Modeling Competitive Reaction Mechanisms of Peroxynitrite Oxidation of Guanine

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5-Guanidino-4-nitroimidazole is a stable product from the peroxynitrite induced one-electron oxidation of guanine. Reaction mechanisms to form the 5-guanidino-4-nitroimidazole as well as 8-nitroguanine, through the combination of the guanine radical cation and nitrogen dioxide radical and through the combination of the deprotonated neutral guanine radical and nitrogen dioxide radical, have been investigated by the use of the B3LYP method of density functional theory. Our calculations suggest that the guanine radical cation mechanism is preferred over the neutral guanine radical mechanism and that a water molecule is involved in the reaction as a catalyst or as a reactant.

Introduction

Oxidative damage to DNA is responsible for aging and many diseases, including cancers.[1] Among the four nucleic acid bases in DNA, guanine has the lowest oxidation potential (1.29 V vs NHE)[2,3] and is therefore the easiest to oxidize to form the guanine radical cation ($G^{\bullet+}$). In the presence of ionizing radiation, photosensitizers or reactive oxygen species (ROS),[2] the electronic hole introduced into the duplex DNA shows a strong tendency to migrate from the site of ionization to the guanine base of 5′-guanine of the 5′-GG-3′ doublets or the more oxidatively sensitive sites 5′-GGG-3′ triplets through DNA π-stacking.[4−6] Guanine radicals can further react with mild reducing agents, such as some redox active amino acids or small peptides,[7−10] or with H_2O and/or O_2, yielding 8-oxoguanine (8-oxoGua) as the most common product.[8,9] The reactions involved in this irreversible lesion are the hydration of guanine followed by one-electron oxidation.

The guanine radical cation, $G^{\bullet+}$, has an oxidation potential (0.53 V vs NHE[10]) even lower than its guanine precursor and therefore could be easily oxidized to give secondary oxidation products.[11−13] Another two-electron oxidation product of guanine is 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FAPY-Gua),[14] a minor derivative compared to 8-oxoguanine. Certain DNA-repairing enzymes are able to recognize those lesions and catalyze the cleavage of DNA at the sites of damage.[15] (Four significant products are given in Figure 1.)

$G^{\bullet+}$ is a much stronger acid ($pK_a = 3.9$) than guanine itself ($pK_a = 9.4$).[16] Once formed, $G^{\bullet+}$ undergoes rapid deprotonation to generate the neutral radical denoted by $\mathbf{G}^{\bullet}(-H)$.[17] In the DNA duplex, the proton will be transferred to N3 of cytosine of the GC base pair. Because the pK_a (4.3) of N3-protonated cytosine is only slightly higher than that of $G^{\bullet+}$, $G^{\bullet+}$ formed in the DNA duplex should retain more cationic character than would an isolated G nucleoside or nucleotide, which means the guanine radical cation would persist long enough to react with oxidizing or nitrating reagents.

Peroxynitrite ($ONOO^-$) is a powerful oxidizing and nitrating agent that can damage a variety of biomolecules.[18] $ONOO^-$ can be formed in the diffusion-limited combination reaction of $NO^{\bullet}$ and $O_2^{\bullet}$, produced by macrophages and neutrophils (two types of inflammatory cells) upon immune response activation.[19−21] In vivo, $ONOO^-$ rapidly combines with CO_2 yielding nitrosoperoxycarbonate ($ONOOCO_2^-$) that undergoes homolysis to produce the carbonate radical ($CO_3^{\bullet-}$) and nitrogen dioxide radical ($NO_2^{\bullet}$).[22−24] $CO_3^{\bullet-}$ and $NO_2^{\bullet}$ are believed to be responsible for the oxidation and nitration of DNA when it is exposed to $ONOO^-$.

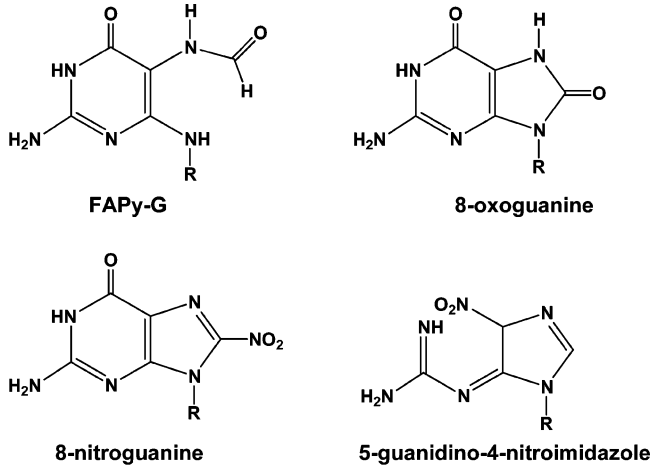

Figure 1. Four significant products from guanine oxidation reactions.

One-electron oxidation products of guanine and $ONOO^-$ include 8-oxoguanine (8-oxoGua), 8-nitroguanine (8-NO_2Gua)[25] and 5-guanidino-4-nitroimidazole.[26] Unlike the first two intermediates that readily undergo further oxidation, the last one is a stable and significant reaction product formed only through peroxynitrite-related chemistry. Thus, 5-guanidino-4-nitroimidazole may be meaningful for understanding the DNA damage induced by peroxynitrite.

To describe the mechanisms of the reactions, two competitive reaction pathways (Figure 2) have been proposed for the formation of 5-guanidino-4-nitroimidazole and 8-NO_2Gua.[26] In the first one, $NO_2^{\bullet}$ combines with the guanine radical cation at the C5 (Figure 2a) or C8 position (Figure 2b) of the purine ring. Subsequent reactions lead to the formation of the final products 5-guanidino-4-nitroimidazole and 8-NO_2Gua. At the beginning of the second reaction pathway, the guanine radical cation, $G^{\bullet+}$, deprotonates to yield the neutral radical $\mathbf{G}^{\bullet}(-H)$, and then a radical combination reaction takes place between $\mathbf{G}^{\bullet}(-H)$ and $NO_2^{\bullet}$. The following reaction steps are analogous

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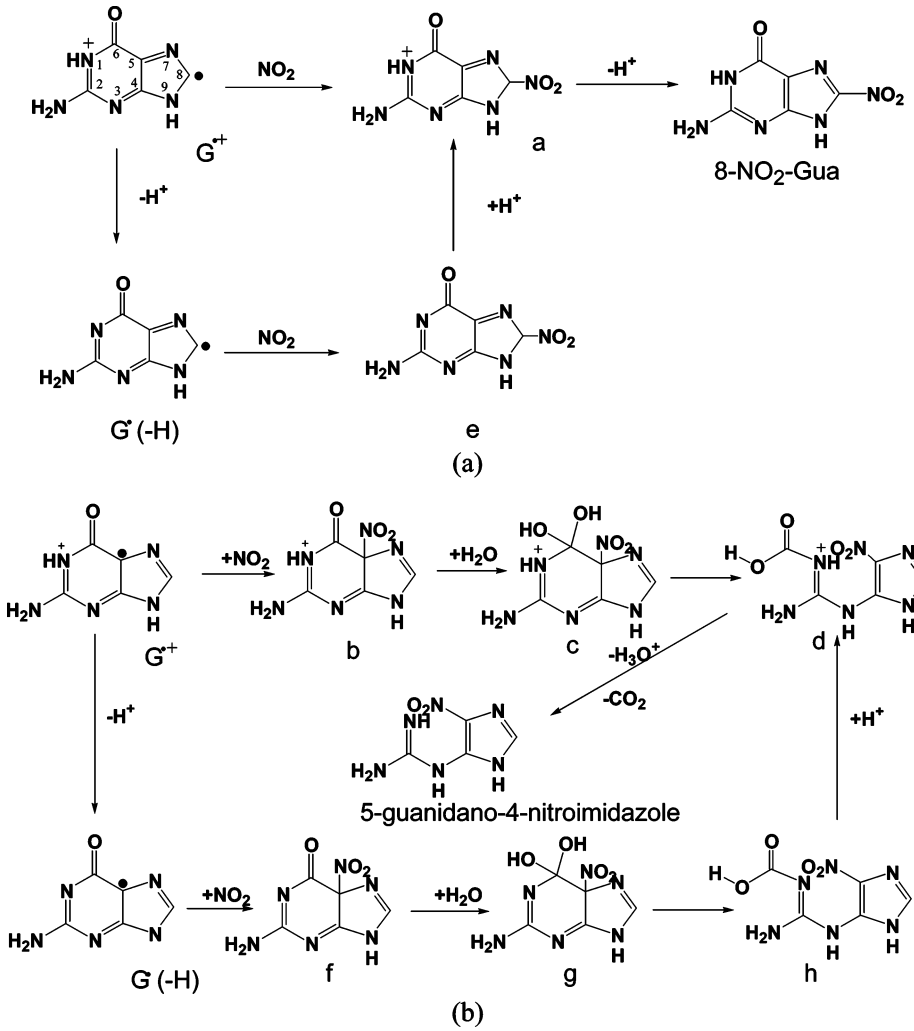

Figure 2. Reaction mechanisms leading to the formation of (**a**) 8-NO_2Gua and (**b**) 5-guanidino-4-nitroimidazole.

to those in the guanine radical cation reaction pathway. Intermediates in both reaction mechanisms are labeled **a** though **h**, as shown in Figure 2.

The present paper describes a computational investigation of the guanine oxidatively generated lesion introduced by peroxynitrite. Comparison of the two proposed reaction mechanisms that lead to the formation of two significant products (8-NO_2-Gua and 5-guanidino-4-nitroimidazole) indicates that the guanine radical cation mechanism is more favorable than the neutral guanine radical mechanism. The important role of a water molecule in all reaction steps is also presented.

Computational Methods

All geometry optimizations and frequency calculations were performed with the B3LYP hybrid density functional in conjunction with the 6-31G(d,p) basis set using the Gaussian 03 suite of programs.[27] Single point calculations were performed with the B3LYP level of theory and Pople's 6-311++G(2df,2p) basis set using the above geometries. Gibbs free energy corrections derived from the frequency calculations were added to the single point electronic energies to obtain relative free energies for all reactants, products, intermediates and transition states.

The PCM and CPCM solvation models were used initially to treat solvent effects in the present work. However, due to convergence problems in the optimization of several structures,

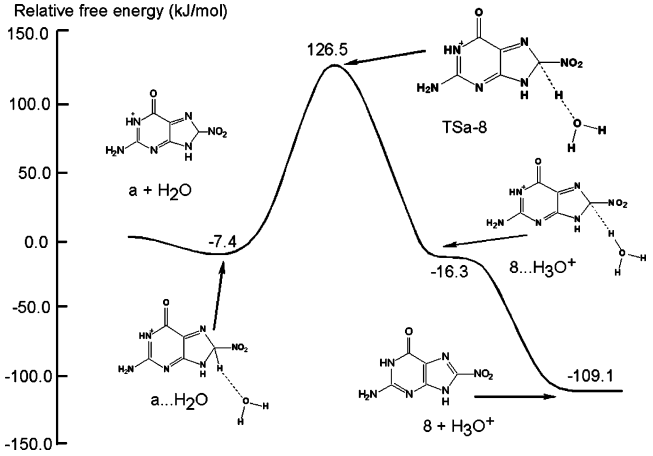

Figure 3. Schematic free energy profile at 298.15 K for the route **a** → **8**-NO_2Gua with one water involved in the deprotonation process.

the more robust Onsager solvation model[29] was utilized in all geometry optimization and frequency calculations. Convergence difficulties with the PCM and CPCM solvation models are well-known (see, for example, the work of Vallet et al.[28] and Wu et al.[29]). All cavity sizes, including that of H_3O^+, were taken to be the default values assumed by Gaussian 03. All energies are in kJ/mol.

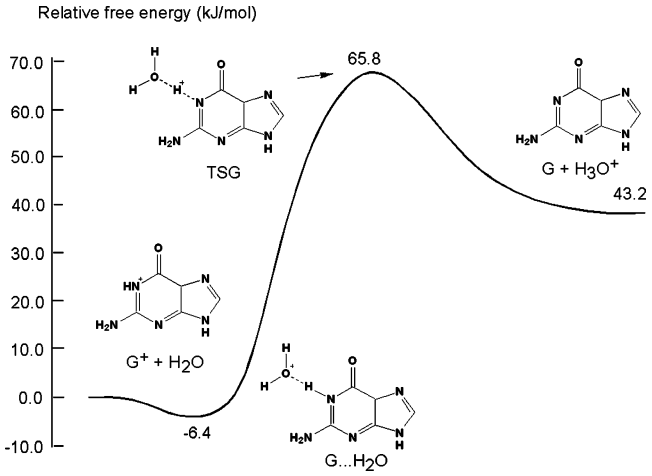

Figure 4. Schematic free energy profile at 298.15 K for the route $G^{\bullet+} \rightarrow G^{\bullet}(-H)$ with one water molecule involved. $G^{\bullet}(-H)$ has significant unpaired electron density at the O6, C5, and C8 positions[30] and therefore NO_2 may combine with $G^{\bullet}(-H)$ at either C5 or C8.

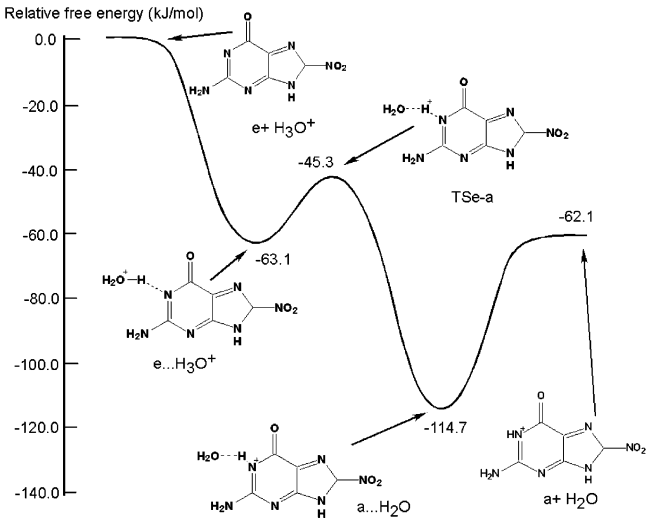

Figure 5. Schematic free energy profile at 298.15 K for the route **e** → **a** with one water involved.

Results and Discussion

Formation of 8-NO_2Gua. In the guanine radical cation pathway, the reaction is initiated by the radical combination reaction of the guanine radical cation and NO_2 at the C8 position of the purine ring, whereas in the neutral guanine radical reaction mechanism, the neutral guanine radical is formed as a result of the deprotonation reaction that takes place at the N4 position of the guanine radical cation.

1. Guanine Radical Cation Reaction Mechanism. The guanine radical cation, formed by the one-electron oxidation of guanine has significant unpaired electron density[30] at the O6, C5, and C8 positions. When the NO_2 radical is available, it may immediately combine with the guanine radical cation at the C8 or C5 position to form 8-nitroguanine (**8**) or 5-guanidino-4-nitroimidazole (**6**). As indicated in Figure 3, intermediate **a** is formed as the NO_2 radical combines with the guanine radical cation at the C8 position. In aqueous solution, deprotonation takes place at the C8 position and leads to the formation of 8-nitroguanine. The energy of complex **a**...H_2O lies lower than that of **a**+H_2O by 7.4 kJ/mol. A transition state **TSa-8** is found with a relative energy of 126.5 kJ/mol. A complex **8**...H_2O is derived after the transition state and followed by the isolated system 8-NO_2Gua + H_2O and their relative energies are −16.3 and −109.1 kJ/mol, respectively.

2. Neutral Guanine Radical Reaction Mechanism. As mentioned previously, the guanine radical cation is a relatively strong acid and will readily lose the proton at N1 in the purine ring to an available proton acceptor. This reaction yields the neutral guanine radical that initiates a series of reactions leading to the formation of the final products 5-guanidino-4-nitroimidazole and 8-NO_2Gua. In this deprotonation process (Figure 4), the transition state **TSG** and the reactant complex **G**...H_2O have energies 65.8 and −6.4 kJ/mol relative to the energy of the isolated system of H_2O and guanine radical cation. A complete transfer of the proton to H_2O leads to a relative energy of 43.2 kJ/mol for $H_3O^+ + G^{\bullet}(-H)$.

Intermediate **e** is derived once the nitrogen dioxide radical combines with the guanine radical at the C8 position. Unlike intermediate **a** in the guanine radical cation mechanism, **e** is not able to form 8-NO_2Gua in a single-step reaction because one proton has to be removed from the C8 position and another proton attached to N1. It is not likely that these two events could be involved in a single-step reaction due to the planar structure of the compound and the distance between these two positions. Our results show that after a proton is attached to give intermediate **a**, the reaction follows the same pathway as is described in the radical cation reaction mechanism to yield

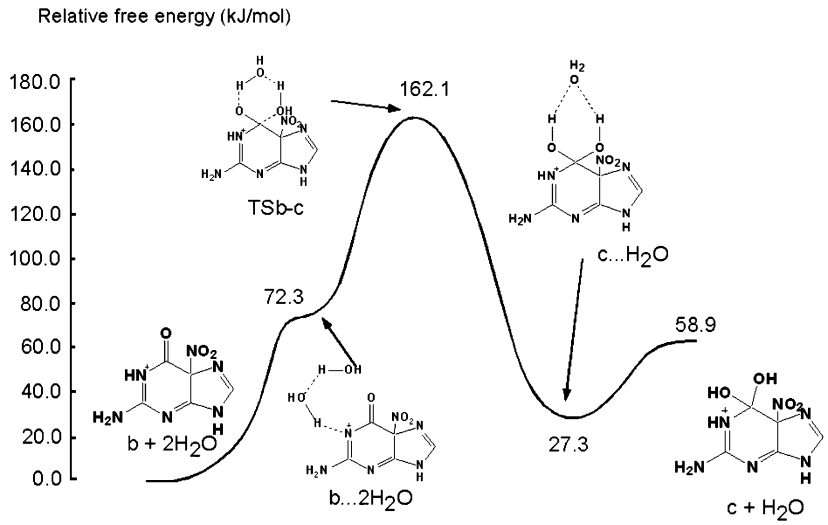

Figure 6. Schematic free energy profile at 298.15 K for the route **b** → **c** with two waters involved in this process.

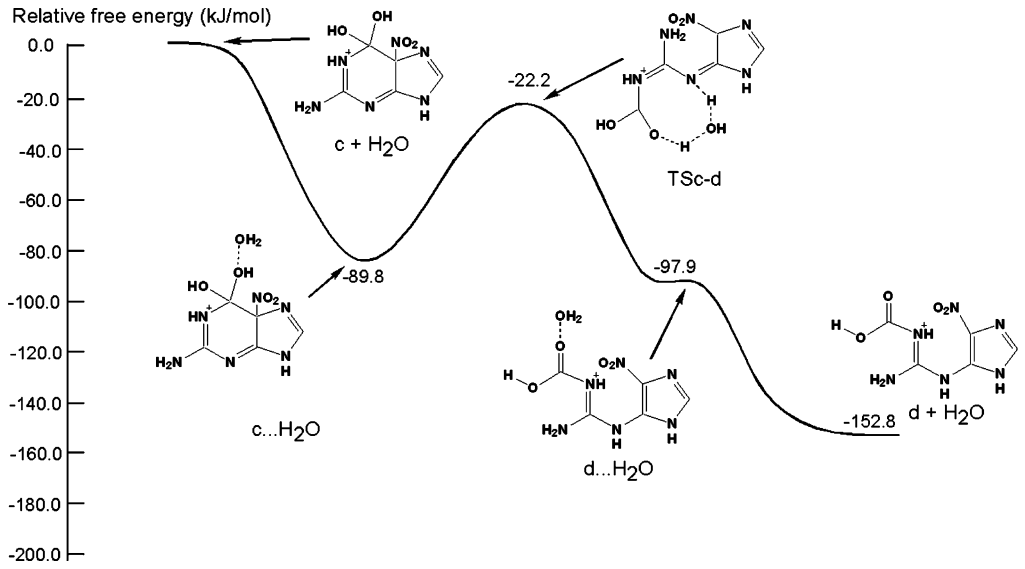

Figure 7. Schematic free energy profile at 298.15 K for the route **c** → **d** with a water molecule involved in this process.

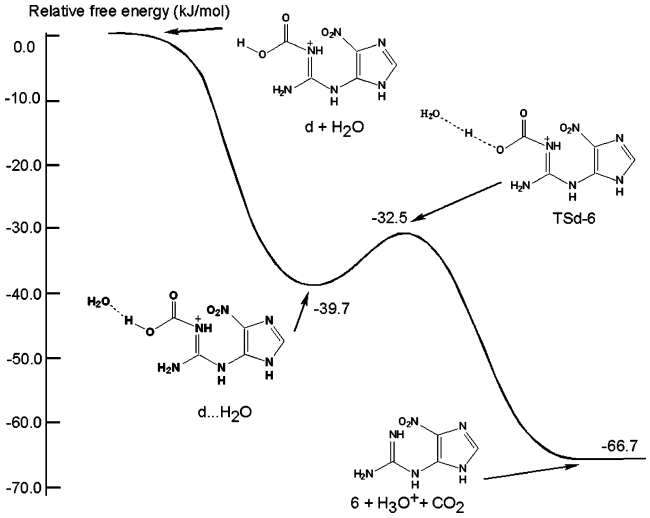

Figure 8. Schematic free energy profile at 298.15 K for the route **d** → **5**-guanidino-4-nitroimidazole with one water molecule involved.

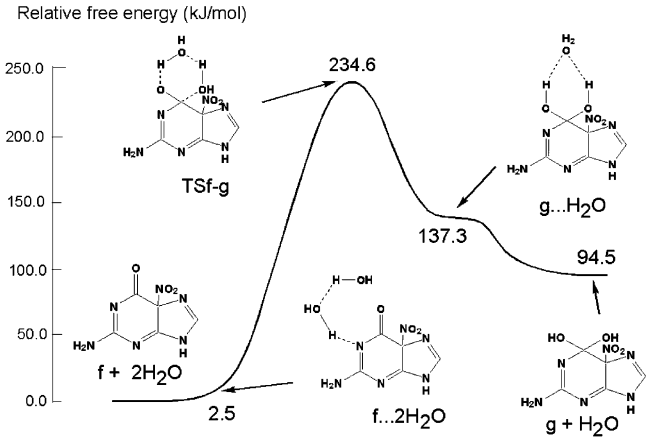

Figure 9. Schematic free energy profile at 298.15 K for the route **f** → **g** with two waters involved.

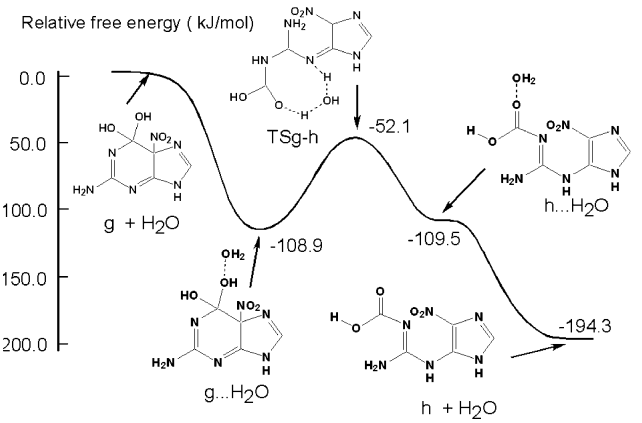

Figure 10. Schematic free energy profile at 298.15 K for the route **g** → **h** with one water involved.

8-NO_2Gua (Figure 5). Relative to the isolated **e**+H_3O^+ system, the transition state **TSe-a** has an energy of −45.3 kJ/mol. The relative energies of the complexes **e**...H_3O^+ and **a**...H_2O are −63.1 and −114.7 kJ/mol, respectively. The isolated system a+H_2O has a relative energy of −62.1 kJ/mol.

Formation of 5-Guanidino-4-nitroimidazole. *1. Guanine Radical Cation Reaction Mechanism.* In the case of C5 addition (Figure 6), intermediate **b** is formed, which can be attacked by water at the C6 position to yield **c**. In this process, two solvent water molecules are involved: one water is connected to the electrophilic C6 position via its oxygen atom and the other water molecule serves as a water bridge to facilitate the proton transfer from the first water molecule to the oxygen originally connected to the C6 position. Therefore, a six-membered ring is formed at transition state **TSb-c**. Complex **b**...2H_2O has an energy of 72.3 kJ/mol relative to that of **b**+2H_2O, the relative energy of **TSb-c** is 162.1 kJ/mol, the complex formed after the transition state, **c**...H_2O, has a relative energy of 27.3 kJ/mol, and the relative energy of separated system **c**+H_2O is 58.9 kJ/mol.

In the next step (Figure 7), an intramolecular proton transfer takes place. The proton is removed from the hydroxyl group that is connected to the C6 position and attached to the N3 position. As shown in Figure 7, the proton transfer is an intramolecular process, which shifts the proton from the hydroxyl group at the C6 position directly to the N3 position with the help of a water bridge. The cleavage of the C5−C6 bond happens simultaneously. Compared to the energies of the isolated group **c**+H_2O, the relative energies of the complexes **c**...H_2O and **d**...H_2O are −89.8 and −97.9 kJ/mol, respectively;

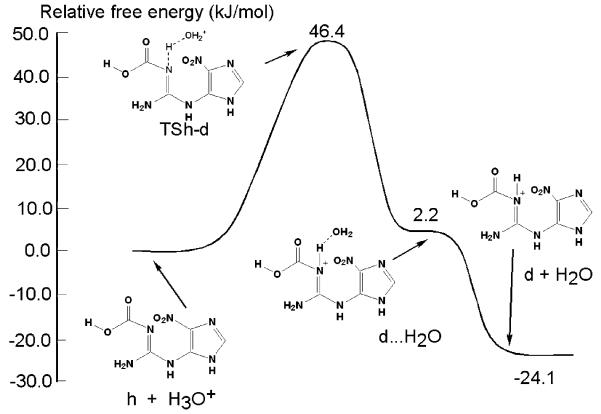

Figure 11. Schematic free energy profile at 298.15 K for the route **h** → **d** with one H_3O^+ involved.

transition state **TSc-d** has a relative energy of −22.2 kJ/mol, whereas the relative energy of the product group **d**+H_2O is −152.8 kJ/mol.

The final product 5-guanidino-4-nitroimidazole is derived through decarboxylation of intermediate **d** (Figure 8). A water molecule acts as a proton acceptor and helps remove the proton from the carboxyl group of the carbamate **d**. Meanwhile, the C−N bond breaks and a CO_2 molecule is released. In this manner, intermediate **d** is decomposed into H_3O^+, CO_2, and 5-guanidino-4-nitroimidazole without the formation of any complex after the transition state **TSd-6**. Relative to the **d**+H_2O system, the complex **d**...H_2O has an energy of −39.7 kJ/mol, the transition state has an energy of −32.5 kJ/mol, and the products 5-guanidino-4-nitroimidazole + CO_2 + H_3O^+ are much lower at −66.7 kJ/mol.

2. Neutral Guanine Radical Reaction Mechanism. Analogous to what happens in the radical cation reaction mechanism, the nitrogen dioxide radical may also combine with the **G**•(−H) at the C5 position and form an intermediate **f**, which can be attacked by a water molecule at the electrophilic C6 position to yield **g**. In the transition state (Figure 9), a six-membered ring is formed with the help of a water bridge to facilitate the proton transfer. Total energy changes relative to the isolated

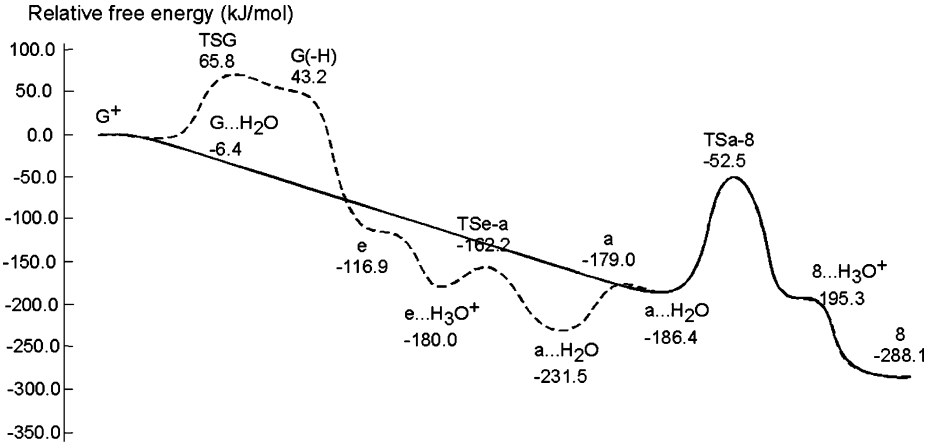

Figure 12. Schematic free energy profile at 298.15 K for the complete reaction G^+ → **8**-NO_2Gua. The solid line represents the guanine radical cation reaction mechanism, and the dashed line represents the neutral guanine radical mechanism.

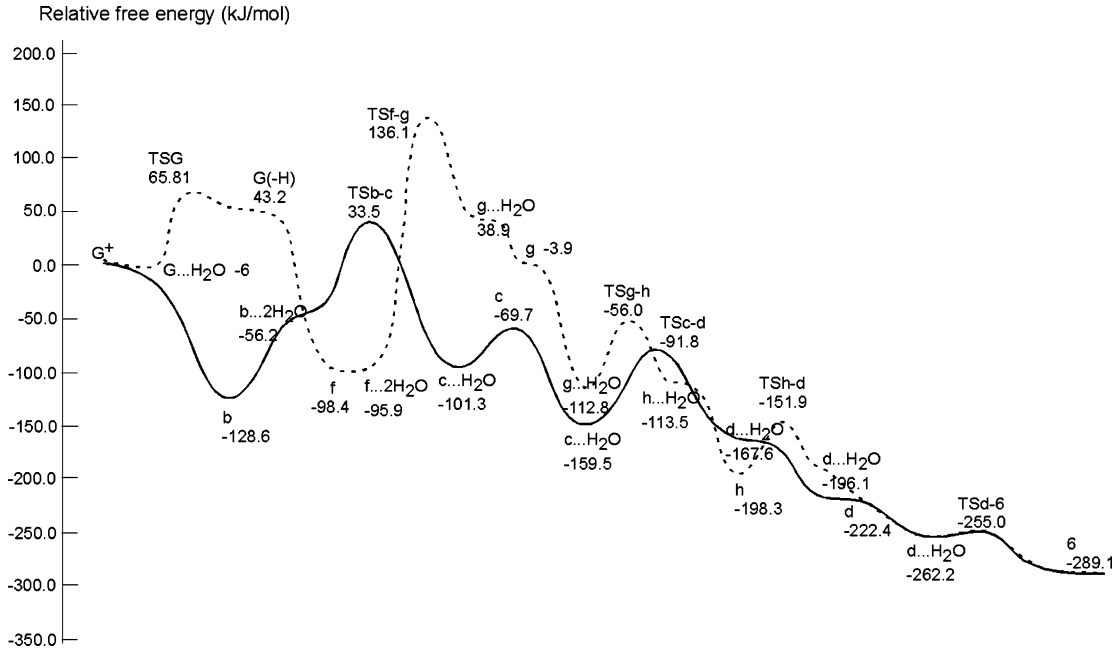

Figure 13. Schematic free energy profile at 298.15 K for the complete reaction G^+ → 5-guanidino-4-nitroimidazole. The solid line represents the guanine radical cation reaction mechanism, and the dashed line represents the neutral guanine radical mechanism.

system of **f**+$2H_2O$ are as follows: the complex **f**...$2H_2O$ has an energy of 2.5 kJ/mol, the transition state **TSf-g** has a relative energy of 234.6 kJ/mol; the relative energies of complex **g**...H_2O and **g**+H_2O are 137.3 and 94.5 kJ/mol, respectively.

Subsequently, an intramolecular proton transfer takes place in the newly formed intermediate **g**. A water molecule is involved as well to form the water bridge across the purine ring plane (Figure 10). This process is accompanied by the cleavage of the C5−C6 bond and followed by the formation of the carbomate derivative **h**. Relative to **g**+H_2O, complex **g**...H_2O has an energy of −108.9 kJ/mol and transition state **TSg-h** has a relative energy of −52.1 kJ/mol; and the relative energies of complexes **h**...$2H_2O$ and **h**+$2H_2O$ are −109.5 and −194.4 kJ/mol, respectively.

In the **G•**(−H) mechanism, however, 5-guanidino-4-nitroimidazole cannot be derived directly from intermediate **h**. A proton must be attached to the N1 position to form intermediate **d** and then the final product 5-guanidino-4-nitroimidazole is derived as a result of the decomposition of **d** (Figure 11). In the protonation process, a H_3O^+ serves as a proton donor. Transition state **TSh-d** is formed with a relative energy of 46.4 kJ/mol and no complex is located before that. The relative energies of the **d**...H_2O complex and products (**d**+H_2O) are +2.2 and −24.1 kJ/mol, respectively.

Immediately following the formation of the guanine radical cation, the radical−radical combination reaction takes place at a diffusion-controlled rate and the intermediate **a** is derived. Otherwise, guanine radical cations will undergo deprotonation to yield the neutral radical **G•**(−H).

For the formation of 8-NO_2Gua, the overall free energy profiles of the guanine radical cation reaction mechanism and the neutral guanine radical mechanism are shown in Figure 12. To produce the intermediate **a**, the single-step radical−radical combination reaction in the radical cation reaction mechanism is more favored than the two steps of the reactions in the neutral guanine radical mechanism. The radical−radical combination reaction has no barrier to overcome and is thermodynamically favored by 179.0 kJ/mol. However, the first deprotonation reaction of the neutral guanine radical mechanism has to overcome a barrier of 65.8 kJ/mol via the transition state **TSG**. In addition, the derived neutral guanine radical **G•**(−H) has to overcome a second barrier of 17.8 kJ/mol to form intermediate **a**. Because the last step of the reactions to form 8-NO_2Gua in the two mechanisms are exactly the same, our results suggest that the guanine radical cation reaction mechanism is preferred over the neutral guanine radical mechanism for the formation of 8-NO_2Gua.

For the formation of the 5-guanidino-4-nitroimidazole, the overall free energy profiles of the guanine radical cation reaction mechanism and the neutral guanine radical mechanism are shown in Figure 13. Overall, the radical cation mechanism is a downhill reaction and has a free energy surface smoother than that of the neutral guanine radical mechanism. In particular, the first-step radical combination reaction in the radical cation mechanism is more favored than the first step deprotonation reaction to form the neutral guanine radical in the neutral guanine radical mechanism. In addition, the **f** → **g** step of the reaction in the neutral guanine radical pathway has the largest barrier of 234.6 kJ/mol. Therefore, the guanine radical cation reaction mechanism is preferred over the neutral guanine radical reaction mechanism for the formation of the significant oxidation product 5-guanidino-4-nitroimidazole.

Conclusions

Our computational study suggests that the guanine radical cation reaction mechanism is more favorable than the neutral guanine radical reaction mechanism in terms of the formation of both 8-NO_2Gua and 5-guanidino-4-nitroimidazole. Furthermore, our calculations suggest that a water molecule is involved in the guanine radical cation mechanism as a catalyst or as a reactant. Given the role of water in the preferred mechanism, we propose that the guanine radical cation reaction mechanism operates in vivo.

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Supporting Information Available: Archive entries of all B3LYP/6-31G(d,p) optimized structures and total energies of all molecules in aqueous solution. This material is available free of charge via the Internet at http://pubs.acs.org.

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