Communications

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-



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 \approx 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 ($k = 5.0 \times 10^4 \text{ s}^{-1}$) to produce the one-electron-oxidized guanosine (dashed line).^[11] In the present work we studied the kinetics in the temperature range of 5.8–50.3 °C and obtained the following Arrhenius parameters: $\log(A/\text{ s}^{-1}) = 8.7 \pm 0.4$ and $E_a = 23.0 \pm 2.5 \text{ kJ mol}^{-1}$ (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-

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Figure 1. Absorption spectra obtained from the pulse radiolysis of Arpurged solutions containing 1 mm 1a at $pH \approx 7$ with 0.25 m tBuOH, recorded 2 μ s (solid line) and 45 μ s (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_{ac}^{-} 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 $\mathbf{3} \rightarrow \mathbf{2}$ 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

$$H \xrightarrow{V}_{i_{1263}} (H_{i_{1263}} (H_{i_{126$$

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

activation energy measured for the tautomerization $5\rightarrow 6$. In analogy with the keto–enol tautomerization in guanine^[12] and 8-oxo-7,8-dihydro-guanine,^[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]

To gain insight into the different tautomeric forms, the 8-bromo derivatives **7** and **10** (Scheme 2) were prepared, since the analogous tautomeriza-



Scheme 2. Reactions of e_{aq}^{-} with 8-bromoguanosine derivatives 7 and 10, of Br_2^{-} with 9, and of SO_4^{-} 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).

tion $5 \rightarrow 6$ could not occur as a result of alkylation at NH and NH₂ moieties. γ -Radiolysis of **7** and **10** in aqueous solutions at pH \approx 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 $8.0 \times 10^9 \text{ m}^{-1} \text{ s}^{-1}$ were determined at pH \approx 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 $G = 0.27 \,\mu\text{mol J}^{-1}$, 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-



Figure 3. Absorption spectrum (**■**) obtained from the pulse radiolysis of Ar-purged solutions containing 0.5 mm **7** at pH \approx 7 and 0.25 m tBuOH, recorded 10 µs after the pulse. Absorption spectrum (\odot) is taken from reference [10] and refers to the reaction of Br₂⁻⁻ with **9**. The lines show the calculated vertical optical transitions for radical **8** (R = Me) (λ (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 \approx 7 and 0.25 M tBuOH, recorded 2 µs after the pulse. Absorption spectrum ($_{\bigcirc}$) obtained from the pulse radiolysis of Ar-purged solutions containing 0.1 mm **12** and 10 mm K₂S₂O₈ at pH \approx 7 with 0.1 m tBuOH, recorded 6 µs after the pulse. The lines show the calculated vertical optical transitions for radical **11** (R = Me) (λ (f): 290 (0.149), 390 (0.070), 480 (0.029), 1030 nm (0.027)).

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^{-10} and of **12** by SO_4^{-1} at pH \approx 7 (Figures 3 and 4).

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.

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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', H', and e_{aq}^{-} were generated with concentrations of 1–20 μ M. Continuous radiolyses were performed at room temperature using a ⁶⁰Co-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 $E_{\rm a}$ 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 \approx 7 were irradiated under stationarystate conditions with a total dose of 2 kGy at a dose rate of ca. 15 Gymin⁻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(-7) = 0.36, G(9) = 0.33, G(-10) = 0.33and $G(12) = 0.30 \,\mu\text{mol J}^{-1}$. Taking into account that $G(e_{aq}^{-}) + G(H^{-}) = 0.33 \,\mu\text{mol J}^{-1}$, 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[•] (0.28), and H[•] (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[•] is scavenged efficiently ($k = 6.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$), whereas H[•] reacts only slowly ($k = 1.7 \times 10^5 \text{ m}^{-1} \text{ s}^{-1}$).^[17]
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