Guanosine Tautomers

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Tautomers of One-Electron-Oxidized Guanosine**

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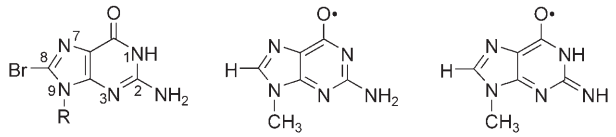
8-Bromoguanine derivatives, such as **1a** and **1b**, capture electrons (e_{aq}^-) with quantitative formation of the corresponding debrominated nucleosides[1,2] and, therefore, they are efficient detectors of excess electron-transfer processes.[3] Indeed, in two recent papers 8-bromo-2′-deoxyguanosine (**1b**) was incorporated in a variety of single- and double-



1a: R = ribose
1b: R = 2′-deoxyribose
1c: R = methyl

2 **3**

stranded oligonucleotides and G-quadruplexes, and the reaction with e_{aq}^- indicated that excess electron transfer is effective.[2,4]

The reaction of e_{aq}^- with **1a** was previously studied by pulse-radiolysis techniques in some detail.[1] These experiments revealed the formation of two short-lived intermediates at pH ≈ 7. Figure 1 shows the absorption spectrum of the first observable species (solid line) obtained 2 μs after the pulse. This species of uncertain structure decays by first-order kinetics ($k = 5.0 \times 10^4\ s^{-1}$) to produce the one-electron-oxidized guanosine (dashed line).[1] In the present work we studied the kinetics in the temperature range of 5.8–50.3 °C and obtained the following Arrhenius parameters: $\log(A/s^{-1}) = 8.7 \pm 0.4$ and $E_a = 23.0 \pm 2.5\ kJ\,mol^{-1}$ (errors correspond to one standard deviation).

What is the structure of the first observable species, which has a characteristic absorbance around 600 nm? We found previously that time-dependent (TD) DFT calculations at the B3LYP/6-31G* level[5,6] provide reliable optical transitions for nucleoside radicals.[7,8] In neutral solution, the initial electron adduct of **1** should be rapidly protonated.[9] Therefore, TD-B3LYP/6-31G* calculations were carried out on 8-

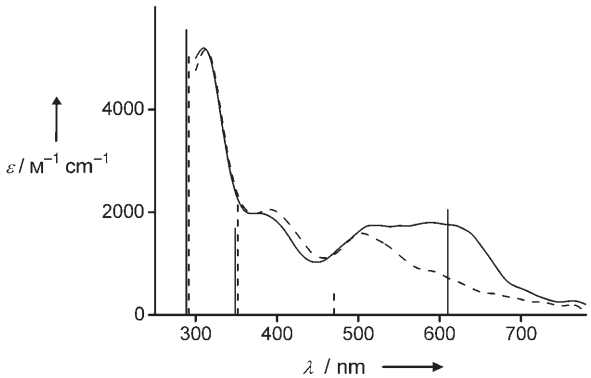
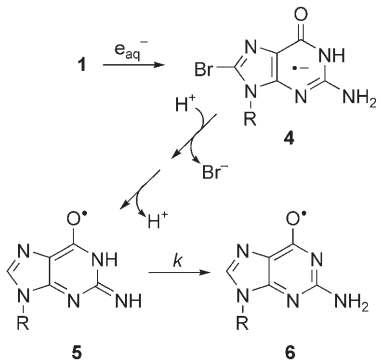


Figure 1. Absorption spectra obtained from the pulse radiolysis of Ar-purged solutions containing 1 mM **1a** at pH ≈ 7 with 0.25 M *t*BuOH, recorded 2 μs (solid line) and 45 μs (dashed line) after the pulse (adapted from ref. [1]). The lines show the calculated vertical optical transitions for radical **2** (dashed lines, $\lambda(f)$: 290 (0.128), 350 (0.054), 470 nm (0.014)) and its tautomer **3** (solid lines, $\lambda(f)$: 290 (0.138), 350 (0.042), 610 nm (0.051)).

bromo-9-methylguanine (**1c**) radical anion, protonated at N7 (as suggested previously[1]) and at any other possible sites; no optical transition was predicted to exist above 500 nm. Interestingly, protonation at C8 produces loss of Br^-, and it could be hypothesized that the first observable species in pulse radiolysis had already lost Br^-. Consequently, we computed the optical spectra (transition wavelengths λ and oscillator strengths f) of the deprotonated 9-methylguanine radical cation (i.e., oxidized 9-methylguanine **2**) and its tautomers bearing an iminic substituent at C2. The computed data for the radical **2** are reported in Figure 1 and are in good accord with values assigned with certainty to the oxidized guanosine.[10] More interestingly, calculations showed that a band is computed at wavelengths longer than 500 nm only for the tautomer **3**. The computed optical transitions are also reported in Figure 1 and are in good agreement with the experimental spectrum recorded 2 μs after the pulse; these data correspond to the transient of uncertain structure.

On the basis of these findings, we propose a revised mechanism for the reaction of e_{aq}^- with **1** (Scheme 1). The



Scheme 1. Proposed mechanism for the reaction of e_{aq}^- with 8-bromoguanosine (**1a**). The initial adduct **4** is rapidly protonated to give the first observable species **5**. The tautomer **6** is assigned to the second transient species observed in the pulse-radiolysis studies (see Figure 1).

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initial electron adduct **4** is rapidly protonated at the C8 position to release Br^- and the first observable transient species **5**,[11] which should have a pK_a value similar to that of oxidized guanosine ($pK_a = 3.9$).[10] The subsequent tautomerization **5**→**6** occurs with a $\log(A/s^{-1}) = 8.7$, which suggests a complex transition state, and an activation energy of 23.0 kJ mol^{-1}. Another point supporting a complex transition state is also the previously reported kinetic isotope effect $k(H_2O)/k(D_2O) = 8.0$.[1]

The reaction barrier (E_a) for the direct tautomerization **3**→**2** is computed to be 183.7 kJ mol^{-1} at the B3LYP/6-31G* level. Such a large value is in evident contrast with the activation energy measured for the tautomerization **5**→**6**. In analogy with the keto–enol tautomerization in guanine[12] and 8-oxo-7,8-dihydroguanine,[13] the water-assisted proton transfer is computed to occur with a much lower reaction barrier (the calculated transition-state structure is given in Figure 2). Indeed, the reaction barrier is computed to be as small as 18.8 kJ mol^{-1}, in good accord with experiment.[14]

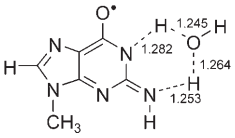
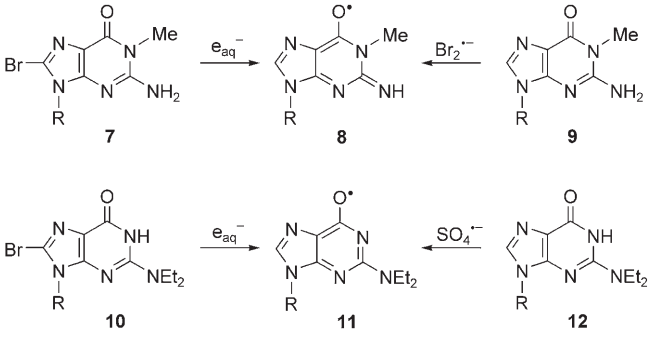


Figure 2. Structure of the transition state for the water-assisted tautomerization **3**→**2** computed at the B3LYP/6-31G* level. Distances are in Å.

To gain insight into the different tautomeric forms, the 8-bromo derivatives **7** and **10** (Scheme 2) were prepared, since the analogous tautomerization **5**→**6** could not occur as a result of alkylation at NH and NH_2 moieties. γ-Radiolysis of **7** and **10** in aqueous solutions at pH ≈ 7 was followed by product studies, which showed the quantitative formation of debrominated derivatives **9** and **12**, respectively.[15] Rate constants of 1.1×10^{10} and 8.0×10^9 M^{-1} s^{-1} were determined at pH ≈ 7 for the reactions of e_{aq}^- with **7** and **10**, respectively, by measuring the rate of the optical density decrease of e_{aq}^- as a function of the concentration of the added nucleoside.[18] Figures 3 and 4 show the optical absorption spectra obtained from these reactions. The ε values were calculated using $G = 0.27$ μmol J^{-1}, since HO• species are scavenged by the added *t*BuOH, and the H• atoms follow another path and therefore do not contribute to the reaction.[16] The decay of these transients follows second-order kinetics and their disappearance does not lead to other transients. Furthermore, these spectra are identical to those obtained after the oxidation of **9** by $Br_2^{\bullet-}$ [10] and of **12** by $SO_4^{\bullet-}$ at pH ≈ 7 (Figures 3 and 4).



Scheme 2. Reactions of e_{aq}^- with 8-bromoguanosine derivatives **7** and **10,** of $Br_2^{\bullet-}$ with **9**, and of $SO_4^{\bullet-}$ with **12**. The radicals **8** and **11** are assigned to the transient species observed in the pulse-radiolysis studies (see Figures 3 and 4, respectively).

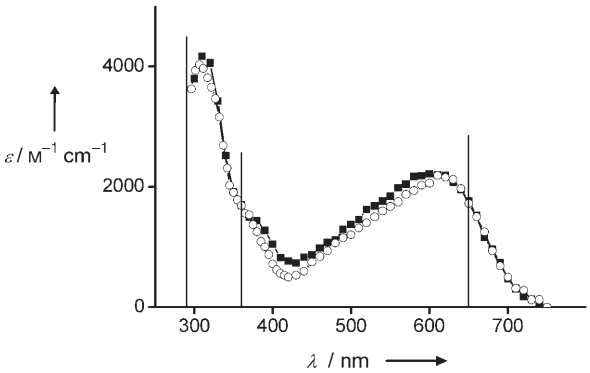


Figure 3. Absorption spectrum (■) obtained from the pulse radiolysis of Ar-purged solutions containing 0.5 mM **7** at pH ≈ 7 and 0.25 M *t*BuOH, recorded 10 μs after the pulse. Absorption spectrum (○) is taken from reference [10] and refers to the reaction of $Br_2^{\bullet-}$ with **9**. The lines show the calculated vertical optical transitions for radical **8** (R = Me) ($\lambda(f)$: 290 (0.063), 360 (0.036), 650 nm (0.040)).

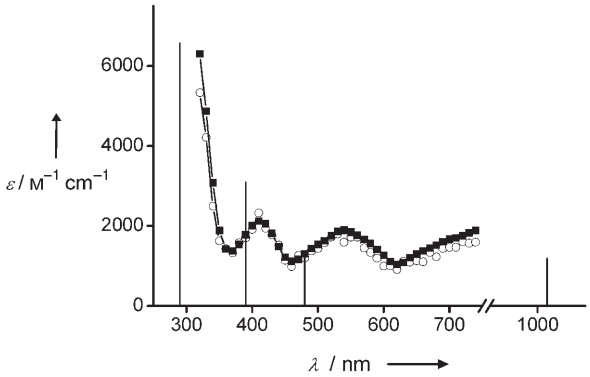


Figure 4. Absorption spectrum (■) obtained from the pulse radiolysis of Ar-purged solutions containing 0.5 mM **10** at pH ≈ 7 and 0.25 M *t*BuOH, recorded 2 μs after the pulse. Absorption spectrum (○) obtained from the pulse radiolysis of Ar-purged solutions containing 0.1 mM **12** and 10 mM $K_2S_2O_8$ at pH ≈ 7 with 0.1 M *t*BuOH, recorded 6 μs after the pulse. The lines show the calculated vertical optical transitions for radical **11** (R = Me) ($\lambda(f)$: 290 (0.149), 390 (0.070), 480 (0.029), 1030 nm (0.027)).

Therefore, the spectra in Figures 3 and 4 are assigned to radicals **8** and **11**, respectively, which can be obtained either from the reduction of 8-bromoguanine derivatives or from the oxidation of the debrominated compounds as illustrated in Scheme 2. TD-B3LYP/6-31G* calculations carried out on the corresponding 9-methyl derivatives of radicals **8** and **11** fit very well with our assignment. Indeed, the computed optical spectra for the radical **8** and for the radical **11**[19]] are in good agreement with the spectra shown in Figures 3 and 4, respectively. Interestingly, both the spectrum of radical **8** and that of radical **5** have a band at 610–620 nm, which is consistent with an iminic substitution at the C2 position. Obviously, radical **5** can tautomerize to **6**, whereas radical **8** is blocked in one form.

In conclusion, our work here demonstrates the first directly observed differences of the two tautomeric forms of oxidized guanosine. The energetically most stable form **6** is the one that is obtained directly by oxidation of guanosine. The less stable form **5** is obtained from the protonation of the 8-bromoguanosine electron adduct, and its tautomerization to **6** has an activation energy of 23.0 kJ mol^{-1}.

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. Continuous radiolyses were performed at room temperature using a ^{60}Co-Gammacell, with a dose rate of ca. 15 Gy min^{-1}. Compounds **7**, **10**, and **12** were prepared following known procedures.[20]

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[14] A detailed computational investigation of the water-assisted tautomerization including the effect of the basis set and of a polarizable continuum model for the aqueous solution will be presented in a full account; preliminary calculations indicate that E_a should increase by only 3–4 kJ mol^{-1} at the higher level of theory.
[15] Deaereated aqueous solutions containing ca. 1.5 mM **7** (or **10**) and 0.25 M *t*BuOH at pH ≈ 7 were irradiated under stationary-state conditions with a total dose of 2 kGy at a dose rate of ca. 15 Gy min^{-1} followed by HPLC analysis.[16] Compound **9** (or **12**) was the only detectable product, and the mass balances were close to 100%; analysis of the data, in terms of radiation chemical yield, gives $G(-\mathbf{7}) = 0.36$, $G(\mathbf{9}) = 0.33$, $G(-\mathbf{10}) = 0.33$ and $G(\mathbf{12}) = 0.30$ μmol J^{-1}. Taking into account that $G(e_{aq}^-) + G(H^{\bullet}) = 0.33$ μmol J^{-1}, our results lead to the conclusion that solvated electrons and hydrogen atoms react with **7** and **10** to yield the observed product.
[16] Radiolysis of neutral water leads to e_{aq}^- (0.27), HO$^{\bullet}$ (0.28), and H$^{\bullet}$ (0.062), the values in parentheses represent the radiation chemical yields in units of μmol J^{-1}; in the presence of 0.25 M *t*BuOH, HO$^{\bullet}$ is scavenged efficiently ($k = 6.0 \times 10^8$ M^{-1} s^{-1}), whereas H$^{\bullet}$ reacts only slowly ($k = 1.7 \times 10^5$ M^{-1} s^{-1}).[17]
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