Chemical Research in Toxicology

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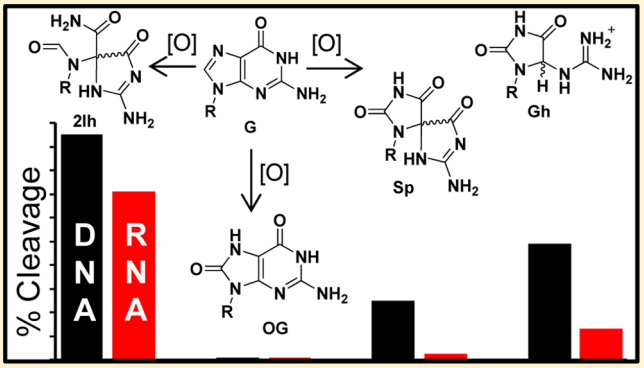
# Rates of Chemical Cleavage of DNA and RNA Oligomers Containing Guanine Oxidation Products

Aaron M. Fleming, Omar Alshykhly, Judy Zhu, James G. Muller, and Cynthia J. Burrows*

Department of Chemistry, University of Utah, 315 South 1400 East, Salt Lake City, Utah 84112-0850, United States

Supporting Information

**ABSTRACT:** The nucleobase guanine in DNA (dG) and RNA (rG) has the lowest standard reduction potential of the bases, rendering it a major site of oxidative damage in these polymers. Mapping the sites at which oxidation occurs in an oligomer via chemical reagents utilizes hot piperidine for cleaving oxidized DNA and aniline (pH 4.5) for cleaving oxidized RNA. In the present studies, a series of time-dependent cleavages of DNA and RNA strands containing various guanine lesions were examined to determine the strand scission rate constants. The guanine base lesions 8-oxo-7,8-dihydroguanine (OG), spiroiminodihydantoin (Sp), 5-guanidinohydantoin (Gh), 2,2,4-triamino-2*H*-oxazol-5-one (Z), and 5-carboxamido-5-formamido-2-iminohydantoin (2Ih) were evaluated in piperidine-treated DNA and aniline-treated RNA. These data identified wide variability in the chemical lability of the lesions studied in both DNA and RNA. Further, the rate constants for cleaving lesions in RNA were generally found to be significantly smaller than for lesions in DNA. The OG nucleotides were poorly cleaved in DNA and RNA; Sp nucleotides were slowly cleaved in DNA and did not cleave significantly in RNA; Gh and Z nucleotides cleaved in both DNA and RNA at intermediate rates; and 2Ih oligonucleotides cleaved relatively quickly in both DNA and RNA. The data are compared and contrasted with respect to future experimental design.




## INTRODUCTION

Mapping nucleobases in DNA or RNA modified by alkylation or oxidation often relies on the ability to specifically cleave the oligonucleotide at the site of modification, followed by gel electrophoresis to develop the map. A method established by Maxam and Gilbert utilizes base-specific alkylation followed by hot piperidine cleavage as the key steps in sequencing the DNA bases.[1] The success of this method lies in the ability to specifically alkylate the DNA bases, as well as having high yields in the hot piperidine cleavage step. A similar base-specific labeling protocol followed by chemical cleavage has been advanced for sequencing RNA;[2,3] however, the inherent alkaline instability of RNA requires substitution of piperidine at pH 11 with aniline at pH 4.5. These methodologies developed a framework for identifying other labile sites of modification in DNA and RNA, for example, those resulting from base oxidation.[4,5] Site-specific oxidation of nucleobases, if left unrepaired, can lead to mutations that are proposed to cause a wide range of diseases such as Alzheimer's disease,[6] melanoma,[7] prostate cancer,[8] and diabetes.[9] Many research efforts have focused on understanding the mechanisms and products that result from oxidation of the nucleobases in DNA oligomers to develop hypotheses that explain cellular mutagenesis.[4,10−14]

The nucleobase guanine stands out as a prominent site of oxidation because it has the lowest standard reduction potential.[15] In DNA, oxidation of 2′-deoxyguanosine (dG) is modulated by the context of the reaction (i.e., single-stranded, duplex, or G-quadruplex contexts).[16−18] Moreover, in duplex DNA, the reactivity of dG toward oxidation shows a subtle sequence context effect.[19] Locating site-specific oxidation at dG nucleotides in DNA oligomers is determined by hot piperidine work up followed by gel electrophoresis to read the damaged sites in comparison to a control ladder. Another approach to identifying damage sites utilizes the base excision repair enzyme Fpg for cleavage at the lesion.[19] In RNA, oxidation of guanosine (rG) has been studied, but to a lesser degree than DNA oxidation.[20,21] For the chemical cleavage methods to be successful at mapping oxidation sites, it is critical to know the rates and extent to which scission of the strand occur for different oxidation products of dG and rG.

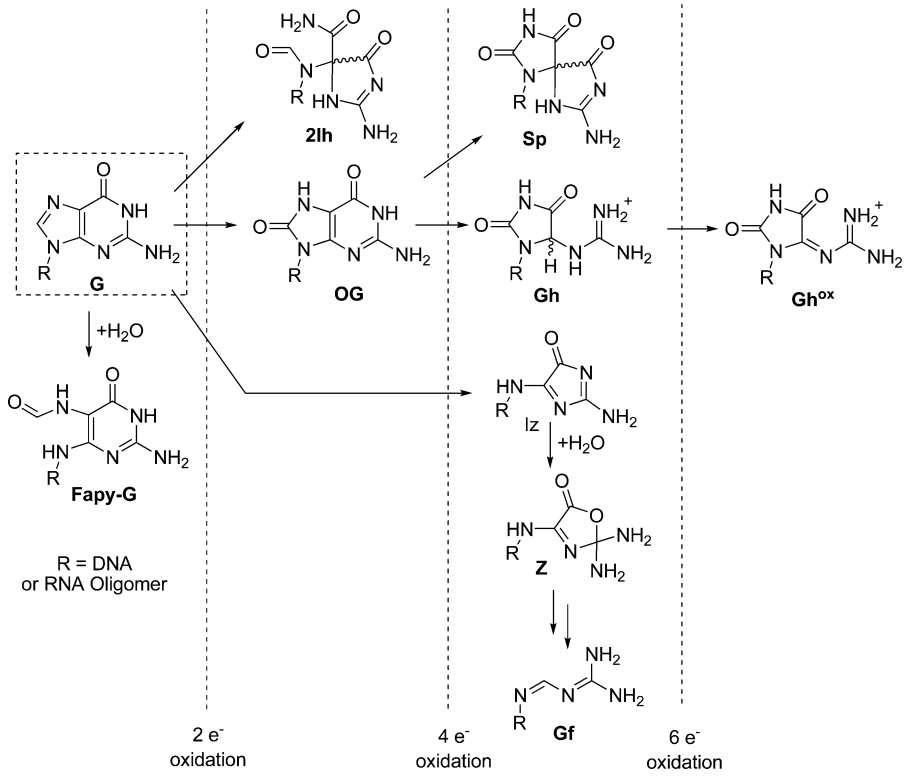
Oxidation of the guanine heterocycle yields classes of products based on the extent of oxidation. Most studies characterized products in DNA models, e.g., nucleosides. Two-electron oxidation of dG yields 8-oxo-7,8-dihydro-2′-deoxyguanosine (dOG) and 5-carboxamido-5-formamido-2-iminohydantoin-2′-deoxyribonucleoside (d2Ih),[16,22−30] while four-electron oxidation of dG yields two diastereomers of spiroiminodihydantoin-2′-deoxyribonucleoside (dSp), two dia-

Received: March 8, 2015
Published: April 8, 2015

DOI: 10.1021/acs.chemrestox.5b00096
Chem. Res. Toxicol. 2015, 28, 1292−1300

**Scheme 1. Pathways for Oxidation of the Guanine Heterocycle**




stereomers of 5-guanidinohydantoin-2′-deoxyribonucleoside (dGh), and 2,5-diaminoimidazolone-2′-deoxyribonucleoside (dIz) that readily hydrolyzes to 2,2,4-triamino-2*H*-oxazol-5-one-2′-deoxyribonucleoside (dZ).[22,31−42] The six-electron oxidation of dG yields dehydroguanidinohydantoin-2′-deoxynucleoside (dGh$^{ox}$), a compound of limited stability that ultimately decomposes to a ribosyl-urea lesion.[14,43−45] Another product that is formed during certain oxidations, but is not formally oxidized, is 2,6-diamino-4-hydroxy-5-formamidopyrimidine-2′-deoxyribonucleoside (Fapy·dG), a ring-opened hydrolysis product of dG (Scheme 1).[28,46] The same products have been observed in oxidations of rG.[38,47] Many of these lesions resulting from dG oxidation have been observed in genomic samples of biological origin,[48−52] except d2Ih and dGh$^{ox}$, whose cellular existence is awaiting confirmation. In cellular RNA samples, rOG is the only lesion that has been characterized thus far.[49] Detection of these lesions in cellular samples typically occurs by nuclease digestion that leads to complete loss of sequence information, followed by mass spectrometry.[49,51,52] Therefore, *in vitro* model studies with DNA or RNA oligomers allow understanding the sequence context effects on lesion formation. The standard approach for identification of lesion sites requires strand cleavage by piperidine (DNA) or aniline (RNA) followed by gel electrophoresis;[17,19,20] another method utilizes ESI-MS,[43] but this approach is limited to short oligomers with only one possible site of modification.

Oxidations conducted on DNA oligomers of known sequences have yielded a wealth of information concerning the sequence context-dependent reactivity of dG toward oxidants and the products.[4,10−14] For interpretation of these data from the point of view of biology, the oxidation reactions are conducted under low product conversion (i.e., single-hit chemistry).[4] Typically, the reaction yield is determined by hot piperidine cleavage of the oligomers using a standard protocol (0.2−1 M piperidine, 30 min, 90 °C);[4] however, the yields and rates for cleavage of the consistently observed dG oxidation products have not been established under identical reaction conditions. Other amines have been substituted for piperidine with variable results.[53,54] Ligation-mediated PCR is an approach for determining lesion sites in genomic samples that relies on the ability to site-specifically cleave the lesion for ligation on a primer sequence used to identify the original lesion location.[55] In this method, chemical cleavage is one approach for effecting strand scission at the lesion site. In RNA, cleavage at sites of base modification sites employs aniline following a standard procedure (0.2−1 M, pH 4.5, 60 °C, 20 min).[5] As for DNA, strand cleavage efficiencies are not known for rG oxidation products in RNA oligomers. Because rates of chemical strand scission for dG and rG lesions have not been established, the extent of an oxidation reaction on DNA or RNA polymers is inaccurately determined. In the present study, the rates of cleavage for OG, Sp, Gh, 2Ih, and Z were determined in DNA oligomers treated with piperidine, and in RNA oligomers treated with aniline under standard reaction conditions. These data determined that lesion cleavage rates using typical reaction conditions are quite variable, and some are not significantly cleaved, even at long incubation times. These data will be paramount for researchers that oxidize oligomers of DNA or RNA with known sequences and then interpret the reaction yields from a biological prospective, for which oxidation events on these polymers are a rare occurrence.

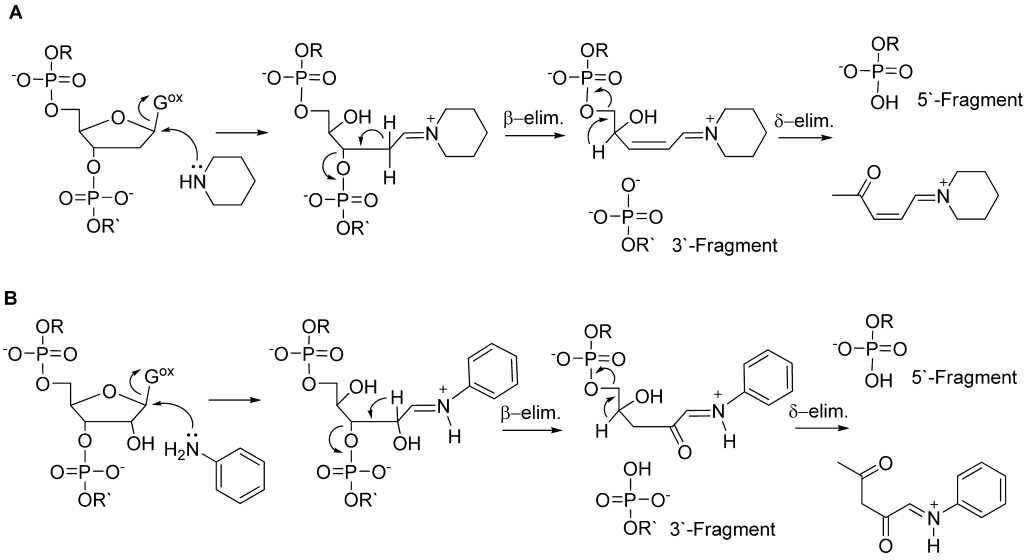
## MATERIALS AND METHODS

**Oligomer Synthesis.** The dG oxidation products dOG, (*S*)-dSp, (*R*)-dSp,[56,57] and a mixture of the dGh diastereomers were synthesized in the 18-mer DNA oligomer 5′-TCA TGG GTC **X**TC GGT ATA-3′ (where **X** = lesion site). The DNA oligomer was synthesized via solid-



DOI: 10.1021/acs.chemrestox.5b00096
*Chem. Res. Toxicol.* 2015, 28, 1292−1300

**Scheme 2. Proposed Pathways for (A) Piperidine Cleavage of DNA Lesions and (B) Aniline Cleavage of RNA Lesions**




phase synthesis by the DNA/peptide core facility at the University of Utah with a dOG phosphoramidite (Glen Research, Sterling, VA) at the position of **X** following standard methods. Analytical ion-exchange HPLC-purified samples provided the dOG-containing DNA oligomer for the piperidine studies. This strand also provided the starting material for the synthesis of the hydantoins. Syntheses of the dSp diastereomers and dGh diastereomers were conducted following literature protocols.[58] The dSp diastereomers were individually purified while the diastereomers of dGh were studied as a mixture because they readily interconvert.[59] Syntheses of dZ and the (*R*)- and (*S*)-d2Ih[60] diastereomers were achieved in a 15-mer DNA oligomer containing a single dG site (5′-AAT CCA CGA CAC CTC-3′) following literature methods.[17,24] All product oligomers were purified using an analytical ion-exchange HPLC column that resolves diastereomeric products (Figure S1). The DNA oligomer products were characterized by ESI-MS (dOG: calcd 5560.6, found 5560.8; mixture of the dSp diastereomers: calcd 5576.6, found 5576.4; mixture of the dGh diastereomers: calcd 5550.6, found 5550.2), or MALDI-MS was used to characterize a mixture of the d2Ih diastereomers as well as for dZ (d2Ih: calcd 4498.8, found 4498.2; dZ: calcd 4445.8, found 4445.3). The dZ-containing oligomer was found to have limited stability due to further decomposition of dZ to guanidinoformimine-2′-deoxyribonucleoside (dGf).[61] The dGf-containing strand was characterized by MALDI-MS (dGf: calcd 4398.8; found 4398.2). Because of the limited stability of dZ in the DNA oligomer leading to dGf, the dZ sample was studied immediately after purification and a 2 h dialysis to remove the purification salts.

The rG-oxidation products rOG, (*R*)-rSp, (*S*)-rSp, rZ, (*R*)-r2Ih, (*S*)-r2Ih, and rGh diastereomers were synthesized in the 7-mer RNA oligomer 5′UUU**G**UUU-3′. Selective conversion of the single rG to the rSp diastereomers, rGh diastereomers, r2Ih diastereomers, and dZ was achieved via literature protocols.[17,24] These strands were then purified by the same method as stated for the lesions in the DNA oligomers (Figure S2). The 7-mer containing rOG was synthesized using the phosphoramidite of rOG (Chem Genes, Wilmington, MA) following the manufacturer's protocol. The RNA oligomer products were characterized by MALDI-MS (rOG: calcd 2136.3, found 2136.7; (*S*)-rSp and (*R*)-rSp diastereomer mixture: calcd 2152.3, found 2153.0; rGh diastereomer mixture: calcd 2126.3, found 2126.1; rZ: calcd 2099.3, found 2099.4; (*R*)-r2Ih and (*S*)-r2Ih diastereomer mixture: calcd 2154.4, found 2154.3).

**Piperidine Cleavage Reaction Conditions for Lesions in DNA Oligomers.** To monitor the cleavage reactions, the lesion-containing DNA oligomers were 5′-labeled with $^{32}$P for visualization by gel electrophoresis followed by phosphorimager autoradiography. The labeling reactions were conducted following literature protocols.[37] Piperidine cleavage reactions for lesion-containing DNA oligomers were conducted by mixing 100 pmol of unlabeled strand with 20 000 cpm of $^{32}$P-labeled strand in 50 μL of $ddH_2O$. For each lesion, nine tubes were prepared for taking time points at 0, 5, 10, 20, 30, 45, 60, 90, and 120 min. To these samples were added 50 μL of freshly prepared aqueous piperidine (2 M) and *β*-mercaptoethanol (BME, 0.5 M) to give a final concentration of 1 μM DNA with 1 M piperidine and 0.25 M BME. Next, the samples were incubated at 90 °C, and at each time point, a sample was removed from the heat source. The samples were lyophilized to dryness and resuspended in 10 μL of gel loading dye (0.25% xylene cyanol, 0.25% bromophenol blue in an aqueous 30% v/v glycerol solution). To a 20% denaturing polyacrylamide gel electrophoresis (PAGE), 5 μL of sample in loading dye was added. The samples were electrophoresed at 45 W for 2 h, after which the gel was loaded into a phosphor screen and visualized by phosphorimager autoradiography after an 18 h exposure. The band intensities were inspected and quantified using ImageQuant software. One reaction was also monitored by ion-exchange HPLC on a sample that was not $^{32}$P labeled that allowed observation of all species in solution during the reaction. The method utilized was the same as the one used for product purification.

**Aniline Cleavage Reaction Conditions for Lesions in RNA Oligomers.** To monitor the cleavage reactions, the lesion-containing RNA oligomers were 5′-labeled with $^{32}$P for visualization by gel electrophoresis followed by phosphorimager autoradiography. The labeling reaction was conducted following literature protocols.[37] Aniline cleavage reactions for lesion-containing RNA oligomers were conducted by mixing 100 pmol of unlabeled strand with 20 000 cpm of $^{32}$P-labeled strand in 50 μL of $ddH_2O$. For each lesion, seven tubes were prepared for taking time points at 0, 10, 20, 30, 40, 60, and 90 min. To these samples was added 50 μL of freshly prepared aniline (pH 4.5, 2 M) to furnish a final concentration of 1 μM RNA and 1 M aniline. The aniline at pH 4.5 was prepared by taking a solution of reagent grade aniline and adjusting the pH to 4.5 with glacial acetic acid to obtain a 2 M stock solution. Next, the samples were incubated at 60 °C in the dark, and at each time point, a sample was removed from the heat source. After completion of the reactions, the samples were processed in an identical fashion as previously described for the lesion-containing DNA oligomers.

## RESULTS AND DISCUSSION

**Efficiency of Piperidine Cleavage of dG Lesions in DNA Oligomers.** Scheme 2A shows the proposed mechanism for piperidine-induced strand scission at the site of a DNA lesion.[4] In the first step, piperidine nucleophilically displaces


DOI: 10.1021/acs.chemrestox.5b00096
*Chem. Res. Toxicol.* 2015, 28, 1292−1300

the modified base yielding a Schiff-base intermediate. This intermediate increases the acidity of H2′ that accelerates β-elimination of the 3′-phosphate terminus in the second step. In the third step, δ-elimination of the 5′-phosphate terminus occurs to yield complete cleavage at the site of the lesion. The correct kinetic rate equation for this complex reaction should take into account all intermediate reactions; however, in practice, piperidine cleavage reactions are followed by PAGE, in which only the intact strand and the cleaved strand are observed. By monitoring loss of the starting material and the appearance of the product, the rate equation can be simplified to a pseudo-first-order rate equation. The assumptions in this simplified approach are that the piperidine concentration does not change, as is validated by the large excess of piperidine used to effect strand scission ($10^6$-fold excess). The second simplifying assumption was the use of the steady-state approximation, in which the concentrations of the reaction intermediates were assumed to reach a steady state, and their concentrations were limited by the rate of the first reaction step. Verification of this assumption was applied to a test reaction that monitored the piperidine cleavage of a (*R*)-dSp-containing DNA oligomer by HPLC. This approach allowed inspection of all species in solution, which is not possible by PAGE. In this analysis, the intact strand, cleavage products (5′ and 3′ fragments), and intermediates were observed. The intermediates were not characterized due to their instability. In support of the steady-state approximation, the intermediate peak ratios did not significantly change during the 90 min reaction profile, while the starting material decreased and the product strands increased (Figure S3). To monitor cleavage reactions for all lesions, the traditional approach of monitoring these reactions by PAGE was selected, and a pseudo-first-order reaction rate equation was applied for mathematically modeling. The rate-limiting step was assumed to be formation of the Schiff-base intermediate. Thus, plots of the ln [intact strand] vs reaction time provided linear data points that were described by eq 1, where the slope of the line provides the reaction rate constant.

$$\ln[\text{intact strand}] = -kt \quad (1)$$

Monitoring the time-course cleavage for the guanine oxidation products studied in a DNA oligomer by PAGE gave a wide distribution of reaction course profiles (Figure 1A and Figure S4). For example, the dOG lesion was not significantly cleaved (<1%) by hot piperidine during the 2 h reaction (Figure 1A). Next, the dSp diastereomers were found to have undergone slow hot piperidine strand scission (Figure 1A), with rate constants for the isomers to be nearly identical values of $(9.5 \pm 1.5) \times 10^{-3}$ and $(9.5 \pm 1.2) \times 10^{-3}$ $\text{min}^{-1}$ for the *R* and *S* isomers, respectively (Table 1). For dGh, strand scission by hot piperidine was faster, with a cleavage rate constant of $(22 \pm 3) \times 10^{-3}$ $\text{min}^{-1}$ (Table 1), while the dZ lesions were cleaved with a rate constant of $(51 \pm 5) \times 10^{-3}$ $\text{min}^{-1}$ (Table 1). Finally, the diastereomers of d2Ih cleaved the quickest with rate constants of $(100 \pm 10) \times 10^{-3}$ and $(110 \pm 11) \times 10^{-3}$ $\text{min}^{-1}$ for the *R* and *S* isomers, respectively (Table 1). These studies demonstrate that the dG oxidation products, other than dOG, cleave with hot piperidine at variable rates, and near-quantitative reaction yields can be obtained if the reactions are allowed to progress long enough.

The pseudo-first-order kinetic rate constants allow determination of the half-life ($t_{1/2}$) of each lesion under the reaction conditions. These values will allow researchers to calculate the reaction time required to achieve the yield they would desire. The dOG lesion in DNA cannot be found by piperidine cleavage reactions, and this point is further discussed below. As for the other dG lesions in DNA, the $t_{1/2}$ values were 73 min for the dSp diastereomers, 32 min for the dGh diastereomers, 14 min for dZ, 6.8 min for (*R*)-d2Ih, and 6.0 min for (*S*)-d2Ih (Table 1). On the basis of these results, all dG-lesions, besides dOG, can be cleaved in high yield as long as the reactions are allowed to progress for a sufficiently long time period.

**Efficiency for Aniline Cleavage of rG Lesions in RNA Oligomers.** Scheme 2B shows the proposed mechanism for aniline-induced strand scission at the site of a RNA lesion.[5] Amine-assisted cleavage at sites of DNA and RNA lesions are similar. Aniline nucleophilically displaces the lesion yielding a Schiff-base intermediate. This intermediate increases the acidity of H2′ that accelerates β-elimination of the 3′-phosphate terminus. Finally, δ-elimination of the 5′-phosphate terminus occurs to yield complete cleavage at the site of the lesion. Because of the similarity in the cleavage mechanisms, and the fact that the same concentration of aniline was used to cleave RNA lesions as was used for piperidine to cleave DNA lesions, the same assumptions were used to fit the data to determine the pseudo-first-order reaction rate constants (eq 1).

The rates of aniline-mediated cleavage (1 M, pH 4.5, 60 °C) for all rG lesions were assayed by following strand cleavage via PAGE analysis. Under these reaction conditions, rOG showed <1% cleavage after a 90 min reaction (Figure 1B). The diastereomers of rSp gave slightly more cleavage than rOG with <10% strand scission. The fitting equations to these data determined the cleavage rate constants for the *R* and *S* diastereomers of rSp to be $(1.2 \pm 0.2) \times 10^{-3}$ and $(1.4 \pm 0.2) \times 10^{-3}$ $\text{min}^{-1}$, respectively (Table 1). Next, the diastereomers of rGh were found to give more strand scission under these conditions (Figure 1B). For rGh, the aniline cleavage rate constant was $(6.9 \pm 0.5) \times 10^{-3}$ $\text{min}^{-1}$ (Table 1). The rZ reaction preceded more quickly giving a reaction rate constant of $(51 \pm 6) \times 10^{-3}$ $\text{min}^{-1}$ (Table 1). Finally, the r2Ih diastereomers cleaved the fastest (Figure 1B) with the *R* and *S* isomers having reaction rate constants of $(61 \pm 7) \times 10^{-3}$ and $(63 \pm 7) \times 10^{-3}$ $\text{min}^{-1}$, respectively (Table 1). These studies demonstrate that site specific cleavage of rG oxidation lesions in RNA were slower than the analogous cleavage reactions in DNA with piperidine.

The pseudo-first-order reaction rate constants for cleavage of rG lesions in RNA allow calculation of the $t_{1/2}$ values for these lesions by aniline. On the basis of these rate constants, the $t_{1/2}$ for the rSp diastereomers were calculated to be 580 and 500 min for the *R* and *S* isomers, respectively (Table 1). These lifetimes are too long to be usable for detection of rSp lesions in RNA, and therefore, support rSp as not being cleavable by aniline in any practical applications. For the other rG lesions, the $t_{1/2}$ values were determined to be 100 min for rGh, 14 min for rZ, and 11 min for the diastereomers of r2Ih.

**Implication of These Reaction Rate Constants on Future Experimental Design.** In the current studies, dOG was not piperidine labile, an observation that has stirred some debate in the literature.[4,62−64] The BME added during the reaction is hypothesized to quench unwanted oxidation of dOG.[63,64] To confirm this observation, piperidine reactions were conducted without BME. After a 2 h incubation (1 M piperidine, 90 °C), ∼10% of the dOG-containing strand cleaved based on PAGE analysis, thus supporting the


DOI: 10.1021/acs.chemrestox.5b00096
Chem. Res. Toxicol. 2015, 28, 1292−1300

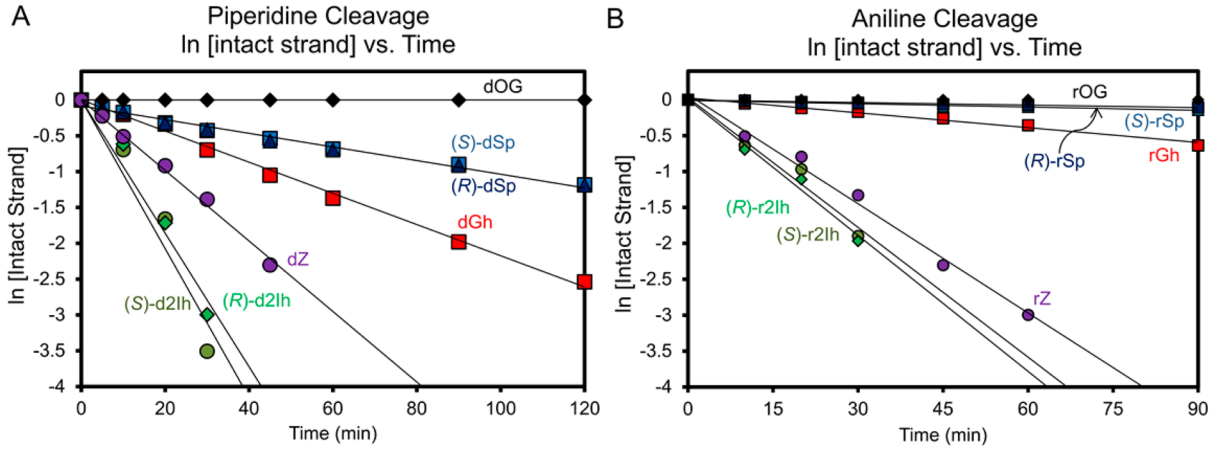



**Figure 1.** Plots of ln[intact strand] vs time for dG oxidation products in DNA (A) and rG oxidation products in RNA (B) oligomers. (A) Time-dependent cleavage yields for the DNA lesions, dOG, (*R*)-dSp, (*S*)-dSp, dGh, dZ, (*R*)-d2Ih, and (*S*)-d2Ih are plotted. The reaction yields were monitored on 5′-$^{32}$P-labeled strands that were separated by PAGE and quantified by phosphorimager autoradiography. Cleavage reactions for these damaged DNA oligomers were conducted with fresh, aqueous piperidine (1 M) and BME (0.25 M) at 90 °C. (B) Time-dependent cleavage yield for the RNA lesions, rOG, (*R*)-rSp, (*S*)-rSp, rGh, rZ, (*R*)-r2Ih, and (*S*)-r2Ih are plotted. The reactions were monitored in the same fashion as those for the DNA oligomers. Cleavage reactions for the damaged RNA oligomers were conducted with fresh, aqueous aniline (pH 4.5) at 60 °C in the dark. Based on triplicate trials, errors for the measurements were ±8%.

**Table 1. Rate Constants for Chemical Cleavage of Guanine Oxidation Products in DNA and RNA Oligomers and the Half-Life for Cleavage of Each Product**

| | DNA oligomer | | RNA oligomer | |
|---|---|---|---|---|
| lesion | rate constant ($\times 10^{-3}$ min$^{-1}$) | $t_{1/2}$ (min) | rate constant ($\times 10^{-3}$ min$^{-1}$) | $t_{1/2}$ (min) |
| OG | – | – | – | – |
| (*R*)-Sp | 9.5 ± 1.5 | 73 | 1.2 ± 0.2 | 580 |
| (*S*)-Sp | 9.5 ± 1.2 | 73 | 1.4 ± 0.2 | 500 |
| Gh | 22 ± 3 | 32 | 6.9 ± 0.5 | 100 |
| Z | 51 ± 5 | 14 | 51 ± 6 | 15 |
| (*R*)-2Ih | 100 ± 10 | 6.8 | 61 ± 7 | 11 |
| (*S*)-2Ih | 110 ± 11 | 6.0 | 63 ± 7 | 11 |

hypothesis that dOG can be oxidized during the cleavage reaction, albeit slowly. Analysis of a test reaction by HPLC confirmed dOG to slowly oxidize to dSp under the hot piperidine reaction conditions (Figure S5), an expected result because at high pH oxidation of dOG yields dSp.[36] Because dOG is poorly cleaved by hot piperidine, one approach for sequencing this lesion via chemical cleavage has been developed by our laboratory.[64] In this method, dOG is further oxidized to dGh with a selective and mild one-electron oxidant, such as $Na_2IrCl_6$, and the corresponding secondary hydantoin lesion is much more labile to piperidine (Figures 1A and Table 1). The current results provided additional support that dOG is not piperidine labile.[63]

The hydantoins dSp and dGh were both found to be labile to piperidine, but with considerably different rate constants (dSp = $9.5 \times 10^{-3}$ min$^{-1}$; dGh = $22 \times 10^{-3}$ min$^{-1}$; Table 1). Further, the *R* and *S* isomers of dSp were studied individually and shown to give similar cleavage rates with hot piperidine (Figure 1A and Table 1). The standard piperidine reaction time is 30 min that will give 25% and 48% strand scission for the diastereomers of dSp and dGh, respectively (Figure 2A); the incomplete strand scission currently observed for these lesions after a 30 min piperidine reaction is consistent with previous literature reports.[64,65] To effect strand scission in a yield >90% for the hydantoins, the piperidine reaction times must be >242

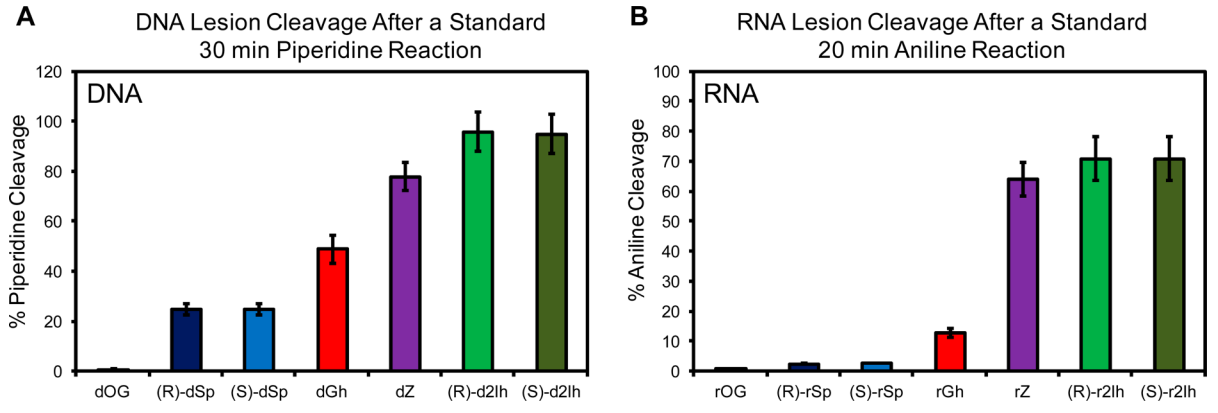



**Figure 2.** Cleavage efficiency for dG lesions (A) and rG lesions (B) following the standard chemical cleavage protocols. (A) The dG lesion-containing oligomers were incubated following the standard cleavage recipe that includes fresh, aqueous 1 M piperidine and 0.25 M BME at 90 °C for 30 min. (B) The rG lesion-containing oligomers were incubated following the standard cleavage recipe that includes fresh, aqueous 1 M aniline (pH 4.5) at 60 °C for 20 min in the dark.



DOI: 10.1021/acs.chemrestox.5b00096
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min and >106 min for dSp and dGh, respectively (Figure 3A). As for dZ after a 30 min reaction, the cleavage yield would be

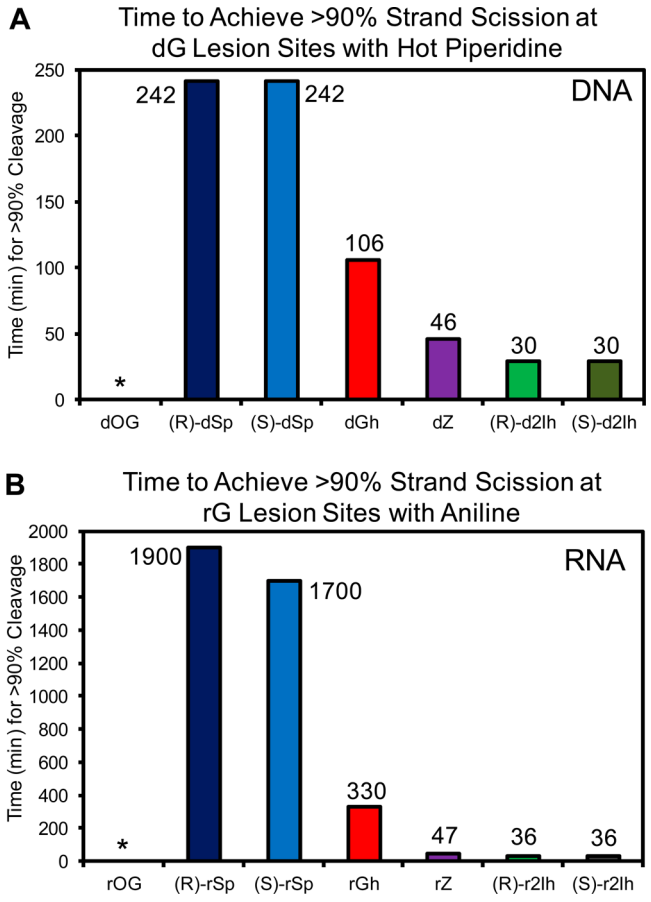


**Figure 3.** Minimum reaction time to achieve >90% cleavage at dG lesions in DNA oligomers (A) and at rG lesions in RNA oligomers (B). The chemical cleavage of lesion-bearing DNA oligomers was conducted with freshly prepared, aqueous piperidine (1 M) and BME (0.25 M) at 90 °C, and the lesion-bearing RNA oligomers was conducted with freshly prepared, aqueous aniline (pH 4.5) at 60 °C in the dark. The reaction times were computed from the strand scission rate constants values for these lesions provided in Table 1. *These lesions were shown to be resistant to chemical cleavage (Table 1).

78%, an observation that is nearly identical to literature reports (80% cleavage after 30 min).[66] Furthermore, extension of the reaction time to >46 min will lead to >90% cleavage at dZ sites (Figure 3A). Finally, the d2Ih diastereomers will cleave by >95% for both diastereomers (Figure 2A), and the standard 30 min piperidine cleavage reaction time will sufficiently identify d2Ih sites (Figure 3A). Only one sequence context was studied for each G lesion in DNA, and sequence context effects on reaction rates may exist; however, it is anticipated that these effects will be minimal at pH 11 and 90 °C. Other reagents that cleave DNA lesions at lower pH have been utilized, such as *N*,*N*′-dimethylethylenediamine (0.1 M) at pH 7.4 and 37, 65, or 90 °C.[54] Studies were conducted to determine if this reagent could cleave dOG, dSp, or dGh under the reported conditions (0.1 M, pH 7.4, and 65 °C). These reactions were monitored by HPLC and found that *N*,*N*′-dimethylethylenediamine was incapable of cleaving all three lesions at reaction times <2 h (Figure S6). Studies with d2Ih and dZ were not conducted. The current results provide data that can benefit future experimental design.

Chemical cleavage of rG lesions in RNA was determined to have lower rate constants for all lesions compared to dG lesions in DNA. For example, the reaction rate for aniline cleavage of the rSp diastereomers was very slow, and almost undetectable; these reaction rate constants determined that after a standard 20 min aniline reaction time, ∼3% of the rSp diastereomers would cleave (Figure 2B). The aniline reaction time would need to be increased to 1900 and 1700 min for the *R* and *S* diastereomers of rSp, respectively, to effect >90% strand scission (Figure 3B). These reaction times are not practically useful, effectively causing the rSp isomers to be resistant to aniline treatment. In comparison, the rGh isomers will cleave 13% after the standard aniline reaction time (Figure 2B), and the reaction time would need to be extended to >330 min to effect >90% strand scission yield at these sites (Figure 3B). Next, rZ would cleave by 64% under the standard reaction time (Figure 2B), and extension of the cleavage reaction to >47 min will facilitate >90% cleavage at these sites (Figure 3B). Finally, 71% of the r2Ih diastereomers will cleave under the standard reaction time (Figure 2B); for these lesions, extending the reaction time to >37 min will lead to >90% strand scission at these sites (Figure 3B).

In contrast, rOG did not give detectable amounts of strand scission with aniline, even after a 90 min reaction. The only rG lesion detected *in vivo* so far is rOG, an observation that validates rG oxidation occurrence in the cell. This observation calls for a need to better understand RNA oxidation processes. This is particularly striking because there exists ∼3 times more RNA in a cell than DNA, and RNA takes on a variety of secondary structures that expose the bases to solvent. These exposed bases will be much more prone to oxidation than those in duplex structures,[37] and a major rG oxidation product will likely be rSp.[17,37] However, the use of RNA oligomers and chemical cleavage agents will not provide an accurate measure of the sites and yields for the rG oxidation product rOG, and likely rSp. Therefore, to study the formation of these lesions in RNA will require a different assay. Either a polymerase stop assay can be performed, as has been previously shown,[67] or rOG can be further oxidized with a mild oxidant ($Na_2IrCl_6$) to yield a hydantoin (e.g., rGh) that is labile to aniline, an approach that has also been previously proposed.[64] These studies in RNA were only conducted in one sequence context; however, at pH 4.5 and 65 °C, it is anticipated that sequence context effects will be minimal.

## CONCLUSIONS

The current research determined the chemical cleavage rate constants for many lesions derived from oxidation of the nucleobase guanine in DNA and RNA oligomers. In these studies, the base damages OG, Sp, Gh, Z, and 2Ih were site-specifically synthesized in DNA and RNA oligomers. These strands were then subjected to the standard chemical cleavage reagents for DNA (1 M piperidine, pH 11, 90 °C) or RNA (1 M aniline, pH 4.5, 60 °C), and the pseudo-first-order rate constants for stand scission were determined. In DNA, dOG was not cleaved, and the rate for the dSp diastereomers was found to be slow (Table 1). The rates of strand scission for dZ, dGh, and d2Ih were faster, and only d2Ih will cleave in a >95% yield after the standard reaction time of 30 min (Figure 2A). The current results highlight that most lesions may require long reaction times to obtain high strand scission yields (Figure 3A).


DOI: 10.1021/acs.chemrestox.5b00096
*Chem. Res. Toxicol.* 2015, 28, 1292−1300

These results taken together identify a great variability in the cleavage yield for the dG lesions. The alternative approach for identifying lesion sites in DNA utilizes a base excision repair enzyme, such as Fpg; however, Fpg cleavage also suffers from lesion-dependent cleavage kinetic differences.[68−70] In addition, in RNA strands, all lesions were found to cleave at a slower rate than their analogous DNA counterparts (Table 1). More specifically, rOG and rSp gave insignificant cleavage with aniline at long reaction times (Figure 1B), while rGh and rZ could be cleaved at very long reaction times (Figure 3B). Finally, these results identify OG and Sp lesions in DNA and RNA strands to be the most stable (Table 1), and they support a hypothesis that these lesions in the genome or transcriptome would be much longer lived than the other guanine base lesions.

## ■ ASSOCIATED CONTENT

### Supporting Information

HPLC and PAGE analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION


### Corresponding Author

*Phone: (801) 585-7290. E-mail: burrows@chem.utah.edu.



### Funding

This work was supported by the NIH (R01 CA090689). The oligonucleotides were provided by the DNA/Peptide core facility at the University of Utah that is supported in part by a NCI Cancer Center Support Grant (P30 CA042014).


### Notes

The authors declare no competing financial interest.

## ■ ABBREVIATIONS

2Ih, 5-carboxamido-5-formamido-2-iminohydantoin; d, 2′-deoxyribose; Fapy·G, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; G, guanine; Gf, guanidinoformimine; Gh, 5-guanidinohydantoin; Iz, 2,5-diaminoimidazolone; OG, 8-oxo-7,8-dihydroguanine; PAGE, polyacrylamide gel electrophoresis; r, ribose; SNP, single nucleotide polymorphism; Sp, spiroiminodihydantoin; Z, 2,2,4-triamino-2*H*-oxazol-5-one

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DOI: 10.1021/acs.chemrestox.5b00096
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DOI: 10.1021/acs.chemrestox.5b00096
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