Computational Study of the Oxidation of Guanine to Form 5-Carboxyamido-5-formamido-2-iminohydantoin (2Ih)

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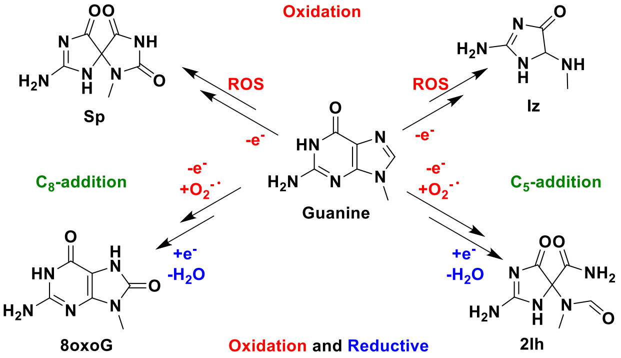
TOC Figure


Oxidation
ROS
-e$^-$
Sp
ROS
-e$^-$
Iz
Guanine
C_8-addition
-e$^-$
$+O_2^{-\cdot}$
C_5-addition
-e$^-$
$+O_2^{-\cdot}$
+e$^-$
$-H_2O$
+e$^-$
$-H_2O$
8oxoG
2Ih
Oxidation and Reductive

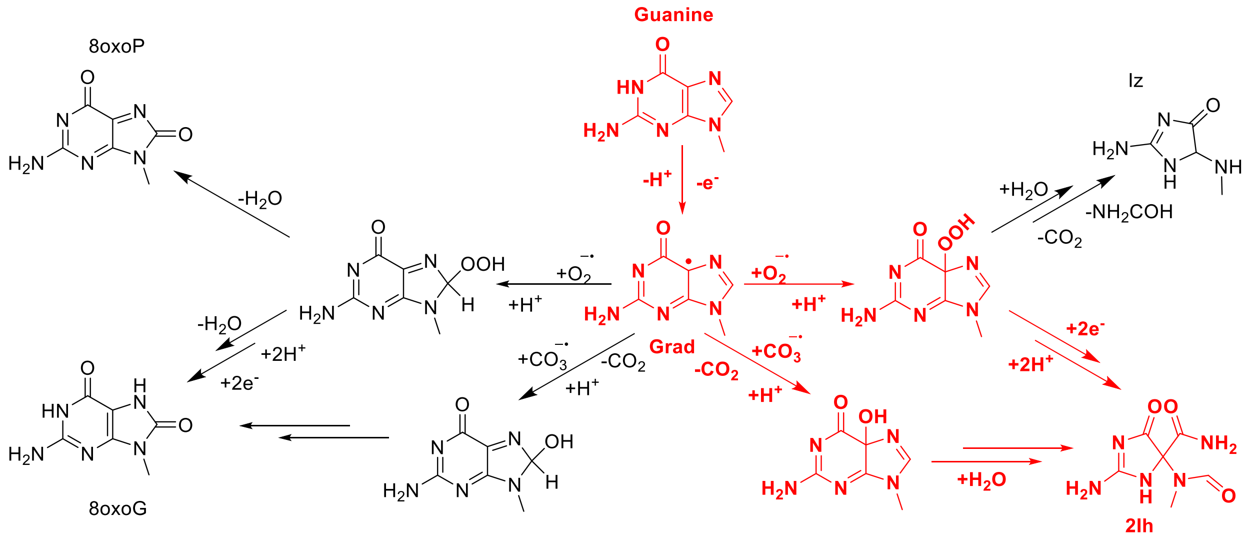
ABSTRACT:

Oxidative damage to DNA leads to a number of two-electron oxidation products of guanine such as 8-oxo-7,8-dihydroguanine (8oxoG). 5-carboxyamido-5-formamido-2-iminohydantoin (2Ih) is another two-electron oxidation product that forms in competition with 8oxoG. The pathways for the formation of 2Ih have been studied by density functional theory using the ωB97XD functional with the 6-31+G(d,p) basis set and SMD implicit water solvation plus a small number of explicit water molecules positioned to help stabilize charged species and facilitate reaction steps. For oxidative conditions that produce hydroxyl radical, such as Fenton chemistry, hydroxy radical can add at C4, C5 or C8. Addition at C4 or C5 followed by loss of H_2O produces guanine radical. Guanine radical can also be produced directly by oxidation of guanine by reactive oxygen species (ROS). A C5-OH intermediate can be formed by addition of superoxide to C5 of guanine radical followed by reduction. Alternatively, the C5-OH intermediate can be formed by hydroxy radical addition at C5 and oxidation by $^3O_2$. The competition between oxidative and reductive pathways depends on the reaction conditions. Acyl migration of the C5-OH intermediate yields reduced spiroiminodihydantoin (Sp^{red}). Subsequent water addition at C8 of Sp^{red} and N7-C8 ring opening produces 2Ih. Hydroxy radical addition at C8 can lead to a number of products. Oxidation and tautomerization produces to 8oxoG. Alternatively, addition of superoxide at C5 and reduction results in a C5, C8 dihydroxy intermediate. For this species, the low energy pathway to 2Ih is N7-C8 ring opening followed by acyl migration. Ring opening occurs more easily at C8-N9 but leads to a higher energy analogue of 2Ih. Thus, the dominant pathway for the production of 2Ih depends on the nature of the reactive oxygen species and on the presence or absence of reducing agents.

1. INTRODUCTION

Investigations into DNA oxidation find a significant number of oxidative products arising from guanine, many of which have been probed experimentally.[1-50] Among the products of guanine oxidation, 8-oxo-7,8-dihydroguanine (8oxoG) is a 2-electron oxidation product that is consistently found in cellular environments and *in vitro* experiments.[1-19] Depending on reaction conditions, 5-carboxyamido-5-formamido-2-iminohydantoin (2Ih)[20] is another 2-electron oxidation product that is formed in yields comparable to 8oxoG.[2-7] Experimental work has provided evidence that the competition between 8oxoG and 2Ih formation is related to competition between the C5 and C8 positions of guanine radical as the site of addition of reactive species.[2-3] Among other product channels, imidazolone (Iz) has been found to compete with 2Ih formation in the presence reducing agents via superoxide combination with C5 of guanine radical.[4-6] Scheme 1 summarizes conversion of guanine to 2Ih, as well as some other competing pathways.

Scheme 1. Comparison of pathways of the formation of 2Ih and other oxidative products.



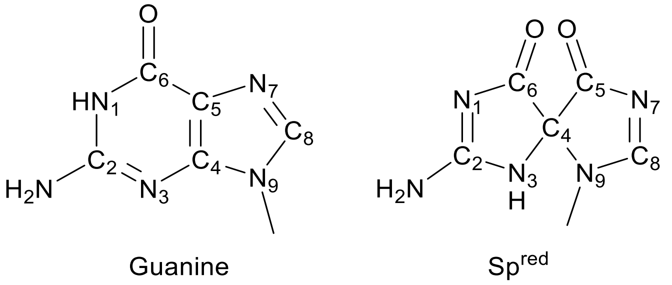
In studying pathways for DNA oxidative damage by hydroxyl radical, Burrows and co-workers have considered the role of biologically relevant reducing agents, such as N-acetylcysteine (model for glutathione) and ascorbic acid in the formation of the observed products.[4-5] They found that there is a competition between formation of Iz and 2Ih in the presence of superoxide, with biologically appropriate concentrations of reducing agents strongly favoring 2Ih. By contrast, 8oxoG and spiroiminodihydantoin (Sp) formation are minimally affected by the presence or absence of reducing agents, suggesting that an alternative pathway for their formation is possible. Other studies relating to guanine oxidation in the presence of superoxide found Iz as a primary product,[11-13, 21] suggesting hydroperoxyl reduction is a key step in formation of 2Ih from superoxide-guanine radical adducts. Meunier and co-workers used a Mn-porphyrin complex, Mn-TMPyP, in the presence of $KHSO_5$ as a two electron oxidant of guanine and found a compound reported to be very similar to 2Ih, but did not find any 8oxoG.[40-41] Further oxidation products, Iz and Gh_{ox} (oxidized guanidinohydantoin) were both reported, suggesting that the mechanistic pathway of oxidation via Mn-TMPyP / $KHSO_5$ bypasses the formation of 8oxoG. Rokhlenko et al. found 2Ih was formed in yields similar to Sp / 8oxoG when guanine was oxidized by carbonate radicals produced from bicarbonate and sulfate radicals generated via single pulse persulfate photodissociation.[2] In the absence of bicarbonate, sulfate radicals can oxidize guanine directly, resulting in 8oxoG under acidic conditions, but no 2Ih.[2] This can be explained by the lower barrier for C8 addition of water to guanine radical cation compared to C5 addition.[51] Ghude et al. found that guanine oxidation, in the presence of Ni (II) coordinated with a tetraazamacrocycle (NiCR) and $KHSO_5$, results in 2Ih as the predominant, and in some cases exclusive, oxidation product of guanine.[48] This was proposed to be due to coordination of the nickel complex with guanine, potentially favoring the C5 center for nucleophilic addition over the C8 center. Oxidation with meta-chloroperoxybenzoic acid (m-CPBA), peracetic acid, or dimethyldioxirane (DMDO) results in a C4-C5 epoxide which leads to 2Ih by an alternate mechanism.[47]

While many studies have found 2Ih,[2-6, 20, 45-48] the mechanistic pathways for its formation are not well understood. In previous studies, we have used density functional calculations to explore mechanisms for guanine oxidation to form 8oxoG, Sp, Gh and FAPyG,[52-55] formation of guanine-lysine crosslinks mediated by type I and type II photosensitizers[56-57] and by sulfate radical[51] and formation of guanine-thymine crosslink products.[55] We have also calculated pK_a's and reduction potentials for nucleobases and intermediates in the guanine oxidation mechanism. [58-61] In this computational study, we address pathways leading to 2Ih. To model the conditions in the study by Burrows and co-workers,[4-5] hydroxyl radical was selected as the initiator. Early experimental investigations of hydroxyl radical reacting with guanine include studies by O'Neill and co-workers[62-63] and by Steenken and co-workers.[29, 44] The initial reaction of hydroxyl radical with guanine was recently re-examined experimentally by Chatgilialoglu and co-workers,[64-65] and computationally by Sevilla and co-workers.[66] In the present study, we start with the addition of hydroxyl radical to C4, C5 and C8 of guanine. Water loss from the C4 and C5 hydroxyl addition intermediates yields guanine radical which can also be generated with milder oxidants, such as carbonate radical. We also consider hydroxyl radical abstracting a hydrogen atom from N1 or N2 of guanine, and electron transfer from guanine to hydroxyl radical to yield guanine radical. The reaction then proceeds by superoxide combining with guanine radical followed by reduction and acyl migration to produce 2Ih. Oxidation of radical intermediates by $^3O_2$ was considered for systems without significant concentrations of superoxide or hydroperoxyl radical.

2. METHODS

2.1 Electronic structure calculations. All calculations were performed using the development version of the Gaussian series of programs[67] and the ωB97XD density functional[68] with the 6-31+G(d,p) basis set.[69-73] SMD implicit water solvation[74] was used to model aqueous conditions. Explicit waters were included as a supplement to the implicit model. Guanine was capped with a methyl group in place of the N9-bound sugar moiety. The numbering of the atoms in guanine and reduced spiroiminodihydantoin, Sp^{red}, is shown in Scheme 2.

Scheme 2. Atom numbering for guanine and Sp^{red}



Guanine

Sp^{red}

2.2 pK_a calculations. The pK_a's for reactants and intermediates are calculated from the free energy differences for deprotonation in solution

$$pK_a = \frac{G_{deprotonated} + G_{H^+_{(aq)}} - G_{protonated}}{2.303RT} \qquad (1)$$

where R is the gas constant (1.987 cal K^{-1} mol^{-1}), T is the temperature (298.15 K), and the free energy of the proton in water is -270.3 kcal/mol[75-78], defined as

$$G_{H^+_{(aq)}} = G_{H^+_{(g)}} + G^{1atm\to 1M} + \Delta G_{H^+_{(solv.)}} \qquad (2)$$

where the gas phase free energy of a proton is $G_{H^+_{(g)}}$ = -6.287 kcal/mol, the conversion from 1 atm to 1 mol/L is $G^{1atm\to 1M}$ is 1.89 kcal/mol[79], and energy of solvation of a proton is $\Delta G_{H^+_{(solv.)}}$ = -265.9 kcal/mol.[75]

Experimentally, the reactions are carried out in buffered solutions. Computationally, the relative free energies of different protonation states of a compound in a buffer can be determined by calculating the free energy for the transfer of a proton to / from a proton acceptor / donor that has a pK_a equal to the pH of the buffer. The relative concentrations of the different protonation states can be determined from the Boltzmann distribution and the relative free energies. Imidazole (pK_a = 7.7 calc., 6.95 exp.[80]) was used to model pH 7 conditions.

2.3 Reduction potential calculations. The standard state reduction potentials E_0 are calculated by

$$E_0 = -\frac{G_{reduced} - G^{\circ}_{(g)} - G_{oxidized}}{nF} - SHE \qquad (3)$$

where SHE is the absolute potential of the standard hydrogen electrode (4.281 V[76-77, 81]), F is the Faraday constant (23.06 kcal/(mol V)), n is the number of electrons (n=1 in all cases in the present study), and $G^{\circ}_{(g)}$ = -0.867 kcal/mol[82-83] is the gas phase energy of the electron at 298 K.

Reduction of hydroperoxyl groups resulting from superoxide addition to radical intermediates was modelled with two explicit CH_3SH molecules acting as reducing agents. This yields CH_3SSCH_3, H_2O and the corresponding alcohol (see Schemes 6 and 7).

2.4 Implicit – Explicit Treatment of Water Solvation. In addition to implicit solvation, explicit waters are important for water addition / elimination reactions, for proton transfer steps and for stabilization of charged species. The optimal number of explicit waters for intermediates and transition states along a pathway can vary. Some studies have used up to 12 water molecules to solvate guanine oxidation products.[50, 66] A smaller, more suitable number of explicit waters was chosen for each step instead of including the same number of explicit waters for every step along the pathway. This reduces the computational cost and complexity in calculating the pathways, and results in a reaction path energy profile that is the sum of multiple segments, as illustrated by the example shown in Figure 1. For water addition steps, waters were placed and oriented to facilitate proton transfer from the water molecule acting as the nucleophile to the heteroatom acting as a proton acceptor. For charge migration steps, waters were positioned and oriented in a manner which would intuitively best stabilize the flow of charge in the various intermediates for progress along the reaction path. Positions of the explicit waters are included in the Cartesian coordinates listed in the Supporting Information.

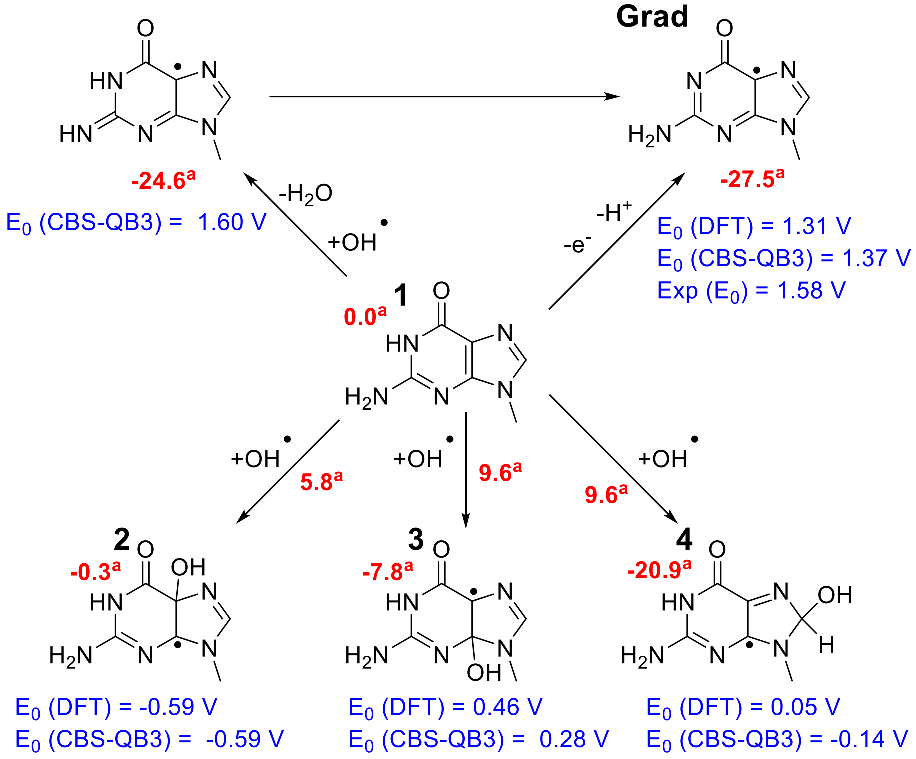
Figure 1. Combined reaction energy profile from reactant **A** to product **E**. Intermediate **C** is -28.6 kcal/mol relative to guanine and hydroxyl radical. Transition state **D** is at 19.1 kcal/mol relative to intermediate **C**, and 0.2 kcal/mol relative to reactants **A**.

3. RESULTS AND DISCUSSION

The oxidative degradation of guanine can be initiated by direct oxidation via reactive oxygen species or by the addition of radicals such as hydroxyl radical. A subsequent oxidation or reduction of intermediate radicals leads to closed shell species which undergo acyl migration and ring opening to form 2Ih. Density functional calculations at the ωB97XD/6-31+G(d,p) level of theory have been used to examine each of these steps and to explore possible branches in the reaction paths.

3.1 Hydroxyl radical addition. Scheme 3 shows the first step in Fenton chemistry with hydroxyl radical addition to the C4, C5, and C8 positions of guanine. C5 addition of hydroxyl radical to guanine was calculated to have a barrier of 5.8 kcal/mol and results in C5-hydroxyl radical intermediate **2**. C4 and C8 addition were calculated to both have barriers of 9.6 kcal/mol and result in intermediates **3** and **4**, respectively. Hydroxyl radical can also abstract a hydrogen atom from N1 or from N2 to produce guanine and water, or transfer an electron to yield guanine radical and hydroxide. In a detailed study, Sevilla and coworkers found that hydrogen atom abstraction and hydroxyl radical addition followed by water elimination are competitive in aqueous solution.[66] Our calculations are in agreement with their results.

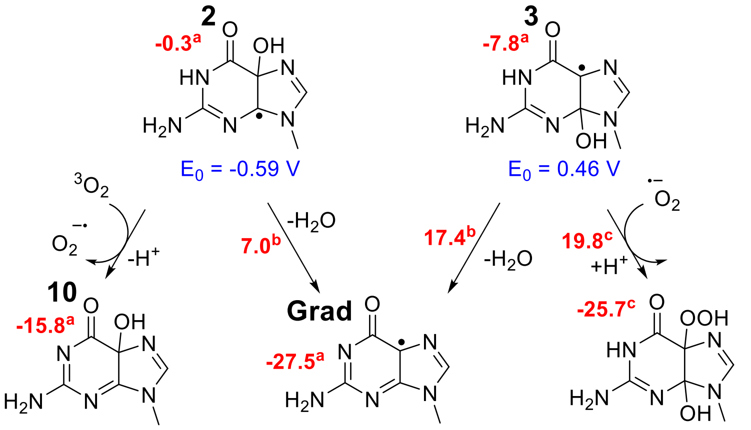
Scheme 3. Hydroxyl radical addition to guanine at C4, C5 and C8, abstraction of hydrogen atom from N2, and single electron transfer (relative energies and barriers (kcal/mol) in red, calculated reduction potentials in blue).



[a]No explicit water molecules were used in calculation of these energies.

Scheme 4 consider the possible fates of the C4 and C5 hydroxyl radical addition intermediates. Formation of **2** is the most favorable; however, **2** is also the least stable. The calculated reduction potential of **2** is -0.59 V, indicating that oxidation by $^3O_2$ (E_o = -0.16 V) to form **10** is favorable. The calculated reduction potential of **3** is considerably higher (0.46 V) and cannot be oxidized by $^3O_2$, but superoxide can add at C5 with a barrier of 19.8 kcal/mol to give C4-OH, C5-OOH substituted guanine. Alternatively, the C4 and C5 hydroxyl radical addition intermediates can lose H_2O to form guanine radical. Guanine radical can also be formed direct by oxidation of guanine by reactive oxygen species (ROS).

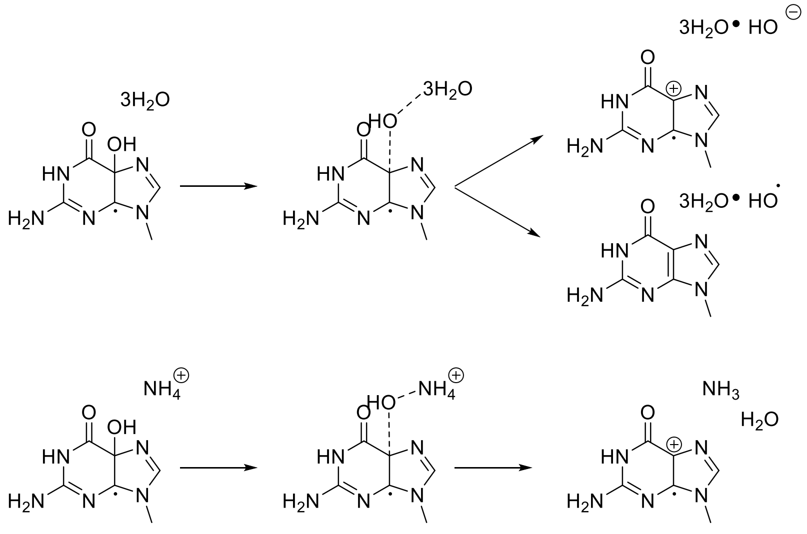
Scheme 4. Reactions involving water loss, oxidation, and radical combination with superoxide for the hydroxyl radical addition intermediates (relative energies and barriers (kcal/mol) in red, calculated redox potentials in blue).



[a]No explicit water molecules were used in calculation of these energies. [b]Three explicit water molecules were used in calculation of these energies. [c]Four explicit water molecules were used in calculation of these energies.

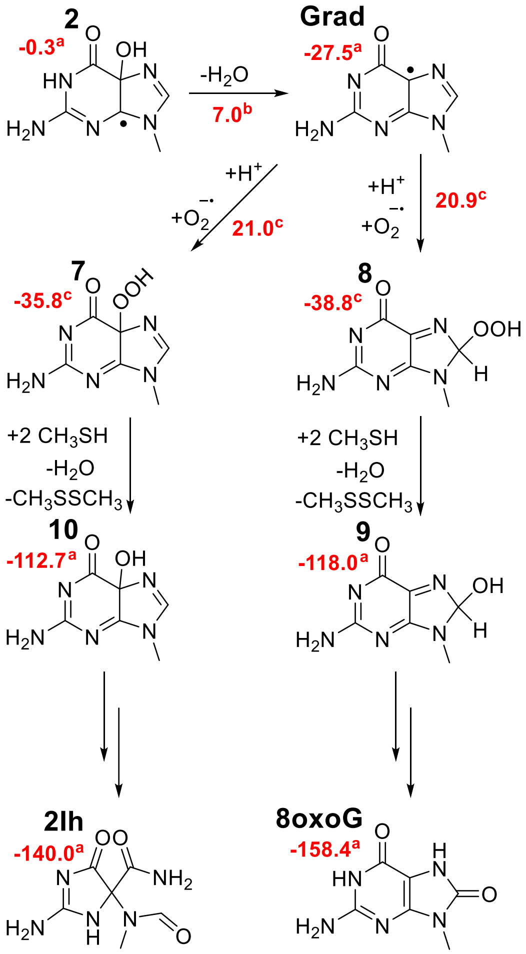
Scheme 5 outlines the calculation of H_2O loss from the C5 hydroxyl radical addition intermediates in the presence of explicit waters and a proton source. The barrier for OH loss is 7.0 kcal/mol calculated with 3 explicit waters and 5.5 kcal/mol when calculated with ammonium. For the former, the spin density on the leaving OH group suggests that this transition state could either revert to guanine and hydroxyl radical (endothermic by 0.3 kcal/mol) or lead to the hydroxide anion and guanine radical cation (exothermic by 8.0 kcal/mol). Tautomerization to neutral guanine radical involves protonation of the OH group and deprotonation of N1 (exothermic by 27.2 kcal/mol relative to **2**).

Scheme 5. Qualitative comparison of OH loss from **2** (explicit water molecules were used to stabilize loss of hydroxide anion; ammonium was used as a proton source for the loss of water).



The formation of **10** from **2** may also occur via a reductive pathway, as shown in Scheme 6, and therefore depends on the reaction conditions. The findings of Alshykhly, Fleming, and Burrows, where reducing agent is required in formation of 2Ih via Fenton oxidation, suggest **2** will favorably undergo water loss to guanine radical, **Grad**, followed by superoxide combination and protonation to **7**, and subsequent reduction to **10**, favoring the reductive pathway over oxidative.[4-5]

Scheme 6. Formation of **10** via oxidative and reductive pathways (relative energies and barriers (kcal/mol) in red).

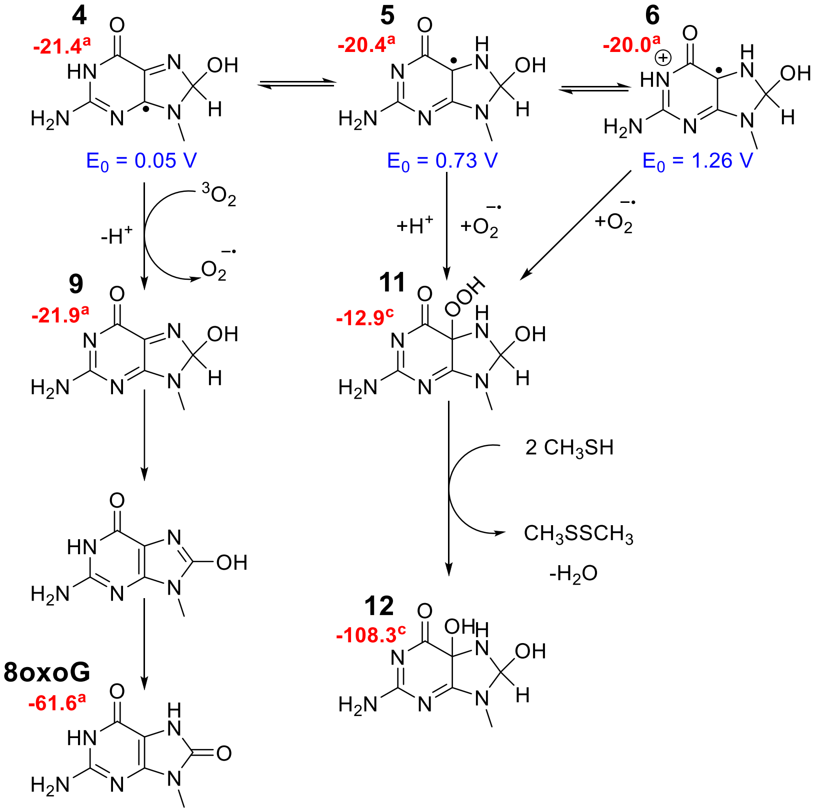


[a]No explicit water molecules were used in calculation of these energies. [b]Three explicit water molecules were used in calculation of these energies. [c]Four explicit water molecules were used in calculation of these energies.

Water loss from intermediate **3** to form **Grad** has a barrier of 17.4 kcal/mol when calculated with 3 explicit waters and 14.6 kcal/mol when calculated with ammonium. The calculated barrier for superoxide addition to C5 of **3** is 19.8 kcal/mol. C4 water loss from the resulting C5-hydroperoxyl, C4-hydroxyl intermediate was calculated to have a barrier of 23 kcal/mol and is not favorable. Reduction of the C5-OOH, C4-OH intermediate to a C4, C5 diol resulted in the C4 water loss barrier being increased to 30.6 kcal/mol. Given that there is no experimental evidence for a C4, C5 diol intermediate, or any product which may reasonable arise from such an intermediate, it is likely that the water loss from **3** to **Grad** is the dominant step after C4 addition of OH radical.

Scheme 7 shows the C8 hydroxyl radical addition intermediates, **4**, **5**, and **6**, which were all calculated to be lower energy than the C4 and C5 addition intermediates. Water loss barriers were calculated to range between 19.8-28.5 kcal/mol and suggest water loss to form **Grad** will not be the most likely pathway. The C8 hydroxyl radical addition intermediate, unlike the C4 and C5 adducts, may favorably undergo tautomerization between N1 and N7 protonation, with the N1/N7 pK_a's of **6** being calculated as 7.3/7.0 with ωB97xD and 5.3/6.6 with CBS-QB3. The significance of tautomerization between **4** and **5** along the guanine oxidation pathway is evident when considering the reduction potentials of the pair. **4** was calculated to have a reduction potential of 0.05 V and -0.14 V with DFT and CBS-QB3, respectively, compared to -0.16 V for triplet oxygen, suggesting oxidation with $^3O_2$ would be only slightly endothermic. C8 deprotonation was calculated to be significantly more exothermic after oxidation,[55] allowing the pathway to continue forward to the **8oxoG** product. **5** was calculated to have a reduction potential of 0.73 V calculated with DFT and 0.64 V with CBS-QB3, suggesting $^3O_2$ oxidation would be about 18-23 kcal/mol endothermic and a stronger oxidant would be required. The increase in reduction potential with N7 protonation suggests that, for **5** and **6**, superoxide radical combination may outcompete **8oxoG** formation in the absence of a sufficiently strong oxidant. **8oxoG** may be formed from oxidation of **4** or from guanine radical-superoxide combination to **8**, in a manner analogous to Scheme 6. Radical combination of superoxide with **5** or **6** results in **11**, followed by reduction to **12**, a proposed key intermediate along the **2Ih** formation pathway. The difference between **10** and **12** is hydroxyl substitution at C8; both may lead to **2Ih** but through different pathways. Candeias and Steenken[44] observed that radiolysis-initiated hydroxyl radical addition to guanine radical resulted in an adduct with oxidizing properties as the dominant product, and an adduct with reducing properties as a minor product. Sharma and co-workers found evidence of a transient C4 hydroxyl guanine radical adduct formed in pulse radiolysis experiments, which was supported by their calculated results and further reinforces the hydroxyl radical addition pathway.[84] Chatgilialoglu et al. reported hydrogen atom abstraction as the primary pathway for hydroxyl radical oxidation of guanine.[64-65] While different studies provide evidence for different pathways, there is agreement that guanine radical is produced as the major product. Our calculations show C4 or C5 hydroxyl radical addition and subsequent hydroxide / water loss results in guanine radical. C8 addition was found to be higher energy, resulting in a reducing species as a minor product. These results are in agreement with experimental and other computational findings.

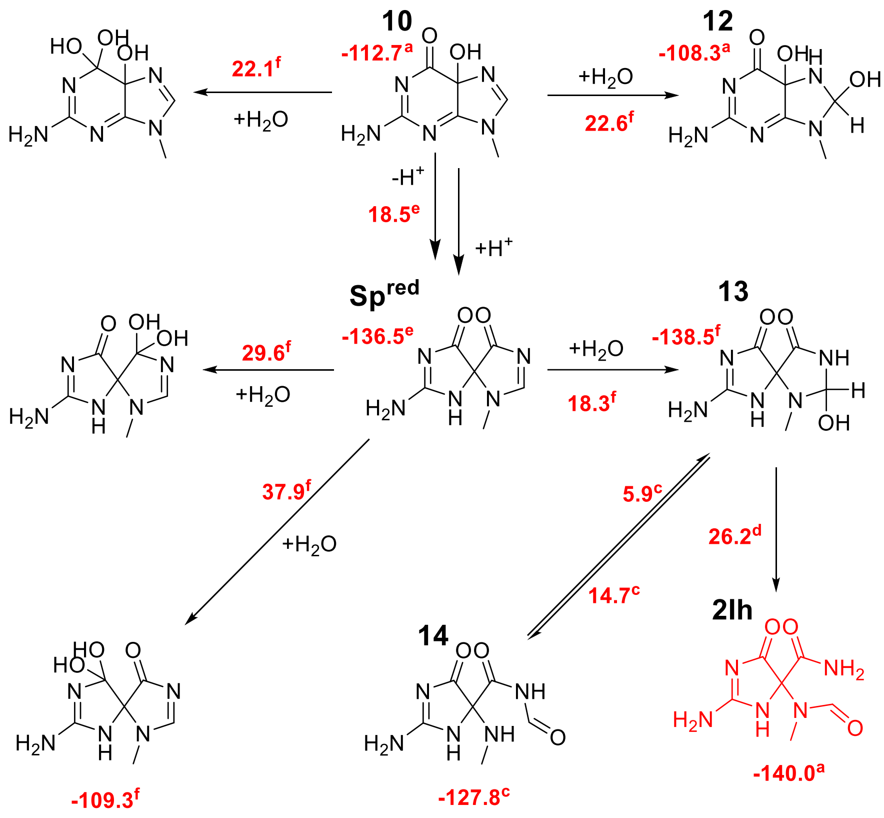
Scheme 7. Water loss, oxidation, and radical combination with superoxide from the hydroxyl radical addition intermediates (relative energies (kcal/mol) in red, calculated redox potentials in blue).



[a] No explicit water molecules were used in calculation of these energies. [b] Three explicit water molecules were used in calculation of these energies. [c] Four explicit water molecules were used in calculation of these energies.

3.3 Acyl Migration and C8 Ring Opening. Scheme 8 shows the conversion of **10** to **2Ih** via acyl migration and ring opening, as well as some potential branching pathways. Conversion of **10** to **12** was considered as a possibility, but the barrier (22.6 kcal/mol) is higher than C5-OH deprotonation and acyl migration to the reduced form of **Sp**, **Sp**$^{\mathbf{red}}$. C8 water addition to this spiroiminodihydantoin intermediate results in **13**, the precursor to **2Ih**. Ring opening may occur via the C8-N9 or N7-C8 bond. C8-N9 ring opening has a lower barrier but formation of product **14** is endothermic, whereas N7-C8 ring opening has a higher barrier but leading to the more stable **2Ih** product.

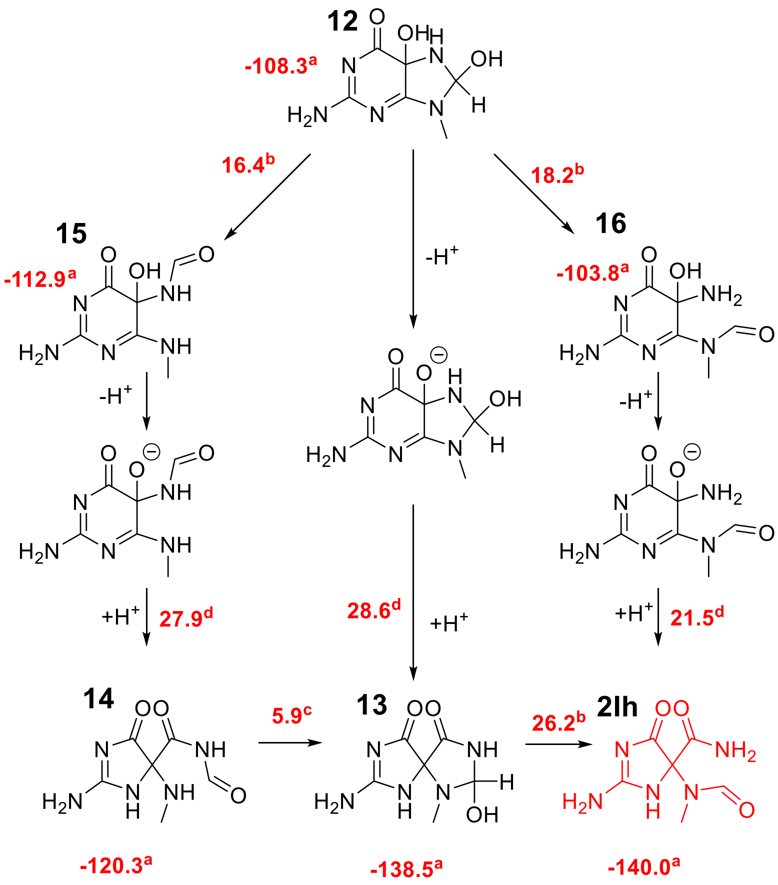
Scheme 8. Conversion of 5-hydroxy-guanine to the **2Ih** product (relative energies and barriers (kcal/mol) in red).



[a] No explicit water molecules were used in calculation of these energies. [c] Four explicit water molecules were used in calculation of these energies. [d] Five explicit water molecules were used in calculation of these energies. [e] Six explicit water molecules were used in calculation of these energies. [f] One explicit water molecule was used to model water addition, with two additional water molecules included to facilitate proton transfer in the water addition process.

Scheme 9 shows conversion of **12** to **2Ih**. Unlike **10**, **12** undergoes ring opening via N7-C8 or C8-N9 bond cleavage before acyl migration. Acyl migration from **12** to **13** prior to ring opening has a significantly higher barrier. For structure **12**, the barrier for C8-N9 ring opening to **15** was calculated to be 2 kcal/mol lower than for N7-C8 ring opening to **16**; however, acyl migration **15** to **14** has a higher barrier than **16** to **2Ih**. While **14** has a low barrier for conversion to **13**, the barrier for **13** to **2Ih** is rather high. Furthermore, water addition to **15** to form a C6 diol (20.0 kcal/mol barrier) is preferred over acyl migration to **14** (27.9 kcal/mol barrier), suggesting that the pathway from **12** through **15** may not result in **2Ih**. Barriers for C6 diol formation in **12** and **16** are higher than ring opening and acyl migration, respectively. Thus, the lowest energy path from **12** to **2Ih** is C8-N9 cleavage followed by acyl migration as shown in Scheme 9.

Scheme 9. Conversion of 5,8-dihydroxy-guanine to the 2Ih product (relative energies and barriers (kcal/mol) in red)



[a]No explicit water molecules were used in calculation of these energies. [b]Three explicit water molecules were used in calculation of these energies. [c]Four explicit water molecules were used in calculation of these energies. [d]Six explicit water molecules were used in calculation of these energies.

4. CONCLUSIONS.

With hydroxyl radical as the initiator, oxidation of guanine to **2Ih** was found to proceed favorably via production of guanine radical as the major pathway, through either C4 or C5 radical addition, with the C8-hydroxyl radical intermediate as the minor pathway (Scheme 3 and 4). Superoxide radical combination with guanine radical at the C5 or C8 positions were found to be competitive, resulting in **2Ih** or **8oxoG** respectively, in the presence of reducing agents (Scheme 6 and 7). Reduction of the C5 hydroperoxyl group to the alcohol and deprotonation of the alcohol is followed by acyl migration to intermediates $\mathbf{Sp^{red}}$ or C8-hydroxy $\mathbf{Sp^{red}}$ (Scheme 8). Water addition to $\mathbf{Sp^{red}}$ results in C8-hydroxy $\mathbf{Sp^{red}}$. C8-N9 ring opening was found to be more favorable than N7-C8 ring opening to **2Ih**, however the resulting intermediate was found to be endothermic and favorably reverts to **2Ih** (Scheme 9).

Candeias and Steenken[44] found that the major pathway of guanine oxidation via hydroxyl radical was addition at C4 or C5 and formation of a radical guanine species with oxidizing properties, with addition of hydroxyl radical C8 hydroxyl being a minor pathway. Our results show that C4, C5, and C8 hydroxyl addition intermediates all have relatively low reduction potentials. Hydroxide or water loss from the C4 and C5 adducts is facile and results in guanine radical, explaining the predominant oxidizing intermediate observed by Candeias and Steenken. The higher barrier for C8 hydroxyl radical addition compared to C5 may also explain the observation of the C8-OH adduct as a minor product. Fenton chemistry studies by the Burrows group[4-6] found that increasing the concentration of reducing agents produced increasing amounts of **2Ih**. In the absence of reducing agents, experiments found **Sp** and **Iz** as the dominant reaction products. Formation of guanine radical from **2**, followed by combination with superoxide to form the hydroperoxyl intermediate, **7**, is a pathway for the formation of **2Ih** that depends on the presence of a reducing agent, in agreement with experiment. Increasing the amounts of reducing agent does not change the observed product yields of **8oxoG** and **Sp**. Formation of **Iz** and **Sp** via a non-reductive pathway is being explored in a separate computational study.[85] Meunier and co-workers investigated the formation of **2Ih** upon two electron oxidation of guanine with Mn-TMPyP and $KHSO_5$ oxidation of guanine.[41] Using HPLC/ESI-MS, they found multiple geometric isomers which they attributed to intermediates along the **2Ih** formation pathway. Among them, **14** was proposed as an explanation for the observed ^{18}O exchange at the C8 position.[41] The computational results support the reversibility of the N9 bond opening, with the low barrier for ring closing explaining the absence of **14** as an isolated intermediate.

Rokhlenko et al. studied guanine oxidation via carbonate radical and found that **2Ih** formed in competition with **8oxoG** and its further oxidation products.[2] The ratio of C5:C8 addition products was about 1.2. Joffe et al. proposed that secondary structure of DNA influences the C5:C8 product distribution, and found ratios ranging 0.8 - 3.4 depending on nucleic acid structure.[32] Radical combination reactions with guanine radical were calculated to have no significant preference for addition to either C5 or C8 positions. However, it should be noted that C8 addition intermediates are consistently lower in energy than C5 addition intermediates. Our calculations have examined only the reactivity of nucleobase and do not take into account the effects of the secondary structure of DNA. The observed range of ratios for C5:C8 addition products may be due to site accessibility or other interactions not considered in the present calculations.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website

> Optimized molecular geometries in Cartesian coordinates and free energies (in Hartree) for all the reactants, intermediates, transition states, and products in PDF format.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

2Ih,5-carboxyamido-5-formamido-2-iminohydantoin; FapyG,2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8oxoG,8-oxo-7,8-dihydroguanine; 8oxoP,2-amino-6,8-dioxo-9-methylpurine; Sp,spiroiminodihydantoin; Sp^{red},reduced spiroiminodihydantoin; Gh,guanidinohydantoin; Iz,imidazolone

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