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Oxidatively Damaged Nucleobases in Duplex DNA Oligomers: Reaction at Thymine−Thymine Mispairs

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Normal aerobic cellular metabolism is estimated to result in the "damage" (chemical transformation) of ~10 000 nucleobases per cell per day.[1] Exposure to light, ionizing radiation, or some drugs greatly increases the rate at which such damage occurs.[2−4] Under some conditions, oxidative damage to cellular DNA occurs at guanines and thymines to a similar extent,[5] which is surprising because experiments have shown that the one-electron oxidation of DNA oligomers in solution results in nearly exclusive reaction at guanines.[3,6,7] In fact, significant reaction at thymine is observed only in DNA oligomers that do not contain guanines.[8,9] The replication of DNA by polymerases results in occasional base substitution errors causing the generation of DNA mispairs.[10] One such error, an A to T transversion, generates thymine−thymine nucleobase mispairs. We report herein that such T−T mispairs are sites of exceptionally high reactivity for oxidative damage to DNA that cause reaction to occur at thymines even when guanine nucleobases are present.

The DNA oligomers studied in this work are shown in Figure 1. Each was prepared by automated DNA synthesis, purified, and then characterized by mass spectroscopy (spectroscopic and physical properties of the oligomers are included in the Supporting Information). Each of these duplex DNA oligomers is linked at a 5′-terminus to an anthraquinone group (Aq), which serves as a photosensitizer for one-electron oxidation, and a [^{32}P] radiolabel (indicated by *) at the 5′-terminus of the strand complementary to the one containing the Aq to facilitate quantitative analysis by radiography.[7] Each oligomer examined was irradiated to low conversion ("single-hit" conditions) at 350 nm (where the Aq absorbs) to cause its one-electron oxidation, then treated with piperidine to reveal damaged nucleobases as strand breaks, and finally analyzed by PAGE. (A description of the experimental procedure and the PAGE results are included in the Supporting Information.)

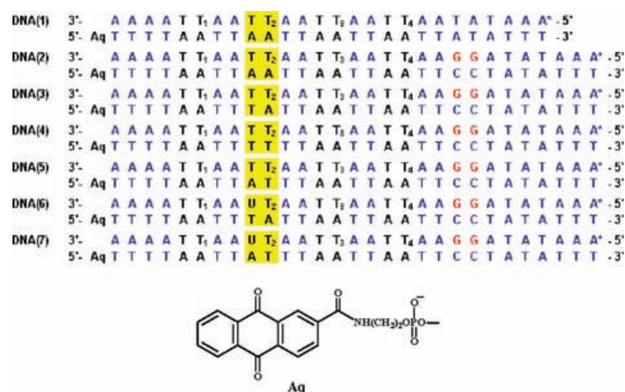

Figure 1. Structures of DNA sequences used in this study. Aq represents the anthraquinone photosensitizer that is covalently attached to a 5′-terminus.

DNA(1) is a duplex oligomer comprised only of fully complementary A/T Watson−Crick base pairs. It contains four TT "steps" (adjacent thymines) on the labeled strand that are each separated by AA sequences. As expected,[8,9] its one-electron oxidation results in essentially exclusive reaction at the TT steps. There is a distance dependence to this reaction. Strand cleavage occurs more frequently at TT steps that are closer to the site of "charge injection" (*i.e.*, initial oxidation of the base pair neighboring the Aq group), and a semilog plot of the strand cleavage yield vs distance is linear with a slope of −0.009 ± 0.001 Å^{-1} indicating that the rate of radical cation hopping (k_{hop}) is greater than that for irreversible trapping (k_{trap}).[11] These results are summarized in the histogram shown in Figure 2. DNA(2) is similar to DNA(1) except that it contains a single GG step *ca.*72 Å from the site of initial charge injection following (*i.e.*, is distal to) the four TT steps. In this case, virtually all of the strand cleavage is observed to occur at the GG step with little reaction at any of the preceding TT steps, see Figure 2. These findings confirm that the radical cation introduced into DNA(2) by one-electron oxidation hops rapidly through the $(TTAA)_4$ sequence and then is trapped irreversibly by rapid reaction at the GG step.[12] A strikingly different result is obtained when there is a single thymine−thymine mispair in the sequence preceding the GG step. DNA(3) is similar to DNA(2) except that the adenine in the complementary strand opposite the 3′-T of the TT_2 step is replaced by a thymine thus creating a thymine−thymine mispair. The one-electron oxidation and subsequent strand cleavage analysis of DNA(3) reveals that reaction occurs predominantly at the TT_2 step, with some strand cleavage detected at TT_1 and much reduced reaction at the remote GG step. There is no detectable reaction at either TT_3 or TT_4, which are between the mispair and the GG step. A related result is obtained from reaction of DNA(4), which contains thymine−thymine mispairs at both thymines of the TT_2 step. However, in DNA(5) where there is a single mispair at the 5′-T of the TT_2 step, there is enhanced reaction at TT_1, and strand cleavage

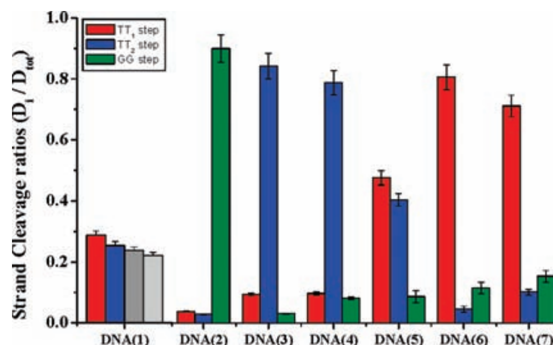

Figure 2. Ratio of strand cleavage at a particular TT step (D_i,) to the total strand cleavage for the entire oligomer (D_{tot}) for DNA duplexes (1)−(7). The gray bars are for TT_3 and TT_4 steps in DNA(1); oligomers DNA(2)−(7) show negligible amounts of strand cleavage at these sites. The corresponding PAGE data are given in the Supporting Information.

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is detected at the remote GG step, but the amount of strand cleavage at the TT_2 step is significantly less than occurs in DNA(3); see Figure 2. The possible reasons for the enhanced strand cleavage yield at the thymine−thymine mispair and its inhibition of reaction at the distal GG step include the imposition of a high barrier to radical cation hopping and that the mispair is an extraordinarily reactive site. A series of experiments was carried out to assess these possibilities.

Previous work has shown that radical cation reaction at TT steps occurs primarily by a tandem process that simultaneously damages both thymines.[8,9,13,14] The major products of this reaction are 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd) and 5-formyl-2′-deoxyuridine (5-FormdUrd), which result from reaction of the thymine methyl group, and the *cis* and *trans* diastereomers of 5,6-dihydoxy-5,6-dihydrothymidine, *c*, *t*-ThdGly, which result from addition to the thymine double bond; see Figure 3. The methyl group of the 3′-thymine in a TT step plays a special role in the tandem reaction. When the 3′-T is replaced by uracil, which lacks the 5-methyl group of thymine, strand cleavage is not detected at either base of the UT step.[9] In contrast, efficient strand cleavage is observed at both bases when a U is substituted for the 5′-thymine of a TU step.[9] These findings highlight the unique role played by the methyl group of the 3′-thymine in the reaction of a radical cation at a TT step. We prepared DNA(6) and DNA(7) to study the effect of uracil substitution for thymine in thymine−thymine mispairs.

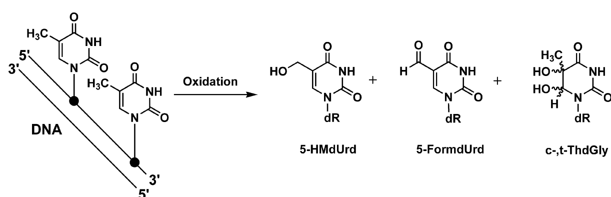

Figure 3. Structures of thymine oxidation products at tandem TT steps.[8]

DNA(6) is similar to DNA(3) except that the mispaired 3′-T at the TT_2 step is replaced by a U. In stark contrast to the one-electron oxidation of DNA(3), there is virtually no reaction at the thymine−uracil mispair of DNA(6) (see Figure 2). In this case, strand cleavage occurs primarily at TT_1, which is located between the Aq and the mispair, and secondarily at the distal GG step. Similarly, in DNA(7), which has a thymine−thymine mispair at the 5′-T and where U is substituted for the 3′-T at the TT_2 step, strand cleavage is detected at the distal GG step and at TT_1, but reaction at the TT_2 step is much reduced compared with DNA(3). Clearly, a thymine−thymine mispair has a different effect on radical cation hopping and reaction when it occurs at the 3′- or the 5′-position of a TT step.

The reaction of a nucleobase radical cation in duplex DNA may occur under either thermodynamic or kinetic control. Under thermodynamic control $k_{ratio} = (k_{hop}/k_{trap}) \gg 1$ at every reactive site. In this circumstance, the probability of reaction at each equivalent position in the oligonucleotide is identical, independent of its distance from the site of charge injection. At intermediate values of k_{ratio}, a semilog plot of the strand cleavage yield against distance from the charge injection site is linear. When $k_{ratio} \ll 1$, the reaction is under kinetic control and its outcome depends on the specific sequence of nucleobases.[11] The introduction of a thymine−thymine mispair into a TT step affects k_{ratio} at that site so that the reaction shifts from thermodynamic to kinetic control.

The value of k_{ratio}, of course, is dependent on both k_{hop} and k_{trap}, and the results reported herein indicate that both are affected by introduction of a thymine−thymine mispair, but the mispair affects the reaction differently depending on its precise location. When the mispair is at the 3′-position of a TT step, as it is in DNA(3), it appears that the rate of irreversible trapping of the radical cation at this site is significantly increased and that its rate of hopping is slowed. Thus, when the 3′-mispair at the TT_2 step is thymine−thymine, *i.e.*, DNA(3), k_{ratio} is small because k_{trap} increases, as evidenced by predominant strand cleavage at the TT_2 step and because k_{hop} decreases, as evidenced by increased reaction at the TT_1 step. When the 3′-mispair at this site is uracil−thymine, *i.e.*, DNA(6), k_{ratio} is small but not because k_{trap} is large (there is little reaction at the TT_2 step due to the absent methyl group) but because k_{hop} is small, as evidenced by much greater strand cleavage at the TT_1 step. When the thymine−thymine mispair is at the 5′-position of a TT step, *i.e.*, DNA(5), k_{ratio} is again small not because k_{trap} is large but because k_{hop} is small since there is significant reaction at the TT_1 step and not much at TT_2. This circumstance is essentially unchanged in DNA(7) where the thymine−thymine mispair is at the 5′-position and U replaces T at the 3′-position of the TT_2 step. Simply put, thymine−thymine mispairs are barriers to long-distance radical cation migration in duplex DNA because they slow hopping and, when in a suitable position, have high reactivity.

Thymine−thymine mispairs have dynamic wobble structures[15] with their methyl groups in the DNA major groove. As such, the high reactivity of thymine−thymine mispairs may be due to increased flexibility enhancing the tandem reaction. Similarly, formation of 5-HMdUrd and 5-FormdUrd requires loss of a proton from the thymine radical cation's methyl group, which may be enhanced in the thymine−thymine mispair due to changes in solvation. In either event, it is clear from this work that thymine−thymine mispairs can increase the amount of oxidative damage to thymines in DNA because they both inhibit radical cation hopping and can be much more reactive than normal Watson−Crick A/T base pairs.

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Supporting Information Available: General experimental methods, PAGE gel images and structural characterization data for the oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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