Steric Effects on Water Accessibility Control Sequence-Selectivity of Radical Cation Reactions in DNA

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The discovery that electron holes (radical cations) migrate long distances in DNA has fostered enormous interest in the mechanism of this processes, due in large part to its apparent similarity with oxidative damage in cells.1,2 Several models have been proposed to account for the extensive experimental observations in this area. Currently, it is generally accepted that holes migrate long distances through duplex DNA by a series of short hops pausing briefly at guanine-containing sites of relatively low oxidation potential (often GG steps) where they may react irreversibly with $H_2O$ or $O_2$.3,4

The two guanines of the GG steps are not equally reactive. A hallmark of the one-electron oxidation of duplex DNA is that the 5′-guanine of GG steps is typically more reactive than the 3′-G.5–7 This finding has been analyzed computationally. Both ab initio and NDDO-G gas-phase model calculations have been interpreted to indicate that electronic effects make the 5′-G of GG steps more easily oxidizable than the 3′-guanine.8,9 It was also suggested that the 5′-G radical cation is stabilized by electronic overlap with the N7 nitrogen atom and O6 oxygen atom of the 3′-G10 and that the reactivity pattern seen in GG steps is controlled by the identity of adjacent nucleobases.11

To assess the influence of electronic structure on the sequence selectivity of guanine oxidation in DNA, we calculated the ionization potentials (IP, evaluated as the difference between the total energies of the neutral and ionized sequences) and the hole spatial distributions of sequences (d(5′-XGGX-3′)/d(3′-YCCY-5′)), where X = A,T,U, and Y is the complementary base. These calculations are quantitatively reliable,12 and the calculated and experimentally measured IPs of the individual nucleobases agree.13 The calculated IPs for these gas-phase, base-paired quartets 14 are 4.02, 4.04, and 4.08 eV for X = A, T, and U, respectively, which is in the expected order. Most significantly, the hole distribution (Table 1) is remarkably insensitive to X. The hole is similarly delocalized over the XGGX sequences with only a modest preference for the 5′-G. This finding suggests that electronic factors may not be the primary determinant of the reaction selectivity for GG steps in these cases.

We also carried out detailed classical molecular dynamics (MD) simulations on the B-DNA oligomers d(5′-GXGXGGXG-3′)/d(3′-GYGGYYGG-5′) that suggest there is an important steric contribution to the preference for reaction at the 5′-G in the GG doublets. These simulations reveal that stacking in these instances is essentially independent of sequence (consistent with X-ray data for DNA and RNA15), and yield pair-distribution functions, g(r; C8), between the reactive sites - C8 of the 5′- and 3′-guanines and the oxygen atoms of neighboring water molecules. These water molecules lie in a cone-shaped region (half opening angle 30°) with the apex at the guanine C8 atom and extending to the 3′-side (reaction at the 5′-side is blocked sterically by the adjacent base13).

Table 1. Hole Occupation Fractions and the Ratio of the Hole Density on 5′ and 3′ Guanines in GG Steps from Gas-Phase Calculations on Duplex B-DNA Quartets: 5′-XGGX-3′/3′-YCCY-5′

<table>
<thead>
<tr>
<th></th>
<th>X = A</th>
<th>X = T</th>
<th>X = U</th>
</tr>
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<tbody>
<tr>
<td>5′-X</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>G</td>
<td>0.50</td>
<td>0.53</td>
<td>0.51</td>
</tr>
<tr>
<td>3′-X</td>
<td>0.43</td>
<td>0.45</td>
<td>0.44</td>
</tr>
<tr>
<td>5′-G/3′-G</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
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</tbody>
</table>

*a* The hole occupations on the complementary strand YCCY and on the sugar−phosphate backbone are negligible.

This cone is oriented in the “tetrahedral bond direction”, that is, that defined by the developing sp3 carbon−oxygen bonding orbital.12 Time-averaged calculated g(r; C8) show (Figure 1) that the probability of finding water molecules in the reactive conical sector of the 5′-G is essentially sequence invariant. In contrast, the g(r; C8) calculated for the 3′-G exhibits a strong sequence dependence with the peak of the distribution highest for 5′-AGGA-3′ sequence and lowest for 5′-TGTT-3′, with the 5′-UGGU-3′ intermediate between the two. The sequence dependence of the water access probability to the C8 site is due to steric hindrance by the thymine methyl group, which, of course, is absent in uracil.13

To assess the validity of this prediction, we undertook a systematic experimental investigation of the one-electron oxidation of DNA oligomers designed specifically to separate steric from electronic effects in the control of reaction selectivity at GG steps. The series of DNA oligomers (Figure 2) was prepared to resolve the affect of neighboring bases on the relative reactivity of the two guanines in GG steps into electronic and steric components. Each duplex contains a covalently linked anthraquinone photosensitizer (AQ) at a 5′-end and is radiolabeled with $^{32}P$ for PAGE analysis and quantitative phosphorimagery. Each duplex also contains a region of regularly repeating nucleobases. For example, in DNA-(1) this region contains the series (5′-AGG-3′)$_n$, which generates a string of six AGGA sequences. Similarly, DNA(2) contains six consecutive TGTT sequences and DNA(3) has successive UGGU sequences. These three DNA duplex oligomers have the expected melting behavior and show circular dichroism spectra characteristic of B-form DNA.

The DNA duplex oligomers were irradiated16 and then treated with piperidine, which causes strand cleavage at the site of an oxidized guanine.1 The samples were analyzed by gel electrophoresis, and the amount of strand cleavage was quantified by phosphorimagery. A typical gel and the phosphorimagery data are shown in Figure 3. As expected for these three DNA duplexes, the amount of strand cleavage at each of the GG steps is the same within experimental error because the rate of hole hopping ($k_{hole}$) is significantly greater than the rate of the irreversible trapping reaction ($k_{trap}$) and thus the distribution of holes among the reaction sites is controlled thermodynamically.
activation by association with a Na⁺ ion and product stabilization by a nearby phosphate group. The results reported here reveal a third important feature—accessibility of reactants (H₂O) to the reaction site, which is sequence-dependent and governed by steric effects. These finding may have implications to oxidative damage in cells where DNA is in a complex molecular environment.

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Supporting Information Available: General experimental methods, calculated ionization potentials, phosphorimetric data, X-ray structural data showing the stacking in TGGT and UGGU sequences, and structural characterization data for the oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(13) See Supporting Information for details.
(15) The MD simulations were carried out at T = 300 K, for 10 ns each with a time step of 1 fs, using the Amber 9b potentials: Cornell, W. D.; Cieplak, P.; Bayly, I. C.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. J. Am. Chem. Soc. 1995, 117, 5179–5197. The partial charges of uracil taken as those fitted for simulations of RNA, TP3P potentials [Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. J. Chem. Phys. 1983, 79, 926.] were used for water. No cutoffs were used for either the electrostatic or Lennard-Jones interactions.
(16) The samples were irradiated for 10 min (single hit conditions) at 350 nm, where only AQ absorbs, in air-saturated sodium phosphate buffer solution (pH 7) at ambient temperature. In the quantitative analyses of relative reactivity, interference caused by end effects was eliminated by considering only the four central “identical” GG steps, but including the others does not change the conclusion.
(18) While the dynamically fluctuating hydrating environment and counterion distribution may influence the IP values and hole distributions (e.g., hole localization) [Barnett, R. N.; Cleveland, C. L.; Joy, A.; Landman, U.; Schuster, G. B. J. Am. Chem. Soc. 2001, 294, 567–571.] they are likely to remain largely sequence independent, leaving our conclusions unchanged.

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Figure 1. Pair distribution functions, g(r; CS), between the reactive sites—the C8 carbon atom of the 5′- and 3′-guanines—and the oxygen atoms of neighboring water molecules. Results shown for 3 sequences.

Figure 2. Structures of DNA oligomers used in this work. The # denotes the position of 32P radioactive label.

Figure 3. Autoradiogram of PAGE gel following the irradiation of DNA-(1)−DNA(3). The graph in the right side shows the ratio of damage for 5′-G and 3′-G for the four GG steps in the middle, which directly shows the difference in reactivity at the 3′-G position for the three sequences used.