bacteriophage mutagenesis studies. Other lesions that may be responsible for G → C transversions include the 2-amino-5-[(2′-deoxy-*β*-D-*erythro*-pentofuranosyl)amino]-4*H*-imidazol-4-one derivative (Iz), which is formed from the oxidation of guanine and 8-oxoG (*59*). Iz is known to pair preferentially with G during primer extension with Pol I and, therefore, may be a contributor to G → C transversions in vivo (*60*).

Single nucleotide primer extension studies carried out using the Klenow fragment (exo^-) recently showed that both Gh- and Sp-containing DNA templates directed insertion of dAMP and dGMP opposite the lesions (*61*). Both Gh and Sp showed nearly the same pattern of insertion of nucleotides opposite the lesions, always with an approximately 2:1 preference for dAMP over dGMP. It is noteworthy that, in vivo, nearly exclusive dGMP incorporation occurs opposite Gh, while Sp lesions exhibit a mixture of dAMP and dGMP insertion opposite the lesion but with an opposite preference of dGMP incorporation with respect to the in vitro primer extension work. Perhaps there is an unknown repair pathway operating on Gh·A or Sp·A base pairs, since recent studies in the David group have indicated that the adenine opposite the lesions is not a substrate for MutY (*34*). An alternative explanation is that the replicative DNA polymerase(s) in *E.*

coli has (have) different dNTP incorporation preferences opposite Gh and Sp than the Klenow fragment. It would be interesting to determine the molecular basis for the dGMP nucleotide insertion preference for the hydantoin lesions with respect to the many oxidative lesions such as Oa, Ca, and Oz, which prefer dAMP incorporation (nearly exclusively for Oa and Ca) in vivo (*26*).

Guanine oxidation results predominantly in the formation of 8-oxoG, Gh, and Sp, as well as a variety of other products. Indeed, oxidation of 8-oxoG has been shown to be a chemically favorable event, and its oxidation products also include Gh and Sp. We report that the hydantoin products Gh, Sp1, and Sp2 are much more mutagenic in vivo than 8-oxoG, thus demonstrating that lesions arising from facile oxidation of dG and 8-oxoG may contribute to the observed G → T and G → C transversions upon replication of oxidized DNA in *E. coli* and mammalian cells.

SUPPORTING INFORMATION AVAILABLE

HPLC chromatograms and mass spectra for oligonucleotides containing each lesion and bypass data for G, Gh, Sp1, Sp2, and AP. This material is available free of charge via the Internet at http://pubs.acs.org.

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