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Thermodynamic Consequences of the Hyperoxidized Guanine Lesion Guanidinohydantoin in Duplex DNA

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Abstract

Guanidinohydantoin (Gh) is a hyperoxidized DNA lesion produced by oxidation of 8-oxo-7,8-dihydroguanine (8-oxoG). Previous work has shown that Gh is potentially mutagenic both *in vitro* and *in vivo* coding for G → T and G → C transversion mutations. In this work, analysis by circular dichroism shows that the Gh lesion does not significantly alter the global structure of a 15-mer duplex, and that the DNA remains in the B-form. However, we find that Gh causes a large decrease in the thermal stability, decreasing the duplex melting temperature by ~ 17 °C relative to an unmodified duplex control. Using optical melting analysis and differential scanning calorimetry the thermodynamic parameters describing duplex melting were also determined. We find that the Gh lesion causes a dramatic decrease in the enthalpic stability of the duplex. This enthalpic destabilization is somewhat tempered by entropic stabilization yet Gh results in an overall decrease in thermodynamic stability of the duplex relative to a control which lacks DNA damage, with a $\Delta\Delta G^\circ$ of -7 kcal/mol. These results contribute to our understanding of the consequences of hyperoxidation of G and provide insight into how the thermal and thermodynamic destabilization caused by Gh may influence replication and/or repair of the lesion.

INTRODUCTION

It is estimated that each cell in the body is exposed to 10,000 to 20,000 reactive radical species every day, with many of these reactive species generated during normal metabolic processes.¹ With regard to DNA, guanine (G) is particularly sensitive to oxidation because it has the lowest reduction potential ($E_0 = 1.3, 1.4, 1.6,$ and 1.7 V versus NHE for G, A, C, and T, respectively).² One oxidized form of G that is detected following exposure of DNA to a variety of oxidizing agents is 8-oxo-7,8-dihydroguanine (8-oxoG) (Figure 1). Indeed, 8-oxoG is present in genomic DNA at steady-state levels of ~1–10 per 10^7 bases.³ It has been shown *in vitro* that 8-oxoG is mutagenic, and can base pair with A during replication, which would yield G → T transversion mutations. When replicated *in vivo*, 8-oxoG is only mildly mutagenic causing G → T transversion mutations with a frequency of <10% in bacterial and mammalian cells.^{4–8} The low mutagenicity of 8-oxoG *in vivo* is due to the presence of an extensive repair system that has evolved to counter its genetic effects.⁹ In mammalian cells, a glycosylase/AP lyase OGG1 excises 8-oxoG from duplex DNA when the damaged G is paired with C. A second glycosylase, MUTYH, removes adenine from an 8-oxoG:A base pair. A third enzyme in this repair system is MTH1, a phosphatase that converts 8-oxodGTP to 8-oxodGMP. This activity removes 8-oxodGTP from the nucleotide pool and prevents incorporation of the oxidized lesion into DNA during replication.

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SUPPORTING INFORMATION AVAILABLE

HPLC chromatograms and ESI-MS spectra for the 8-oxoG and Gh-containing oligonucleotides, van't Hoff plots of the concentration-dependent circular dichroism melting experiments for the G, 8-oxoG, and Gh-containing duplexes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Interestingly, 8-oxoG has a reduction potential that is significantly lower (0.7 V vs. NHE) than G.¹⁰ Thus, 8-oxoG is susceptible to further oxidation, and several hyperoxidized G lesions have been identified.^{11–18} The two most well-studied hyperoxidized G lesions are the diastereomers of guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp) (Figure 1). Furthermore, Sp was detected following treatment of *E. coli* deficient in the base excision repair enzyme endonuclease VIII (Ner) with potassium dichromate.¹⁹ In contrast to the mildly mutagenic 8-oxoG, most of the hyperoxidized lesions studied to date, including Gh and Sp, are potentially mutagenic when replicated *in vitro* yielding G → T and/or G → C transversion mutations.^{20–24} These results reveal the inherent miscoding potential of the hyperoxidized G lesions. Nevertheless, if the hyperoxidized lesions form *in vivo* yet are excised from DNA prior to replication, their mutagenicity would be dramatically reduced.

Experiments performed *in vitro* have shown that Gh and Sp are substrates for several repair glycosylases/AP lyases from *E. coli* including endonuclease III (Nth), Ner, and MutM, where the latter is the bacterial homolog of OGG1.^{25–27} Additionally, both Gh and Sp can be removed from DNA by yeast OGG1 (yOGG1) and yeast OGG2 (yOGG2).²⁸ Finally, the mammalian homologs of Ner, which are designated the ‘Ner-like’ or ‘NEIL’ family of glycosylases, including human NEIL1 (hNEIL1), and murine NEIL1, NEIL2, and NEIL3 (mNEIL1, mNEIL2, and mNEIL3, respectively), recognize and excise Gh and Sp from DNA.^{29–33}

Interestingly, when single-stranded vectors containing a site-specific Gh or Sp lesion were replicated in repair proficient *E. coli*, mutation frequencies of nearly 100% were observed for both lesions.^{34, 35} Such high mutation frequencies suggest that *in vivo* the lesions are not removed from DNA prior to replication and therefore, are not substrates for the *E. coli* repair glycosylases/AP lyases. However, the lack of repair may be a function of the single-stranded nature of the lesion-containing DNA substrates used in the aforementioned studies. It is known that most glycosylases are catalytically active on duplex substrates, but not single-stranded DNA. Notable exceptions are hNEIL1 and the mNEIL enzymes, which have been shown to remove Gh and Sp from single-stranded, bubble, and bulged DNA substrates.^{31, 32} Interestingly, while the ability of *E. coli* Ner to remove Gh or Sp from single-stranded DNA has not been reported, Ner is able to remove uracil and thymine glycol from double-stranded but not single-stranded DNA.³⁶ Thus, it may be that the nearly 100% mutation frequency observed when Gh and Sp-containing DNA were introduced to *E. coli* is due to the inability of *E. coli* glycosylases to excise the hyperoxidized G lesions from single-stranded DNA prior to replication. Ultimately, experiments in which Gh and Sp are replicated in mammalian cells will contribute greatly to our understanding of the mutagenicity and biological impact of these hyperoxidized G lesions.

Despite a growing body of literature that reveals catalytic activity of repair enzymes on substrates containing Gh and Sp, the molecular origin of the ability of glycosylases to recognize and remove these hyperoxidized G lesions from DNA is not well understood. It has been reported that lesion-derived changes in thermodynamic stability of a duplex can affect the repair process and may facilitate recognition of the damaged nucleobase by glycosylases.^{37, 38} Furthermore, thermodynamic stability may also affect replication of a lesion and modulate the miscoding potential of a lesion. These observations underscore the importance of defining the thermodynamic impact of a DNA lesion on duplex stability. The thermodynamic impact of Sp has previously been examined and the lesion was found to be significantly destabilizing to the duplex.³⁹ In this work we used circular dichroism (CD), optical melting, and differential scanning calorimetry (DSC) to determine the effect of Gh on global structure, thermal stability, and thermodynamic stability using a 15-mer duplex containing a Gh:C base pair. Our studies reveal that the Gh:C base pair does not change the global structure of DNA, yet is considerably destabilizing to the duplex relative to G:C and

8-oxoG:C base pairs. We discuss the biological implications of these findings with respect to DNA repair and replication.

EXPERIMENTAL PROCEDURES

Oligonucleotide Synthesis and Purification

Unmodified oligonucleotides were synthesized using standard phosphoramidite chemistry on a BioAutomation MerMade DNA/RNA synthesizer. Oligonucleotides were synthesized with a 5'-dimethoxytrityl group to aid purification. Oligonucleotides were cleaved from the solid support and nucleobase protecting groups were removed by treatment with concentrated NH_4OH at 55 °C for 8 h. HPLC purification was performed using a Microsorb C_{18} reverse-phase HPLC column (Varian; 250 × 10 mm) using mobile phases of 30 mM ammonium acetate (solvent A) and acetonitrile (solvent B). Solvent B was increased from 5% to 25% over 25 min at a flow rate of 3.5 mL/min. The 5'-dimethoxytrityl group was removed by treatment with 80% glacial acetic acid for 12 min at room temperature. The oligonucleotides were then purified again by HPLC using the same mobile phases and solvent B was increased from 0% to 15% over 35 min.

The oligonucleotide containing 8-oxoG was synthesized without the 5'-dimethoxytrityl group to avoid exposing the modified nucleobase to 80% glacial acetic acid. The 8-oxoG phosphoramidite (5'-dimethoxytrityl- N^2 -isobutyryl-8-oxo-deoxyguanosine-3'-[(2-cyanoethyl)- N,N -diisopropyl]-phosphoramidite) was purchased from Glen Research. The DNA was cleaved from the solid support and the nucleobase protecting groups were removed by treatment with 1 M β -mercaptoethanol in NH_4OH at 55 °C for 24 h. HPLC purification was performed on a Dionex DNAPac PA100 anion-exchange column (Thermo Scientific; 4 × 250 mm) using mobile phases of 10% acetonitrile (solvent A) and 0.8 M NH_4Cl in 10% acetonitrile (solvent B). Solvent B was increased from 55% to 75% over 5 min, then 75% to 90% over 15 min, and finally 90% to 100% over 5 min at a flow rate of 1 mL/min. An HPLC chromatogram of the purified 8-oxoG-containing oligonucleotide is provided in the Supporting Information, along with the results of ESI mass spectrometry characterization.

Synthesis and Purification of Gh-containing DNA

The Gh-containing oligonucleotide was prepared as reported previously by incubating the 8-oxoG-containing oligonucleotide (12 μM) in 100 μM $\text{Na}_2\text{IrCl}_6 \cdot 6\text{H}_2\text{O}$ (Sigma), 10 mM sodium phosphate, 100 mM NaCl, pH 6.0 for 30 min at 4 °C.²⁴ The reaction was quenched by addition of EDTA (final concentration was 20 mM EDTA, pH 8.5) and twice dialyzed against H_2O using an ultra centrifugal filter (Amicon; MWCO 3,000). The crude reaction mixture was purified by HPLC using the method described above for the 8-oxoG-containing oligonucleotides and the mass of the Gh-containing oligonucleotide was confirmed by ESI mass spectrometry (Supporting Information).

Circular Dichroism

Circular dichroism spectra were obtained at 20 °C using a Jasco J-815 CD spectropolarimeter with a Peltier temperature controller using a stoppered 1 cm pathlength cuvette. All three duplex samples had a final concentration of 2.6 μM in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0. The samples were incubated for 5 min at 20 °C and scanned from 220 to 320 nm with a 0.2 nm step resolution and a 20 nm/min scan rate. All reported spectra represent an average of three scans.

Optical Melting Analysis

Optical melting experiments were performed using a Beckman Coulter DU800 UV-Visible spectrophotometer equipped with a Peltier temperature controller using a stoppered 1 cm pathlength cuvette. DNA samples had a final concentration of 0.65 to 2.6 μM in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0. Additionally, optical melting experiments were performed using a Jasco J-815 CD spectropolarimeter with a Peltier temperature controller using a stoppered 2 mm pathlength cuvette. For optical melting analysis by CD, DNA samples had a final concentration of 1.3 to 22 μM in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0. For both the UV-visible and CD melting experiments, prior to analysis samples were incubated for 5 min at 95 °C and cooled to room temperature over ~2.5 h. The G and 8-oxoG-containing duplexes were then heated at a rate of 1 °C/min from 25 to 95 °C while monitoring absorbance at 260 nm, held at 95 °C for 5 min, and returned to the starting temperature at a rate of 1 °C/min. Analysis of the Gh-containing duplex was the same except the starting temperature was 10 °C, instead of 25 °C. Using the absorbance versus temperature data the T_m was determined according to previously reported procedures.^{40, 41, 61} By obtaining T_m at several DNA concentrations, and plotting $1/T_m$ versus $\ln(C_T)$, in which C_T is the total DNA concentration, thermodynamic parameters ($\Delta H^\circ_{\text{VH}}$, $\Delta S^\circ_{\text{VH}}$, and $\Delta G^\circ_{\text{VH}}$) were extracted by van't Hoff analysis of the resulting concentration-dependent melting curves obtained by UV-visible spectroscopy and CD.^{40, 41}

DSC Analysis

Calorimetry experiments were performed using a TA Instruments NanoDSC III. The duplexes were prepared at a concentration of 32 μM in 900 μL of 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0 buffer. The samples were degassed *in vacuo* for 12 min at 25 °C prior to analysis by DSC. Data was obtained by continuously monitoring the excess power required to maintain both the sample and the reference cells at the same temperature. The resulting thermograms display excess heat capacity (C_p) as a function of temperature. Each experiment consisted of a forward scan, in which the temperature was increased from 10 to 105 °C at a rate of 1.0 °C/min, and a reverse scan in which the temperature was decreased from 105 to 10 °C at a rate of 1.0 °C/min. The sample was equilibrated for 10 min at 10 and 105 °C between each forward and reverse scan, respectively. In order to confirm that Gh is stable under these conditions of repeated cycles of heating and cooling, the Gh-containing duplex was analyzed by ESI-MS before and after analysis by DSC (Supporting Information).

A buffer sample was analyzed using the same procedure described above and the thermograms for the duplex samples were background corrected using this buffer thermogram. Subsequently, the duplex thermograms were normalized for concentration and the area under the resulting curve was integrated and a baseline correction was performed using a 5th-order polynomial fit in the NanoAnalyze Data Analysis software version 2.2.0 (TA Instruments) in order to obtain ΔH and ΔS corrected for ΔC_p ($\Delta H^\circ_{\text{cal}}$ and $\Delta S^\circ_{\text{cal}}$). $\Delta H^\circ_{\text{cal}}$ was determined by integrating the area under the excess heat capacity curve. $\Delta S^\circ_{\text{cal}}$ was determined by plotting $(C_p)/T$ against the temperature in Kelvin and integrating the area under the resulting curve. The model-independent free energy associated with duplex melting at 37 °C was calculated using the equation $\Delta G^\circ_{\text{cal}} = \Delta H^\circ_{\text{cal}} - T\Delta S^\circ_{\text{cal}}$ (Supporting Information).

Additionally, the area under the excess heat capacity curve was integrated and a baseline correction was performed using a linear fit of the pre- and post-baselines resulting in an integrated thermogram that is uncorrected for ΔC_p . This procedure was followed in order to derive thermodynamic data from the DSC assuming a two-state model (ΔH_{cal}); this data can

then be compared directly with the thermodynamic parameters derived by van't Hoff analysis of optical melting experiments, which also assume a two-state model. In this case, ΔG° at 37 °C was calculated using the equation $\Delta G^\circ(T) = T\Delta G^\circ(T_m)/T_m - [\Delta H_{cal}(T_m) - \Delta C_p T_m]/(1 - T/T_m) + T\Delta C_p T_m \ln(T/T_m)$ in which $\Delta G^\circ(T_m) = -RT_m \ln(4/C_T)$, $\Delta C_p \approx 0$, and C_T is equal to the total strand concentration.^{40, 41, 62} The ΔG° determined from the DSC data by assuming a two-state model is presented in the Supporting Information.

RESULTS AND DISCUSSION

Lesion-Containing DNA Duplexes

Three DNA duplexes were designed to determine the effect of Gh on the thermodynamic stability of a 15-mer DNA duplex (Table 1). Two of the three duplexes are controls in which G or 8-oxoG is located in the center of the DNA and is paired opposite C. The third duplex contains Gh paired opposite C, also located in the center of the duplex.

Analysis of Lesion-Containing DNA by Circular Dichroism

CD was employed to determine the extent to which Gh perturbs the global structure of a DNA duplex. Differences in base stacking, hydrogen bonding, and/or base tilting, relative to B-form DNA, are manifested as changes in the peak maxima, minima, and/or crossover points in the CD spectrum.⁴² The CD spectra for duplexes containing G, 8-oxoG, or Gh paired opposite C are similar to one another, and display patterns commonly observed for B-form DNA (Figure 2);⁴³ all three duplexes have a maximum at ~280 nm, a minimum at ~250 nm, and a crossover point at ~260 nm. Slight differences in the magnitude of the maxima and minima at ~280 nm and ~250 nm, respectively, are observed for the three duplexes. However, these slight differences are likely not due to a major structural change in the duplex, but rather due to subtle changes in the electronic transitions and molar extinction coefficients of G, 8-oxoG, and Gh.⁴⁴ Notably, we do not observe a shift of the crossover point in the damage-containing duplexes, a feature which has been suggested to indicate partial denaturation of the duplex.^{43, 45–47}

As expected based on previously published reports, 8-oxoG does not alter the global structure of the duplex.^{48–53} Furthermore, similar to 8-oxoG, the introduction of Gh to the duplex does not cause a significant change to the CD spectral profile; this result indicates that Gh does not alter the global structure of DNA when it is base paired opposite C and that DNA containing the lesion remains B-form.

Determining the Thermal and Thermodynamic Impact of the Gh Lesion by Optical Analysis

The G, 8-oxoG, and Gh-containing duplexes were analyzed using UV-Visible spectrophotometry in order to generate optical melting curves (Figure 3). The optical melting curve for each duplex contains a sharp increase in absorbance at increased temperatures, which is indicative of duplex melting, and reflects the melting temperature (T_m) or thermal stability of the duplex. As expected, the G-containing duplex has the highest thermal stability with a T_m of 56.5 °C. The presence of 8-oxoG had only a slight effect on the thermal stability of the duplex, decreasing the T_m by 1.3 °C. Notably, upon the introduction of Gh, a significant decrease in the thermal stability is observed, with a ΔT_m of -16.7 °C, with respect to the control duplex lacking damage.

Melting temperatures for duplexes containing the Gh lesion have been reported previously and are ~1 to 8 °C lower relative to the corresponding control duplex containing a G:C base pair.²⁴ In these previous experiments the Gh lesion was four bases from the 5' end of an 18-mer template that was hybridized to a 14 or 16-mer primer; formation of the primer/template pair results in the Gh lesion being paired opposite a base but lacking a base pair on the 5'

side of the lesion, or paired opposite a base and flanked on the 5' side by two base pairs. Interestingly, the Gh lesion was less thermally destabilizing when paired opposite A, as compared to other bases.²⁴ It was also observed that the identity of the base pairs flanking the Gh lesion influenced the T_m of the primer/template pair, with G:C base pairs flanking the Gh lesion yielding constructs that were thermally more stable than when A:T base pairs flanked the lesion.²⁴ Indeed, we speculate that the two A:T base pairs that flank Gh:C in our duplex contribute to the large thermal destabilization we observe relative to the undamaged control.

In addition to thermal stability of the duplexes, thermodynamic parameters associated with duplex melting were derived by van't Hoff (VH) analysis of the UV-visible optical melting curves (Figure 4).⁴⁰ Van't Hoff analysis was also performed on melting curves obtained by CD (Supporting Information), and thermodynamic parameters comparable to the UV-visible derived results were obtained. Table 2 provides the values for change in enthalpy (ΔH°_{VH}), entropy (ΔS°_{VH}), and free energy (ΔG°_{VH}) for the G, 8-oxoG, and Gh-containing duplexes. Consistent with literature reports, relative to the G-containing duplex, the 8-oxoG lesion does not influence the ΔH°_{VH} , ΔS°_{VH} , or ΔG°_{VH} associated with duplex melting.^{39, 48, 52} In contrast to 8-oxoG, the hyperoxidized lesion Gh has a significant impact on the enthalpic stability, entropic stability, and overall free energy of the duplex. Relative to the G-containing duplex, the Gh-containing duplex has a $\Delta\Delta H^\circ_{VH}$ of -31 kcal/mole. Both hydrogen bonding and base stacking interactions are reflected in the value of ΔH° , indicating that Gh disrupts one or both of these interactions. A $\Delta\Delta S^\circ_{VH}$ value of -78 cal/(mole•K) relative to the G-containing duplex describes an increase in disorder when the Gh lesion is present. Notably, the entropic stabilization introduced by Gh is offset by the enthalpic destabilization of the lesion and the $\Delta\Delta G^\circ_{VH}$ for the duplex containing Gh is -6 kcal/mole. Previous reports, using data obtained from van't Hoff analysis of optical melting data, cited values for $\Delta\Delta G^\circ_{VH}$ of -1 to -5 kcal/mole for a duplex containing a Gh:C base pair in various sequence contexts.²⁴

Calorimetric Analysis of the Thermal and Thermodynamic Impact of Gh

The G, 8-oxoG, and Gh-containing duplexes were also analyzed by DSC (Figure 5). DSC provides a direct measurement of the heat released during duplex melting and thermograms obtained by DSC present excess heat capacity plotted as a function of temperature. An advantage of obtaining thermodynamic parameters from calorimetric analysis, as opposed to from optical analysis, is that calorimetry allows for direct measurement of the heat supplied to or released from a system during the melting transition and is independent of the melting model followed. In contrast, deriving thermodynamic parameters from optical melting curves using van't Hoff analysis requires the assumption that duplex melting occurs via a two-state model.^{40, 41} In this two-state model, the DNA exists either as a duplex or two single strands, with no intermediates populating the transition from the structured duplex to the unstructured single-stranded state. One can propose a model for duplex melting by comparing the enthalpic component of thermodynamic stability derived from optical melting experiments (ΔH°_{VH}) to data obtained by calorimetry (ΔH°_{cal}). In cases where duplex melting obeys a two-state model, $\Delta H^\circ_{VH} \approx \Delta H^\circ_{cal}$. However, if $\Delta H^\circ_{VH} < \Delta H^\circ_{cal}$ then intermediates populate the melting transition from duplex to single strands and the two-state model is not followed. Furthermore, if $\Delta H^\circ_{VH} > \Delta H^\circ_{cal}$ then a multi-molecular aggregate state is involved in the melting transition, or there is a difference in helix initiation, and the two-state model is not followed.^{39, 55} While thermodynamic parameters for Gh-containing DNA have been extracted from optical melting data by van't Hoff analysis, prior to this study calorimetric analysis had yet to be performed on a Gh-containing duplex.

When examined by DSC the overall thermodynamic stability of the duplexes is G:C $\approx$ 8-oxoG:C > Gh:C (Table 3), and this trend is consistent with our van't Hoff analysis of the

optical melting curves. Our data also reveal that $\Delta H^\circ_{\text{vH}}$ and $\Delta H^\circ_{\text{cal}}$ are within experimental error for the G and 8-oxoG-containing duplexes, indicating that melting follows a two-state process for these duplexes. Interestingly, $\Delta H^\circ_{\text{vH}}$ for the Gh-containing duplex is greater than $\Delta H^\circ_{\text{cal}}$ indicating that the two-state model is not followed. This phenomenon has been observed for other DNA lesions^{54–56} including Sp, in which $\Delta H^\circ_{\text{vH}}$ was ~12 kcal/mole greater than ΔH_{cal} .³⁹

In addition to the model-independent thermodynamic parameters obtained from calorimetry, a two-state model can also be assumed to obtain model-dependent thermodynamic parameters from the DSC data.^{40, 41, 62} For the G, 8-oxoG, and Gh-containing duplexes, comparison of the van't Hoff analysis of the UV-visible optical melting data and DSC data reveal comparable values for ΔG° (Supporting Information). Furthermore, using both techniques the overall thermodynamic stability of the duplexes is G:C $\approx$ 8-oxoG:C > Gh:C.

With respect to thermal stability, consistent with the results obtained by UV-Vis, when determined by calorimetry the G-containing duplex has the greatest thermal stability. The 8-oxoG-containing duplex has a ΔT_m of -0.9°C relative to the control (Table 3). Furthermore, DSC analysis confirms results obtained by optical melting that Gh causes significant thermal destabilization of duplex DNA with a ΔT_m of -16.6°C .

Thermodynamic parameters for duplexes containing 8-oxoG have been reported and the differences in the magnitudes of $\Delta\Delta H$ and $\Delta\Delta S$ relative to G-containing DNA vary based on the sequence context and location of the lesion.^{24, 39, 48, 51, 52} It has been shown that sequences with higher G:C content can stabilize the lesion-containing duplex. Singh and coworkers also showed that there was a larger decrease in ΔG relative to the control duplex lacking damage for duplexes in which the 8-oxoG was centrally located versus when 8-oxoG was positioned closer to the end of the duplex.⁵¹ This difference can be rationalized by the increased dynamics at the ends of a duplexes due to end fraying. Additionally, as we describe here, in some cases it has been reported that the 8-oxoG:C base pair is thermodynamically equivalent to the G:C base pair.^{24, 52} More importantly for this work, the DSC experiments reveal that the Gh:C pair is thermodynamically destabilizing when present in the duplex with a $\Delta\Delta G^\circ_{\text{cal}}$ of -7 kcal/mole. Thus, while in this 15-mer duplex G:C and 8-oxoG:C base pairs are thermodynamically equivalent, the Gh:C base pair is destabilizing. Notably, given the precedent for the thermodynamic impact of 8-oxoG to be dependent on sequence context and location of the lesion with respect to the end of a duplex, the thermodynamic impact of Gh may also vary depending on the DNA duplex.

It is important to note that the thermal and thermodynamic parameters we report here describe a mixture of the *R* and *S* diastereomers of the Gh lesion. To date, homogeneous populations of each diastereomer have not been attained because the diastereomers interconvert on a time scale that is too fast to allow for isolation.⁵⁷ Thus, while it is possible that one diastereomer may have more of an impact on the thermal and thermodynamic stability of the duplex, our data reflect a population comprised of the two diastereomers.

Comparison of Thermodynamic Parameters for Gh-Containing DNA to Sp-Containing DNA

As previously stated, the two most widely-studied hyperoxidized G lesions are Gh and Sp. Therefore it is pertinent in the discussion of the thermodynamic impact of Gh to describe how it compares to Sp. Using the same 15-mer duplex as in this current work, Chinyenetere and Jamieson reported that a Sp:C pair is thermodynamically destabilizing and decreases the free energy of the duplex by 7.8 kcal/mole relative to the G-containing duplex.³⁹ In our studies with the Gh lesion we observe a comparable decrease in free energy. These results suggest that Gh and Sp, while structurally quite different, have a similar thermodynamic impact on duplex DNA.

Biological Significance

A crystal structure of a replicative DNA polymerase bound to DNA containing a Gh lesion has recently been reported.⁵⁸ In the crystal structure, the *R* configuration of Gh was captured and the lesion was in the *high syn* conformation. Additionally, in the crystal structure the Gh is extrahelical and is not positioned to serve as a templating base. This positioning of the lesion suggests that it would serve as a block to replication. Indeed, Gh has been found to be a block to replication by polymerases both *in vitro* and *in vivo*.^{24, 35} The data provided in the current work suggest that the thermodynamic destabilization of the Gh lesion in DNA may provide the molecular basis for the observed extrusion of Gh.

While our data reveal a detrimental impact of Gh on the thermal and thermodynamic stability of DNA, they do not pinpoint the cause of the observed destabilization. However, a change in buckle angle of the deoxyribose of 8-oxoG relative to G has been described.⁵⁹ It has been proposed that this change in buckle angle decreases the thermodynamic stability of the duplex and facilitates recognition by glycosylases.⁵⁹ The thermal and thermodynamic destabilization induced by Gh that we report here may contribute to the high buckle angle at the site of the lesion in the crystal structure of polymerase bound Gh-containing DNA.⁵⁸

It has been suggested that the stability of the duplex may contribute to the ability of DNA repair enzymes to recognize lesions amongst the vast excess of undamaged DNA. In one such example, *E. coli* mismatch-specific uracil DNA glycosylase, responsible for removing U from U:G mismatches, was shown to have sequence-dependent activity in which U is preferentially removed from sequence contexts that have reduced thermal stability.⁶⁰ Therefore, the thermodynamic impact of the Gh lesion may have significant implications for its removal by repair enzymes. Repair of Gh by the *E. coli* glycosylases Nth²⁵, Nei²⁵, and MutM^{25–27} has been examined and all three enzymes can excise Gh from duplex DNA. Furthermore, using a recognition-competent but catalytically-inactive mutant of MutM (E3Q EcFpg) it was revealed that MutM has an ~1000-fold preference for binding to duplexes containing a Gh:C base pair as compared to a 8-oxoG:C base pair.²⁷ It was proposed that the higher affinity of the repair enzyme for the Gh-containing duplexes was due to destabilization caused by Gh relative to 8-oxoG. These previous studies provide examples of duplex stability modulating the ability of repair enzymes to recognize and excise DNA lesions.

In conclusion, in this work the structural, thermal, and thermodynamic impact of a Gh:C base pair in DNA duplex was examined. We demonstrate that while Gh does not alter the global B-form conformation, the Gh lesion significantly decreases the thermal and thermodynamic stability of the duplex. The differences in thermal and thermodynamic impact between the oxidized G lesion 8-oxoG, and the hyperoxidized G lesion Gh are quite striking and should be considered with respect to the origins of mutagenicity and repair of these DNA lesions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CD	circular dichroism
C_p	excess heat capacity
DSC	differential scanning calorimetry
ESI	electrospray ionization
EDTA	ethylenediaminetetraacetic acid
Gh	guanidinohydantoin
G	guanine
HPLC	high-performance liquid chromatography
Nei	endonuclease VII
NEIL	Nei-like
hNEIL	human Nei-like
NHE	Normal Hydrogen Electrode
Nth	endonuclease III
mNEIL	murine Nei-like
Sp	spiroiminodihydantoin
T_m	melting temperature
VH	van't Hoff
yOGG	yeast oxoguanine glycosylase
8-oxoG	8-oxo-7,8-dihydroguanine

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Figure 1. Schematic representation of oxidation of G to form 8-oxoG, followed by hyperoxidation to form Gh and Sp.

Figure 2. Circular dichroism spectra for the G, 8-oxoG, and Gh-containing duplexes (2.6 μM) in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0.

Figure 3. Optical melting profiles for the G, 8-oxoG, and Gh-containing duplexes (0.9 μ M) in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0.

Figure 4. van't Hoff plots of the concentration-dependent UV-visible optical melting experiments for the G, 8-oxoG, and Gh-containing duplexes.

Figure 5. DSC thermograms for the G, 8-oxoG, and Gh-containing duplexes (32 μ M) in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0.

Table 1

DNA Sequences Used in this Study

Name	Nucleotide Sequence
G	5'-ACTGATAG <u>G</u> ACGCACT -3'
8-oxoG	5'-ACTGATAG ^{OX} <u>G</u> ACGCACT -3'
Gh	5'-ACTGATAG <u>h</u> ACGCACT -3'
Complement	3'-TGACTATCTGCGTGA -5'

G^{OX} represents 8-oxoG

Table 2

UV-Visible-Derived Thermodynamic Parameters for the Melting of the G, 8-oxoG, and Gh-Containing Duplexes^a

Duplex	T_m^b (°C)	ΔH°_{vh} (kcal/mole)	ΔS°_{vh} (cal/(mole•K))	$\Delta G^\circ_{vh}^c$ (kcal/mole)	ΔT_m (°C)	$\Delta \Delta H^\circ_{vh}$ (kcal/mole)	$\Delta \Delta S^\circ_{vh}$ (cal/(mole•K))	$\Delta \Delta G^\circ_{vh}$ (kcal/mole)
G	56.5 ± 0.1	111 ± 13	308 ± 30	15 ± 0.7	-	-	-	-
8-oxoG	55.2 ± 0.6	108 ± 7	307 ± 12	13 ± 0.9	-1.3	ND ^d	ND ^d	ND ^d
Gh	39.8 ± 0.1	80 ± 7	230 ± 15	9 ± 0.6	-16.7	-31	-78	-6

^aDNA in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0.

^b T_m reported for a duplex concentration of 2.6 μM.

^cValue at 37 °C.

^dNot statistically different from G-containing duplex.

Table 3

DSC-Derived Thermodynamic Parameters for the Melting of the G, 8-oxoG, and Gh-Containing Duplexes^a

Duplex	T _m ^b (°C)	ΔH_{cal}° (kcal/mole)	ΔS_{cal}° (cal/(mole•K))	ΔG_{cal}° (kcal/mole)	ΔT_m (°C)	$\Delta \Delta H_{cal}^{\circ}$ (kcal/mole)	$\Delta \Delta S_{cal}^{\circ}$ (cal/(mole•K))	$\Delta \Delta G_{cal}^{\circ}$ (kcal/mole)
G	64.3 ± 0.2	103 ± 2	305 ± 6	9 ± 0.5	-	-	-	-
8-oxoG	63.2 ± 0.1	101 ± 2	299 ± 5	8 ± 0.4	-0.9	ND ^e	ND ^e	ND ^e
Gh	47.7 ± 0.1	62 ± 6	193 ± 18	2 ± 0.3	-16.6	-41	-112	-7

^aDNA in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0.

^bT_m reported for a duplex concentration of 32 μM.

^cDerived from DSC thermograms in a model-independent manner as described in the experimental section.

^dValue at 37 °C.

^eNot statistically different from G-containing duplex.