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Role of the Guanine N1 Imino Proton in the Migration and Reaction of Radical Cations in DNA Oligomers

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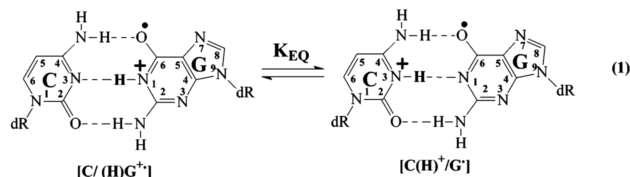
Intensive investigation of the oxidation of duplex DNA has shown that loss of an electron generates a radical cation ("hole") that migrates by a hopping mechanism until it is trapped irreversibly in a chemical reaction with H_2O or O_2, which usually occurs at a guanine or a G_n sequence.[1−6] The relevance of these processes to genetic mutation and to the potential applications of DNA in molecular electronics has fueled interest in understanding the detailed mechanisms of the hopping and trapping reactions.[7] A significant open question concerns the role played by the guanine N1 imino proton in these processes.

Conversion of 2′-deoxyguanosine to its radical cation causes an enormous increase in its acidity.[8] On the basis of solution-phase pK_a data, Steenken[9] concluded that oxidation causes the proton on N1 of guanine in a DNA G/C base pair to shift spontaneously to N3 of the cytosine ($K_{EQ} = 10^{0.4}$, $\Delta G = -1.5$ kcal/mol at room temperature), see eq 1. However, DFT calculations in the gas phase,



which is suggested to be a realistic model of stacked base pairs in DNA (where solvent is excluded), indicate that the structure with a proton on guanine N1 [C/(H)$G^{\bullet+}$] is more stable than [C^+(H)/$G^\bullet$] by 1.4 kcal/mol.[10] In contrast, first-principles calculations on a partially hydrated G/C base pair in DNA indicate that the proton transferred form [C^+(H)/$G^\bullet$] has an energy that is 4.0 kcal/mol below that of [C/(H)$G^{\bullet+}$].[11] Furthermore, an extensive calculation on a related system indicates that charge transfer in oxidized duplex DNA is coupled with proton transfer from guanine to cytosine.[12] This conclusion is consistent with experiments carried out in D_2O that reveal a kinetic isotope effect for guanine oxidation[13] and for charge transfer in DNA,[14] both of which implicate a concerted proton-coupled electron transfer involving the guanine N1 proton, and with experiments that show inhibition of charge transfer when proton loss from guanine is facilitated.[15]

The N1 proton is also thought to play the deciding role in the chemical reactions of oxidized guanines.[16−19] In solution, rapid loss of this proton and subsequent reaction of the resulting guanine radical with O_2 leads eventually to an oxazolone (dZ, see Figure 1). However, proton loss from a guanine radical cation is slowed when it is part of a base pair with cytosine in DNA, and in this form it reacts with H_2O to form 8-oxo-7,8-dihydroguanine (8-oxoG).

We report here an investigation of the one-electron oxidation of a duplex DNA oligomer that contains 5-fluoro-2′-deoxycytidines (F^5dC) in selected positions complementary to GG steps. As expected, F^5dC is a weaker base than 2′-deoxycytidine, with $\Delta(pK_a) = 1.7$ determined by titration in solution (see Supporting Informa-

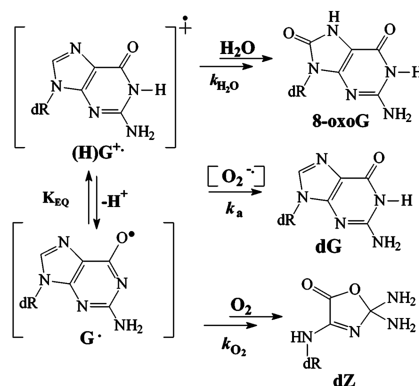


Figure 1. Reaction of oxidized guanine with H_2O, O_2, and O_2^-.

DNA(1)	5'AQ- A A T T G G₁ T T G G₂ T T G G₃ T T G G₄ T T G G₅ T T G G₆ A T A T* -3' 3'- T T A A C C A A C C A A C C A A C C A A C C A A C C T A T A -5'
DNA(2)	5'AQ- A A T T G G₁ T T G G₂ T T G G₃ T T G G₄ T T G G₅ T T G G₆ A T A T* -3' 3'- T T A A C C A A F F A A C C A A F F A A C C A A F F T A T A -5'

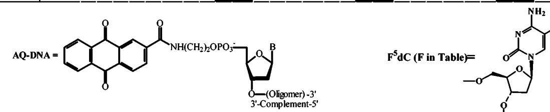


Figure 2. Schematic representation of the DNA oligomers.

tion). Consequently, proton transfer from a guanine radical cation to F^5dC is thermodynamically unfavorable. We find that replacement of dC by F^5dC has no measurable effect on long-distance radical cation transport, but F^5dC partially inhibits the reaction, leading to strand cleavage at the complementary guanine.

The DNA oligomers examined in this work are shown in Figure 2. DNA(1) contains a series of six GG steps separated by TT sequences, an anthraquinone group (AQ) linked covalently to a 5′-terminus, and a ^{32}P radiolabel (* in Figure 2) on the 3′-terminus of the GG-containing strand. As expected, irradiation of DNA(1) (350 nm, only the AQ group absorbs) and its subsequent treatment with formamidopyrimidine glycosylase (Fpg enzyme) results in strand cleavage at each GG step, which is detected by PAGE and autoradiography (see Figure 3A). Importantly, Fpg cleaves DNA at sites that contain either a dZ or an 8-oxoG lesion,[20] and F^5dC does not affect the ability of Fpg to induce strand cleavage (see Supporting Information). The electronically excited state of AQ causes the one-electron oxidation of an adjacent nucleobase to its radical cation, which hops (k_{hop}) through the duplex until it either is trapped (k_{trap}) by reaction with H_2O or O_2 or is consumed (k_a) in an annihilation reaction[21] that leads to regeneration of the guanine.[19] This experiment was carried out under single-hit conditions (low conversion, see Supporting Information), where each DNA molecule reacts once or not at all. In this circumstance, analysis of the linear semilog plot of the distance-dependent distribution of strand cleavage (see Figure 3B) gives the ratio of the rate of radical cation hopping to its consumption by trapping and annihilation $k_{ratio} =$

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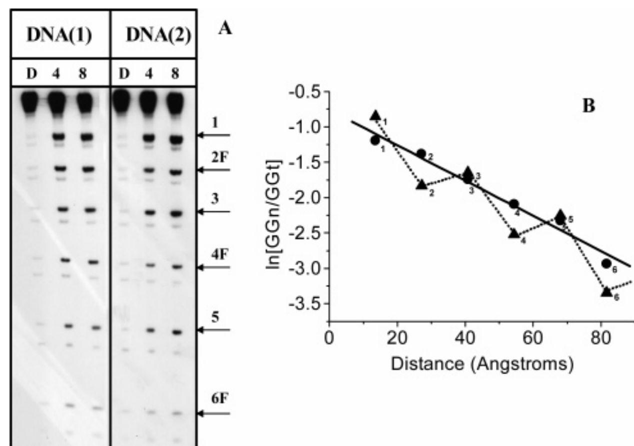

Figure 3. (A) Autoradiogram showing the results of irradiation of DNA-(1) and DNA(2). The six GG steps of these oligomers are indicated by the numbered arrows at the right, and F indicates the presence of F[5]dC at the complementary strand of DNA(2). The three lanes correspond to D (dark control) and 4 and 8 min of irradiation, respectively. (B) Semilog plots of the amount of strand cleavage at the GG steps of DNA(1) determined by phosphorimagery (circles, the solid line is the least-squares fit) and DNA-(2) (triangles, the dotted line connects the data points), as a function of distance from the AQ. Strand cleavage at each GG step (n) is normalized to the total (t) amount of reaction at all GG steps.

10. This indicates that hopping from GG step to GG step in DNA-(1) is approximately 10 times faster than the irreversible reactions that consume the guanine radical cation.[22]

DNA(2) contains F[5]dC bases in place of dC at positions complementary to three of the six GG steps ($GG_{2,4,6}$). Its irradiation and subsequent treatment with Fpg also results in strand cleavage at each of the GG steps (see Figure 3A). Significantly, the relative amount of cleavage observed at the GG steps opposite unmodified cytosines ($GG_{1,3,5}$) in DNA(2) is the same within experimental error as for DNA(1), but strand cleavage is inhibited at the positions that are complementary to F[5]dC (see Figure 3B). These findings show that the efficiency of charge hopping is unaffected by the 50-fold decrease in basicity of cytosine that accompanies its fluorine substitution, but this modification does influence the reactions of the complementary guanine radical cation with H_2O or O_2.

The role played by the imino N1 proton in charge migration in DNA and in reaction of the guanine radical cation is illuminated by these experiments. The reduced basicity of F[5]dC would inhibit hopping of the radical cation from one GG step to the next if this process is strongly coupled to proton transfer from guanine to cytosine. The experiments reported here show that substitution with F[5]dC does not measurably affect the efficiency of hopping. This suggests that the N1 proton remains primarily on the guanine radical cation even in a normal G/C base pair, as was suggested by calculation.[10] In this case, the kinetic isotope effect observed in charge-transfer experiments carried out in D_2O solution must involve other protons of the DNA or may be attributed to participation of water molecules tightly bound to the DNA. In contrast, the irreversible chemical trapping of the radical cation that is revealed by Fpg-induced strand cleavage at damaged guanines is partially inhibited by substitution with F[5]dC. Apparently, proton transfer from the guanine radical cation to its cytosine partner plays an important role when the DNA duplex and its immediate solvent environment are in a conformation that enables reaction to occur. Clearly, the decreased basicity of F[5]dC shifts K_{EQ} and reduces the amount of guanine radical available for reaction with O_2, which may cause a concomitant reduction in the magnitude of k_{trap}, and thus a greater fraction of the radical cation will be consumed by the annihilation reaction (k_a, see Figure 1) that simply regenerates dG and reduces the amount of strand cleavage.

The complexity of DNA is reflected in the analyses of the hopping and trapping reactions. For example, the replacement of dC by F[5]dC may modify the base pair hydration environment, or, operating through the hydrogen bonds, it may affect the electronic structure of the guanine.[23] However, neither of these effects is observed when 5-methyl-2′-deoxycytidines are paired with GG steps.[24] Clearly, the most significant consequence of replacing dC with F[5]dC is the reduction in basicity, and the findings reported here are interpreted on this basis. Additional experiments are underway to test this conclusion.

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Supporting Information Available: Preparation and characterization of DNA oligomers, synthesis of F[5]C, pH titrations of dC and F[5]C, quantitative analysis of irradiation experiments, reactivity of Fpg with DNA containing F[5]dC, melting temperature and CD data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ly, D.; Kan, Y.; Armitage, B.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 8747−8748.
(2) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260.
(3) Giese, B.; Spichty, M.; Wessely, S. *Pure Appl. Chem.* **2001**, *73*, 449−453.
(4) O'Neill, M. A.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 11471−11483.
(5) Lewis, F. D. *Photochem. Photobiol.* **2005**, *81*, 65−72.
(6) Kawai, K.; Majima, T. *Pure Appl. Chem.* **2005**, *77*, 963−975.
(7) Schuster, G. B. *Long-Range Charge Transfer in DNA I, II*; Springer-Verlag: Heidelberg, 2004; Vol. 236, p 237.
(8) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094−1099.
(9) Steenken, S. *Biol. Chem.* **1997**, *378*, 1293−1297.
(10) Li, X.; Cai, Z.; Sevilla, M. D. *J. Phys. Chem. B* **2001**, *105*, 10115−10123.
(11) Gervasio, F. G.; Laio, A.; Iannuzzi, M.; Parinello, M. *Chem. Eur. J.* **2004**, *10*, 4846−4852.
(12) Gervasio, F. G.; Laio, A.; Parrinello, M.; Boero, M. *Phys. Rev. Lett.* **2005**, *94*, 158103.
(13) Weatherly, S. C.; Yang, I. V.; Armistead, P. A.; Thorp, H. H. *J. Phys. Chem. B* **2003**, *107*, 372−378.
(14) Shafirovich, V.; Dourandin, A.; Geacintov, N. E. *J. Phys. Chem. B* **2001**, *105*, 8431−8435.
(15) Giese, B.; Wessely, S. *Chem. Commun.* **2001**, *2001*, 2108−2109.
(16) Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692−9694.
(17) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1154.
(18) Cadet, J.; Douki, T.; Gasparutto, D.; Ravanat, J.-L. *Mutat. Res.* **2003**, *531*, 5−23.
(19) Misiaszek, M.; Crean, C.; Joffe, A.; Geacintov, N. E.; Shafirovich, V. *J. Biol. Chem.* **2004**, *279*, 32106−32115.
(20) Tretyakova, N. Y.; Wishnok, J. S.; Tannenbaum, S. R. *Chem. Res. Toxicol.* **2000**, *13*, 658−664.
(21) Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8353−8358.
(22) Liu, C.-S.; Hernandez, R.; Schuster, G. B. *J. Am. Chem. Soc.* **2004**, *126*, 2877−2884.
(23) Kawai, K.; Wata, Y.; Hara, M.; Tojo, S.; Majima, T. *J. Am. Chem. Soc.* **2002**, *124*, 3586−3590. These authors report a slightly accelerated rate constant for electron-transfer quenching in CH_2Cl_2 solution by a modified Br[5]C/G base pair compared with C/G, which is attributed to a reduced E_{ox} for G in Br[5]C/G. The pK_a of C is reduced by bromine substitution.
(24) Kanvah, S.; Schuster, G. B. *J. Am. Chem. Soc.* **2004**, *126*, 7341−7344.

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