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Cytosine-Gated Hole Creation and Transfer in DNA in Aqueous Solution

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The underlying mechanism of charge generation and transfer in DNA is of fundamental importance to the understanding of free-radical-induced damage[1,2] and the development of both molecular electronic and biosensor devices.[3] Oxidatively generated damage to DNA by radical attack and absorption of ionizing radiation leads to selective strand cleavage at guanine (and multi-guanine) sites through the migration of electron-loss centers (holes) in DNA.[4] It is known from measurements of ionization potentials, IP, that the purine bases in isolation are more easily oxidized than the pyrimidine bases,[5] and theoretical gas-phase calculations give the order of ease of oxidation as guanine,G > adenine,A ≫ cytosine,C > thymine,T.[6] This has been borne out in aqueous solution where the measured value for the one-electron reduction potential of the guanyl radical (for guanosine), $E(G^{\bullet}, H^{+}/G)$, is 1.29 V compared to the radicals of adenosine (1.42 V), 2′-deoxycytidine (ca. 1.6 V), and thymidine (ca. 1.7 V).[7] The guanyl radical cation, $G^{+\bullet}$, has a pK_a of 3.9,[8] and it rapidly loses a proton from the oxidized nucleoside and single-stranded DNA to solvent. It has been postulated that, following deprotonation of $G^{+\bullet}$ in a GC base pair of double-stranded DNA, the proton is stabilized by its shift toward the cytosine.[8,9] However, it is the neutral, deprotonated guanyl radical, $G^{\bullet}$, which is detected by EPR upon oxidation of DNA in aqueous solution at room temperature.[10] Calculations on the effect of base pairing reveal that GC has a much lower IP value than AT and the individual purine bases with that of C being raised and G being lowered.[11] On this basis, it is expected that G in the GC pair will be oxidized by a one-electron oxidant in preference to C.

The sulfate radical anion, $SO_4^{\bullet-}$, is known to be a good one-electron oxidant of all 2′-deoxyribonucleosides,[12,13] due to its high redox potential of ca. 2.43 V,[14] reacting with rate constants of ca. $1-4 \times 10^9$ M^{-1} s^{-1}.[2] The $SO_4^{\bullet-}$ radical oxidizes DNA to give an absorption spectrum which has been interpreted as mainly a mixture of the purine radicals.[15] The selenite radical anion, $SeO_3^{\bullet-}$, of lower redox potential, 1.68 V,[16] has been reported to oxidize 2′-deoxyguanosine at a rate similar to that of the $SO_4^{\bullet-}$ radical.[17] We have observed only the radical−radical reaction of the $SeO_3^{\bullet-}$ radical in the presence of the pyrimidine bases, nucleosides, and 2′-deoxyadenosine (0.2−1 mM), limiting a rate constant for any reaction with these nucleosides to $< 5 \times 10^5$ M^{-1} s^{-1}. The $SO_4^{\bullet-}$ and $SeO_3^{\bullet-}$ oxidizing radicals are conveniently formed using pulse radiolysis, where a short pulse of 4 MeV electrons (in 200 ns) is employed to produce the free radical species of water, and the oxidizing $^{\bullet}OH$ radicals are scavenged by *tert*-butanol to form an inert radical, and the reducing e^-_{aq}'s are scavenged by either added peroxodisulfate (which also scavenges the H-atom) or selenate ions.[18] A typical radiation dose of 2.5 Gy produces 0.85 and 0.70 μM of the $SO_4^{\bullet-}$ and $SeO_3^{\bullet-}$ radicals, respectively, in <1 μs.

$$H_2O \wedge\wedge\wedge\rightarrow e^-_{aq} + {}^{\bullet}OH + H^{\bullet} + H_2O_2 + H_2 + H_3O^+$$

$$^{\bullet}OH + (CH_3)_3COH \rightarrow H_2O + {}^{\bullet}CH_2(CH_3)_2COH$$

$$e^-_{aq}/H^{\bullet} + S_2O_8^{2-}/SeO_4^{2-} \rightarrow SO_4^{\bullet-} + SO_4^{2-}/SeO_3^{\bullet-} + 2OH^-$$

$$SO_4^{\bullet-}/SeO_3^{\bullet-} + DNA \rightarrow DNA^{+\bullet} + SO_4^{2-}/SeO_3^{2-}$$

In Figure 1, we display the radical spectra produced upon oxidation of 2′-deoxycytidine 5′-monophosphate, dCMP, by the $SO_4^{\bullet-}$ radical and that for 2′-deoxyguanosine 5′-monophosphate, dGMP, upon oxidation by the $SeO_3^{\bullet-}$ radical. The weakly absorbing oxidizing radicals were observed to decay to new spectra with the same characteristics of the cytosyl radical and guanyl radicals, respectively, as reported previously using the $SO_4^{\bullet-}$ radical.[8,13] The $SeO_3^{\bullet-}$ radical, measured at 1 μs, oxidizes calf thymus DNA, with $k = 3.5 \times 10^7$ M^{-1} s^{-1},[17] to produce the absorption spectrum of the cytosyl radical by 20 μs (Figure 2), which in turn decayed with $k = 1.5 \times 10^4$ s^{-1} to the more strongly absorbing guanyl radical but at ca. one-half of its intensity as measured in Figure 1. This second phase in absorbance change is independent of the concentrations of DNA, selenate ions, and radiation dose and is therefore an intramolecular process (Figure 2, inset). The fact that the guanyl radical absorbs at ≥650 nm indicates that it is in its neutral form as the guanyl radical cation does not absorb in this region.[8] The carbonate radical of redox potential ca. 1.7 V[14] has been found to oxidize guanine bases in an oligonucleotide on a millisecond time scale,[19] and we have measured its rate constant with DNA to be 9×10^6 M^{-1} s^{-1}, which precludes the faster observations made in the present study. The formation of the cytosyl radical on a shorter time scale is a surprising result as the $SeO_3^{\bullet-}$ radicals are incapable of oxidizing dCMP on the same time scale, if at all. Hole generation by photooxidants, with trapping on distant N_4-cyclopropylcytosine residues in duplex oligonucleotides, has also been observed,[20] supporting the involvement of cytosine in charge transfer along DNA.

These results represent a paradigm shift in our understanding of oxidative damage to DNA. The $SeO_3^{\bullet-}$ radical (acting as DNA probe) has revealed that the full yield of the cytosyl radical is transiently formed in preference to the other DNA base radicals. This is at variance with prediction arising from the IP calculations for the base pair, and it is unlikely that cytosine is directly oxidized by the $SeO_3^{\bullet-}$ radical as the HOMO resides on guanine. The IP calculations are based on accommodating a positive charge in the radical base pair following electron loss from guanine. The guanyl radical cation cannot lose its positive charge through proton donation to the solvent (water in the major groove). We propose a subsequent concerted formation of a cytosyl radical (**1**), with proton loss from the amine substituent of cytosine into the major groove and H-atom transfer to the guanyl radical (Scheme 1). Our data show that **1** undergoes an intramolecular process, giving rise partially to the neutral guanyl radical spectrum (**2**) and, presumably, another nonabsorbing species.

EPR studies on the one-electron oxidation of 1-methylcytosine, cytosine, and 2′-deoxycytidine by the $SO_4^{\bullet-}$ radical have revealed that the lifetime of the initially formed radical cation is <200 ns with the spectrum of the uncharged aminyl radical being observed,

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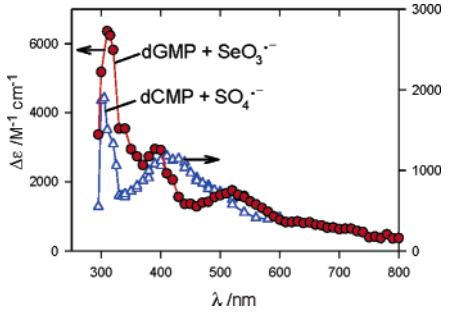


Figure 1. Absorption spectra observed in N_2-saturated aqueous solution of dCMP and dGMP (0.25 mM) at 5 μs after pulse radiolysis in the presence of $K_2S_2O_8$ (15 mM)/Na_2SeO_4 (25 mM), 2-methyl-2-propanol (0.25 M), $NaClO_4$ (0.1 M), and sodium phosphate (5 mM), pH 7.

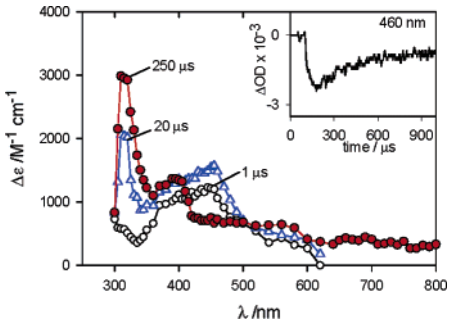
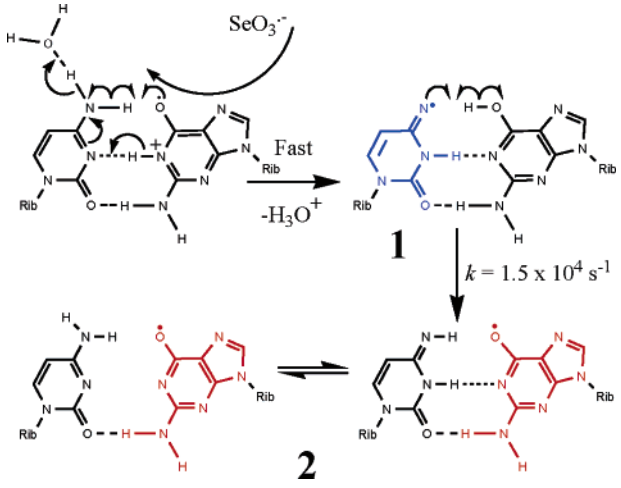


Figure 2. Absorption spectra observed upon pulse radiolysis of N_2-saturated aqueous solution of calf thymus DNA (2 mM in base pairs) containing Na_2SeO_4 (50 mM), $NaClO_4$ (0.1 M), 2-methyl-2-propanol (0.25 M), and sodium phosphate (5 mM), pH 7. Inset: kinetic trace of the observed change in transmittance with time.

Scheme 1. Proposed Sequential Formation of Neutral Cytosyl and Guanyl Radicals in GC Base Pair



which stabilizes in its iminyl form upon migration of the aminyl proton to N3 of the ring within 2 μs.[21] Our spectral studies show that the same UV−vis spectrum, to that produced by the $SO_4^{\bullet-}$ radical reacting with dCMP, is produced by the $SeO_3^{\bullet-}$ radical reacting with double-stranded DNA. This observation supports the contention that the initially produced hole (loss of an electron) in DNA is most likely on guanine followed by the rapid formation of the iminyl form of the cytosyl radical, upon loss of a proton to the solvent. The iminyl radical spectrum undergoes transformation to approximately one-half the absorbance of the neutral guanyl radical, implying that the cytosyl radical undergoes a concurrent competitive reaction to form a radical which has minimal absorbance in the UV−vis region. The loss of one of the hydrogen bonds in the GC base pair increases its flexibility[22] with the possibility of a radical in the base pair abstracting a hydrogen atom from a 2-deoxyribose moiety above or below its plane, as has been suggested from radical studies on AT base pairs and from studies with polyuridylic acid.[23] There is no specific evidence that one-electron oxidation of bases in double-stranded DNA leads to frank strand breaks. However, both strand breaks and damage to guanine have been reported upon the reaction of the $SeO_3^{\bullet-}$ radical with plasmid DNA.[24]

The measured intramolecular rearrangement of the initially formed cytosyl radical to a neutral guanyl radical (and possibly a sugar radical) can be viewed as an alternative mechanism for the fixation of the hole in DNA to that of the guanyl radical cation reacting with water. In this scenario, the cytosyl radical can act as an oxidant in equilibrium with nearby GC pairs (or GC−GC pairs), whereas the formation of radicals of lower reduction potential in the nucleotide pair, at ca. 1.5×10^4 s^{-1}, is in competition with such a process. We have recently measured the same rate constant for a competitive reaction to the reported[17] fast chemical repair of DNA by DNA-bound ligands. Our results show that the cytosyl radical, being of higher one-electron reduction potential than the paired guanyl radical, may well be the pivotal radical driving charge transfer along DNA in a stepwise process to distant GC pairs.

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Supporting Information Available: Comparison spectra of half the guanyl radical and that measured for the DNA radical, as well as spectral changes seen for the $SeO_3^{\bullet-}$ radical reacting with polyGC. This material is available free of charge via the Internet at http://pubs.acs.org.

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