Guanyl Radicals

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A Reevaluation of the Ambident Reactivity of the Guanine Moiety Towards Hydroxyl Radicals**

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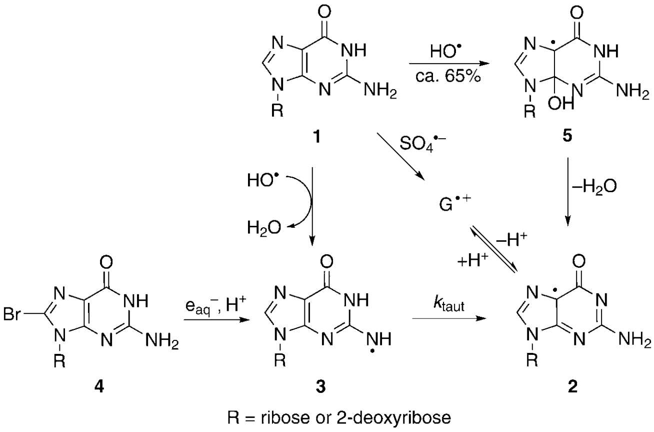
Two tautomers of guanyl radical have been identified experimentally. The most familiar tautomer **2** (see Scheme 1) is produced by one-electron oxidation of guanine derivatives **1** using oxidants, such as $SO_4^{\bullet-}$, $Br_2^{\bullet-}$, or $CO_3^{\bullet-}$ [1–3] whereas the tautomer **3** derives by protonation of the electron adduct of 8-bromoguanine derivatives **4**.[6,7] These two tautomers **2** and **3** showed different visible spectra around 610 nm, which allows the Arrhenius parameters to be measured for the tautomerization **3**→**2**, that is, $\log(A/s^{-1}) = 8.7 \pm 0.4$ and $E_a = 23.0 \pm 2.5$ kJ mol^{-1}.[6] Kinetic isotope effect, phosphate catalysis, and theoretical work support a water-assisted tautomerization.[6,7]



Scheme 1. The tautomerization **3**→**2** related to oxidation of guanine **1** and to reduction of 8-bromoguanine **4** has been studied in some detail.[6,7] The deprotonation of $G^{\bullet+}$ is associated with the $pK_a = 3.9$.[1]

The reaction of hydroxyl radical ($HO^{\bullet}$) with guanine is one of the most important damage processes in DNA. Initially the reaction was studied by O'Neill and co-workers using pulse radiolysis techniques.[8] They found that the reaction produces reducing and oxidizing radicals in equal amounts, the oxidizing radicals being tentatively assigned to radical **5**. Afterward, this concept was further discussed by Steenken in his Review article.[9] A few years later,[10] Candeias and Steenken revisited the reaction of $HO^{\bullet}$ with guanine derivatives in aqueous solution. In particular, they reported that the main reaction (ca. 65%) is the addition at the C4 position with formation of adduct **5**, which undergoes a dehydration reaction to give **2**. The minor path (ca. 17%) is the addition at C8 position and this adduct is the precursor of FapyG and 8-oxoG through reduction and oxidation reactions, respectively.[11] The ambident reactivity of guanine moiety towards the $HO^{\bullet}$ radical has been well assessed (e.g., see recent Reviews[12] and books[13]). Herein, we demonstrate that the main reaction of the $HO^{\bullet}$ radical with guanine moiety is not the addition at the C4 position but a hydrogen abstraction from the NH_2 moiety (**1**→**3**) followed by the tautomerization **3**→**2**.

The spectral changes obtained from the pulse irradiation of a N_2O-saturated aqueous solution of 1 mM guanosine (**1**) are shown in Figure 1.[14,16] The optical absorption spectrum taken 1 μs after the pulse shows a sharp band around 305 nm and a very broad one around 610 nm. By measuring the growth of the band at 305 nm or 610 nm as a function of the

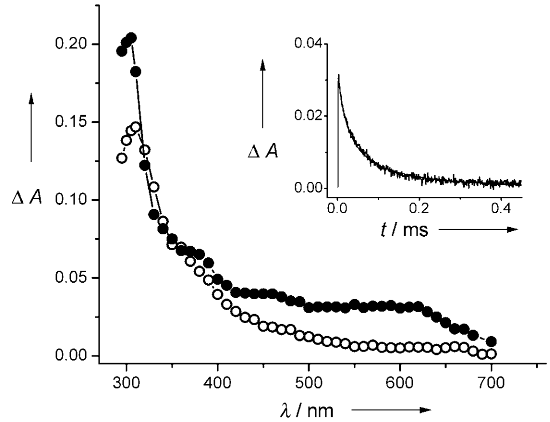


Figure 1. Absorption spectra obtained from the pulse radiolysis of N_2O-saturated aqueous solutions containing 1 mM guanosine at natural pH, recorded 1 μs (●) and 170 μs (○) after the pulse; optical path = 2.0 cm, dose per pulse = 22 Gy. Inset: Time dependence of absorption at 620 nm; the solid line represents the first-order fit to the data.

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concentration of the added nucleoside, a rate constant of $(5.7\pm0.4)\times10^{9}\,\text{M}^{-1}\,\text{s}^{-1}$ is obtained for the reaction of HO$^{\bullet}$ radical with **1**.[16] The spectrum taken 170 µs after the pulse shows the disappearance of the band around 610 nm and the decrease of the absorption around 305 nm. By monitoring the absorbance change at 620 nm, a first-order rate constant of $(2.3\pm0.1)\times10^{4}\,\text{s}^{-1}$ is obtained (inset of Figure 1).[16,17]

The absorption around 610 nm was previously assigned to the HO adduct **5**.[8–10] However, it is not clear why such a radical with limited spin delocalization should absorb in this region. We found previously that time-dependent DFT (TD-B3LYP/6-311G**//B1B95/6-31+G**) calculations provide reliable optical transition for nucleoside radicals.[6,7,18] Table 1 shows the absorption spectra computed for the HO adduct **5**. For this radical the calculations predict a main transition at 333 nm with two shoulders, the first one at lower wavelength (315 nm) and the second one at higher wavelength (353 nm). Figure 2 (right) shows the SOMO of the radical **5** in which the unpaired electron is mainly delocalized at the imidazolyl ring. Therefore, DFT-TD calculations do not support the assignment of 610 nm band to **5**.[19]

Table 1: Vertical optical transitions of **5** computed at TD-B3LYP/6-311G**//B1B95/6-31+G** level.

λ [nm]	f[a]	Transition {spin}
315	0.037	pπ(out-of-phase N2′,N1,O6′)→SOMO (pπ(C5)) {β}
333	0.096	SOMO (pπ(C5))→π*(N7-C8)−π*(C6-O6′) {α}
353	0.027	pπ(N3)→SOMO ((pπ(C5)) {β}

[a] Oscillator strengths (f) for transition ≥0.010.

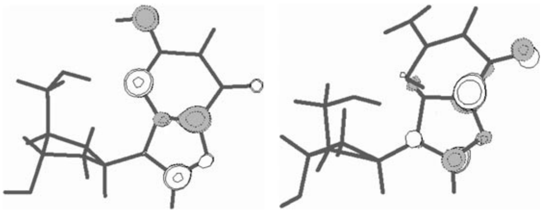
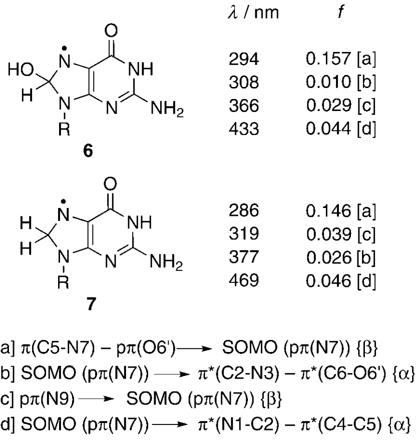


Figure 2. SOMO of the radicals **3** (left) and **5** (right) computed at the B3LYP/6-311G**//B1B95/6-31+G** level.

Since e_{aq}^{-} are scavenged by N_2O, the H$^{\bullet}$ and HO$^{\bullet}$ radicals accounted for 10 and 90 %, respectively, of the initial reactive species.[14] Hydrogen atoms add at the C8 position of guanine moiety[20] selectively affording radical **7** and, as described above, 17 % of the HO$^{\bullet}$ radicals add to C8 position to give radical **6** (Scheme 2). For both species calculations predict the main optical transition around 290 nm and these transitions should contribute substantially to the sharp band around 305 nm of the spectrum taken 1 µs after the pulse (Figure 1). Furthermore, three much weaker transitions in the range 300–500 nm are computed for both conjugated aminyl radical **6** and **7**. By replacing HO with H in these radicals the order of transitions (308 and 366 nm for **6**) is exchanged (377 and 319 nm for **7**) owing to the expected decrease of SOMO energy. The OH adduct **6** is computed to be more stable than the OH adduct **5** by 47.7 kJ mol^{-1} at the B1B95/6-31+G** level, further supporting that the absorption around 610 nm could not be assigned to the HO adduct **5**.



	λ / nm	f
6	294	0.157 [a]
	308	0.010 [b]
	366	0.029 [c]
	433	0.044 [d]
7	286	0.146 [a]
	319	0.039 [c]
	377	0.026 [b]
	469	0.046 [d]

[a] π(C5-N7) − pπ(O6′) ⟶ SOMO (pπ(N7)) {β}
[b] SOMO (pπ(N7)) ⟶ π*(C2-N3) − π*(C6-O6′) {α}
[c] pπ(N9) ⟶ SOMO (pπ(N7)) {β}
[d] SOMO (pπ(N7)) ⟶ π*(N1-C2) − π*(C4-C5) {α}

Scheme 2. Vertical optical transitions of radicals **6** and **7** computed at TD-B3LYP/6-311G**//B1B95/6-31+G** level. Oscillator strengths (f) for transition ≥0.010.

The absorption around 610 nm of spectrum taken 1 µs after the pulse is very similar to the reported spectrum of tautomer **3** obtained by one-electron reduction of 8-bromoguanosine **4** (cf. Scheme 1). Figure 3 shows for comparison the two spectra, that is, the reactions HO$^{\bullet}$+**1** and e_{aq}^{-}+**4**. The ε values of the spectrum arising from the reaction HO$^{\bullet}$+**1** were calculated using $G=0.61\,\mu\text{mol}\,\text{J}^{-1}$ since both HO$^{\bullet}$ and H$^{\bullet}$ species are scavenged by guanosine.[14] The spectrum of e_{aq}^{-}+**4** is the 65 % of the ε value calculated using $G=0.27\,\mu\text{mol}\,\text{J}^{-1}$ of hydrated electrons. The overlap in the range 500–700 nm is excellent. This broad band is predicted very well by the DFT-TD calculations for radical **3**, that is, λ = 616 nm, f = 0.045 and transition {spin}: pπ(out-of-phase N2′, N1, O6′)→SOMO (out-of-phase N2′, N3, C5, C8) {β}.[6,7] Figure 2 (left) shows the SOMO of **3** in which the unpaired

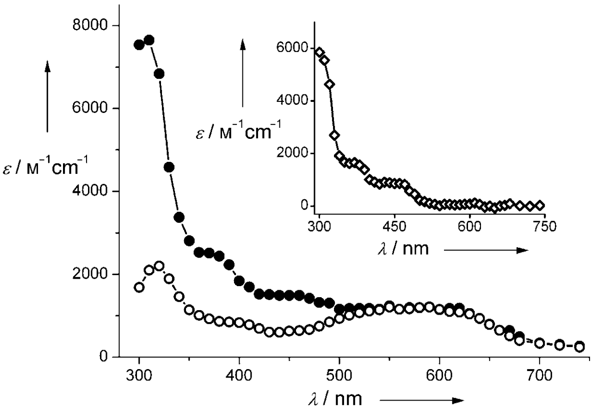


Figure 3. Absorption spectra obtained from the reaction of HO$^{\bullet}$ with guanosine (**1**) recorded 1 µs (●) after the pulse (cf. Figure 1) and 65 % of the intensity of the absorption spectra obtained from the reaction of e_{aq}^{-} with 8-bromoguanosine (**4**) recorded 1 µs (○) after the pulse.[6,7] Inset: The spectrum resulting from the subtraction of (○) from (●).

electron is fully delocalized on the base. On the other hand, the inset in Figure 3 shows the difference of the two spectra which gives a strong band around 300 nm and various smaller broad bands up to 500 nm. This computed spectrum is in good agreement with the calculated transitions of radicals **6** and **7** (Scheme 2), although a small contribution from the sugar-derived radicals is also expected.

The high reactivity of HO$^\bullet$ radicals towards the NH_2 moiety of aromatic amines is well documented. With aniline, for example, it was established that 36 % of the HO$^\bullet$ radicals abstract hydrogen from the NH_2 moiety, whereas the remaining 64 % affords HO$^\bullet$ adducts.[21] We have shown that for X–H + Y$^\bullet$ to give X$^\bullet$ + H–Y, a major factor affecting the energy of activation is X–Y antibonding at the transition state.[22] It was suggested that abstraction from NH would have a low energy of activation because N–O antibonding would be low as a result of the weak N–O bond. Moreover, a Car–Parrinello molecular dynamics study on the reaction of HO$^\bullet$ radical with guanine in the gas-phase indicated that hydrogen abstraction from the NH_2 moiety is more favored than the addition at the C4 and C8 positions, the C4-hydroxylated radical being the less stable.[23]

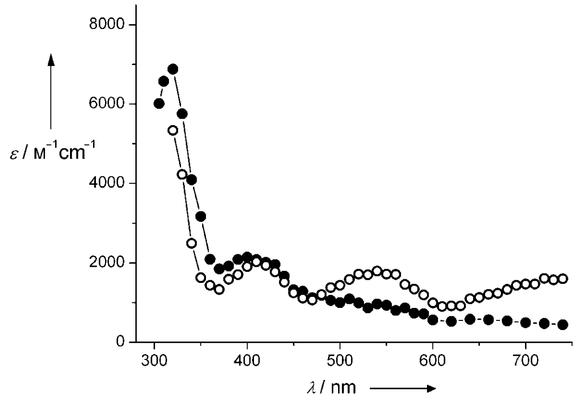
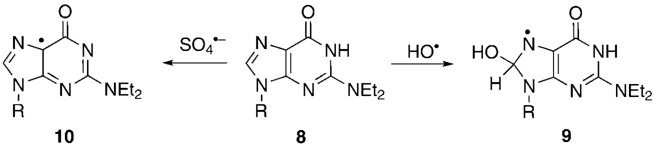


Figure 4. Absorption spectrum (●) obtained from the pulse radiolysis of N_2O-saturated solutions containing 1 mM **8** at natural pH value, recorded 1 μs after the pulse. Absorption spectrum (○) obtained from the pulse radiolysis of argon-purged solutions containing 0.1 mM **8** and 10 mM $K_2S_2O_8$ at natural pH value with 0.1 M *t*BuOH, recorded 6 μs after the pulse.



Scheme 3. Reactions of HO$^\bullet$ and SO$_4^{\bullet -}$ with N^2,N^2-diethylguanosine **8**.

To gain further confirmation of our proposal that HO$^\bullet$ radical abstracts hydrogen from the NH_2 moiety, it was necessary to determine whether dialkylation of the NH_2 group could produce another effect. Figure 4 shows the optical absorption spectra obtained from the reaction of HO$^\bullet$ with N^2,N^2-diethylguanosine (**8**).[16] Some features associated with these spectra are of great importance: 1) the rate constant for the formation of the transient species is found to be identical to that in guanosine case, that is $(5.7 \pm 0.2) \times 10^9\,\mathrm{M^{-1}s^{-1}}$, 2) the band at 610 nm is absent, 3) the transient species decays by second-order kinetics without evidence of unimolecular transformation, and 4) the transient species does not correspond to one-electron oxidized radical **10** (open circles in Figure 4), obtained by the reaction of SO$_4^{\bullet -}$ with **8** (Scheme 3).[6] We suggest that the resulting spectra from the reaction HO$^\bullet$ + **8** are a sum of reactions involving the addition of HO$^\bullet$ to the C8 position to give **9** (main contribution) and the hydrogen abstraction from the sugar and NEt$_2$ moieties.

In conclusion, our work herein demonstrates that the ambident reactivity of the guanine moiety (in guanosine or 2′-deoxyguanosine) towards HO$^\bullet$ radicals is represented by hydrogen abstraction from the NH_2 moiety (main path) and addition at C8 position (minor path). The initially formed guanyl radical **3**, characterized by a broad band around 610 nm, undergoes a water-assisted tautomerization to give the most stable tautomer **2**, which is also formed directly by one-electron oxidation of guanosine or 2′-deoxyguanosine.

Experimental Section

Pulse radiolysis with optical absorption detection was performed by using a 12 MeV linear accelerator, which delivered 20–200 ns electron pulses with doses between 5 and 50 Gy, by which HO$^\bullet$, H$^\bullet$, and e_{aq}^- were generated with concentrations of 1–20 μM. For computational details see the Supporting Information.

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[16] Similar results are obtained by replacing guanosine with 2′-deoxyguanosine (see Supporting Information).

[17] This value is four-times higher than the one reported before.[10] It is worth noticing that in the Figure 1 of Ref. [10], the decay traces at 620 nm does not correspond to the reported spectra because the decay does not reach the zero absorbance as evidenced from the spectra.

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