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One-Electron Oxidation of DNA Oligomers That Lack Guanine: Reaction and Strand Cleavage at Remote Thymines by Long-Distance Radical Cation Hopping

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Damage to DNA is caused by oxidative processes that result in loss of an electron and the concomitant generation of a radical cation that migrates through the nucleobases of the duplex by a hopping mechanism.[1−4] The radical cation is eventually trapped in a reaction with H_2O or O_2 that results in the conversion of a base to a mutated form. A defining characteristic of the one-electron oxidation of duplex DNA is reaction at G_n ($n = 1-3$) sites that is detected as strand cleavage following chemical or enzymatic treatment. It has been generally agreed that reaction occurs primarily at guanines because they have low oxidation potentials (E_{ox}),[5,6] which causes the migrating radical cation to pause there briefly, and this facilitates trapping. We show here that this model is incomplete.

There are reports that reactions of radical cations in DNA occur at bases other than guanine.[7] Analysis of the benzophenone-sensitized oxidation of calf thymus DNA by GC−MS showed that the yield of the adenine oxidation product, 7,8-dihydro-8-oxoadenine, is a few percent of the 7,8-dihydro-8-oxoguanine yield, and cytosine and thymine oxidation products are formed in trace amounts.[8] Similarly, oxidation of DNA by photoionization with 193 nm light results primarily in reaction at G, but in "guanine-poor regions", reaction at adenine is also observed.[9] These findings are consistent with the idea that relative oxidation potential determines the reaction site because the E_{ox} of adenine is somewhat greater than that of guanine,[5] and pyrimidines T and C are considerably more difficult to oxidize than are the purines.[10] Indeed thymine, which has an E_{ox} of ca. 2.1 V vs NHE,[11] is the nucleobase that is most difficult to oxidize. However, Wagner[12] and co-workers report that photosensitized oxidation of DNA by a menadione (2-methyl-1,4-naphthoquinone) group that is linked covalently at an internal position causes reaction at nearby bases, including T. We report here a systematic investigation of the one-electron oxidation of DNA oligomers that do not contain guanine. Surprisingly, reaction occurs primarily at thymidines.

The DNA oligomers examined in this work are shown in Figure 1. They were purified by HPLC and characterized by mass spectrometry, melting temperature, and circular dichroism spectroscopy, which indicates normal B-form DNA in all cases. All of these compounds have an anthraquinone (AQ) group covalently linked to a 5′-end of one strand and a ^{32}P label at the 5′-end of the complementary strand. Irradiation of an AQ-linked DNA oligomer at 350 nm generates the excited singlet state of the AQ, which intersystem crosses rapidly to the triplet. Analysis using the Rehm−Weller equation[13] shows that AQ*[3] has sufficient oxidizing power to form an AQ radical anion and the radical cation of any of the four nucleobases in an exothermic reaction. The ^{32}P-labeled strand of DNA(1) contains an $(A)_5$ segment surrounded by $(AATT)_2$ sequences. On the basis of relative oxidation potentials, we anticipated that its photosensitized one-electron oxidation would result in reaction primarily at the adenines located within the $(A)_5$ segment. That was not the outcome.

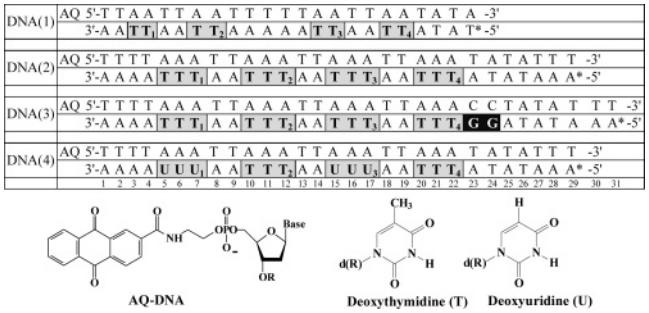

Figure 1. Structure of the DNA oligomers used in this work; $* = {}^{32}P$ radiolabel.

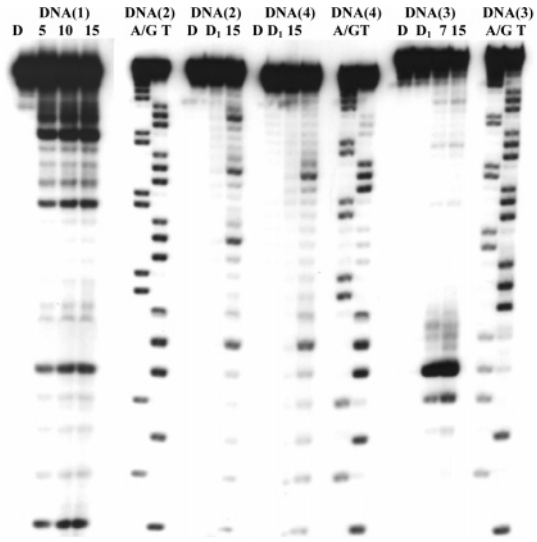

Figure 2. Autoradiograms of DNA (1−4). D and D_1 are control lanes (no UV irradiation, and UV irradiation but no piperidine treatment, respectively). The labels above the lanes identify the DNA oligomer and show the time of irradiation in minutes. Lanes labeled A/G and T are the Maxim−Gilbert sequencing lanes.

Irradiation of DNA(1) in a buffer solution of sodium phosphate (10 mM) and $MgCl_2$ (2 mM) at pH 7 and its subsequent treatment with piperidine reveals that strand cleavage occurs predominantly at thymidines in the four TT steps. An autoradiogram of the high-resolution PAGE gel from this experiment[14] is shown in Figure 2. Control experiments show that no cleavage occurs without UV irradiation (Lane D), and that strand cleavage requires piperidine treatment (Lane D_1), which indicates that reaction occurs at the thymine not at the deoxyribose.[15] Importantly, we showed that no detectable reaction of the radical cation occurs at adenines by treating irradiated samples with Na_2IrCl_6 before their reaction with piperidine.[16] There are four TT steps in DNA(1), the closest (TT_1) is ca. 7.2 Å from the AQ group and the farthest is nearly 65 Å

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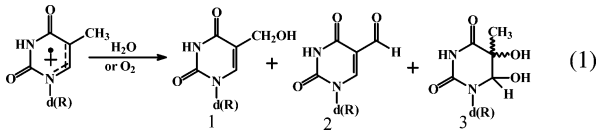
away. The amount of strand cleavage decreases with increasing separation between the AQ and TT step, which is a typical characteristic of long-distance charge transfer by hopping.[17] We examined the other DNA oligomers shown in Figure 1 to explore the reaction mechanism.

DNA(2) is a 29-mer comprised of four regularly spaced triple T sites (TTT) uniformly separated by AA steps on the ^{32}P-labeled strand. Its irradiation leads to piperidine-dependent strand cleavage predominantly at the central T of the TTT sites (Figure 2). There is an approximately equivalent amount of reaction at each of the TTT sites, which indicates that, in this case, the rate of the radical cation hopping reaction is faster than trapping.[18] DNA(3) is similar to DNA(2) except that it contains a GG step positioned directly after the fourth TTT site. Compared with DNA(2), the irradiation of DNA(3) shows a nearly complete absence of strand cleavage at the thymines. Instead, reaction occurs primarily at the 5′-G of the GG step, which is ca. 82 Å from the initial position of the radical cation. Since irradiation times are the same, the relative reaction efficiencies at the GG step and the TTT sites are comparable. In DNA(1) and DNA(2), the radical cation is trapped at a thymine, in DNA(3), the radical cation hops through the A/T base pairs and reacts at the remote guanines. DNA(4) is similar to DNA(2) except that the thymines in $(TTT)_1$ and $(TTT)_3$ have been replaced by uracils. The difference between uracil and thymine is that the latter carries a methyl group at its C5-position, whereas the former has a hydrogen atom at that site.

Figure 2 shows the results of irradiation of DNA(4). Strand cleavage occurs exclusively at $(TTT)_2$ and $(TTT)_4$, and there is no significant reaction at the sites where U has replaced T. The radical cation introduced in DNA(4) by irradiation of the AQ group must pass through the A/U base pairs in the (UUU) sites, but it does not react there. Clearly, the methyl group of the thymine radical cation plays an important role in its reactivity in oxidized duplex DNA.

The reaction of thymidine radical cation in aqueous solution follows two paths to the formation of the three oxidation products shown in eq 1.[19] It can lose a proton from the C5-methyl group forming a radical that is trapped by O_2 and generates 5-(hydroxymethyl)uridine (**1**) and 5-formyluridine (**2**). Or the thymidine radical cation can be attacked by H_2O resulting in formation of 5,6-dihydroxythymidine (**3**). DNA containing 5-formyluracil is cleaved by treatment with piperidine.[20] The reactions of the thymine radical cation in the relatively hydrophobic environment of duplex DNA may differ from those of the nucleoside radical cation in solution (as is the case for guanine radical cations[21]). The results reported here suggest that proton loss from thymine radical cation, possibly leading to 5-formyluracil formation, occurs following the one-electron oxidation of DNA duplexes that do not contain guanine.

It is evident from these findings that sufficient radical cation



(1)

density can reside on a thymine in DNA long enough for it to lose a methyl proton and undergo an irreversible trapping reaction with O_2. The observation that a remote GG step inhibits reaction at preceding thymines shows that hopping from (TTT/AAA) to (TTT/AAA) through an (AA/TT) "bridge" is faster than trapping at T; however, trapping of the radical cation at the GG step is far more efficient than it is at a T.[18] In other words, in the DNA oligomers that lack guanines, the radical cation "visits" each T many times and will eventually react there, but the migrating radical cation much less frequently escapes encounters with the GG step.

Remarkably, for oligomers lacking guanines, one-electron oxidation results in reaction primarily at thymine even though adenine has a significantly lower E_{ox}. This is a circumstance that is readily understood by application of the Curtin−Hammett principle.[22] It is not the most stable (i.e., lowest E_{ox}) species in the equilibrated distribution of the radical cation among various locations on the oligomer that gives the major product but the one with the highest reactivity (i.e., reaction path with the lowest barrier). Evidently, the rate of irreversible radical cation trapping at T is much greater than it is at A, and it is this feature that controls the outcome of the reaction.

These findings clearly have important implications for understanding DNA mutations caused by loss of an electron. They suggest that the generation of a nucleobase radical cation in an extensive guanine-poor region will cause thymine oxidation. Significantly, identification and characterization of a mammalian 5-formyluracil glycosylase was reported recently.[23] This enzyme signals the existence of a repair system for oxidatively damaged thymines. We are currently assessing the generality and significance of these findings.

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