One-electron oxidation of DNA: thymine *versus* guanine reactivity†

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One-electron oxidation of anthraquinone (AQ)-linked DNA oligonucleotides containing A/T base pairs with repeating TT steps results in the distance-dependent reaction of the resulting radical cation and base damage at the TT steps that is revealed by subsequent reaction as strand cleavage. However, the inclusion of a remote guanine or GG step inhibits the reaction at thymine and results in predominant reaction at the guanine bases. For the oligomers examined in this work, the results reveal that the specific sequence of nucleobases determines the distance dependence, location of reaction and the efficiency of radical cation migration. In particular, a sequence of A/T base pairs can behave either as a trap, shuttle or barrier, depending on the context of the entire oligomer. The A/T sequences act as a shuttle when reaction occurs at a remote G or GG step and the same sequence of A/T bases acts as a barrier when there is more than one GG step in the sequence. In contrast, the A/T steps act as a trap in sequences that lack guanines.

Introduction

Cellular DNA is subjected to constant oxidative stress leading to chemical reactions creating lesions at nucleobases and at the deoxyribose sugar units.[1] The one-electron oxidation of DNA generates a radical cation that hops reversibly through the nucleobases of the duplex until it is trapped irreversibly in a chemical reaction.[2] In normal DNA containing all four common bases (G, C, A, T), reactions of the radical cation occurs predominantly at guanine to generate 8-oxo-7,8-dihydroguanine (8-OxoG) as the major product.[1]

Thymine in DNA is known to be prone to oxidative damage at two functional groups. First, reaction at its 5,6-double bond leads to formation of *cis* and *trans* diastereomers of 5,6-dihydroxy-5,6-dihydrothymidine (*c*-, *t*-ThdGly) and other minor products. These lesions are not strongly mutagenic, but they do lead to replication errors.[3–5] The second reaction is at the thymine methyl group resulting in the formation of an intermediate peroxyl group that eventually is converted to 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd) and 5-formyl-2′-deoxyuridine (5-FormdUrd)[6] See Scheme 1. In fact, 5-FormdUrd and 8-OxoG are the most abundant oxidation-induced nucleobase lesion products formed from DNA when it is exposed to ionizing radiation in the presence of molecular oxygen.[7,8] The mutagenic potential of 5-FormdUrd arises from its tendency to mispair with guanine.[7,9] Damage to pyrimidines has been used as a molecular biomarker in the study of oxidative stress and cancer.[10] Clearly, identification of the factors that affect the reactions of thymine in DNA is necessary for a complete understanding of the biological consequences of DNA oxidation, especially in those sequences that have A/T-rich regions.[11–14]

We recently reported that the one electron oxidation of duplex DNA that does not contain guanine leads to reaction at thymine—predominantly at adjacent TT steps by a tandem reaction[15,16] that requires neighboring thymines.[17–19] In this work, we examine the effect of distance and nucleobase sequence on the distribution of nucleobase radical cation reaction products in oligomers containing both TT steps and guanine nucleobases.

Experimental section

DNA oligomers (Fig. 1) were synthesized using an Expedite 8909 DNA synthesizer, purified by reversed phase HPLC and characterized by ESI Mass Spectrometry. The oligomers were radiolabeled at the 5′-end using γ-[^{32}P] ATP with T4 PNK enzyme for autoradiography analysis. DNA duplexes were hybridized in pH 7.0 buffer solution containing 10 mM sodium phosphate and 2 mM $MgCl_2$. Relevant experimental procedures are given in the supporting information.†

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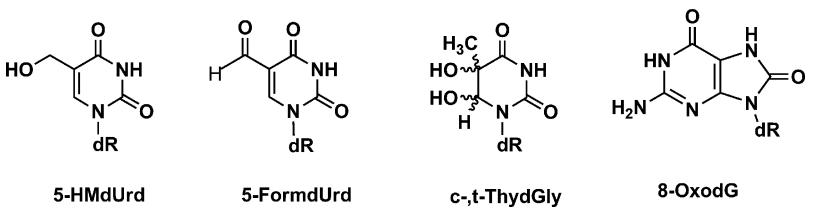
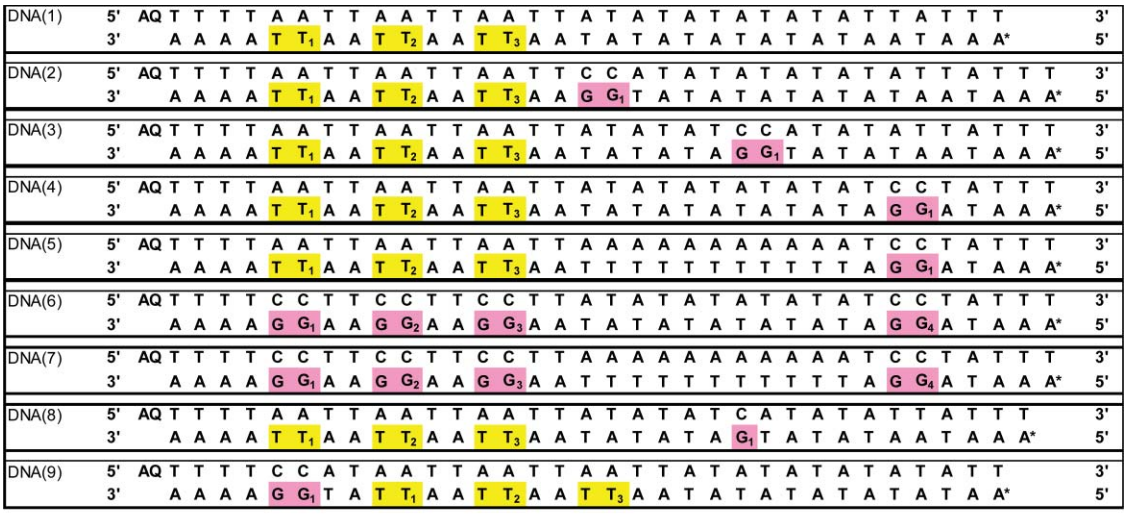
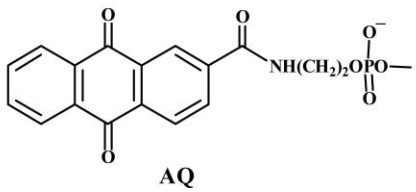
† Electronic supplementary information (ESI) available: General experimental methods, autoradiograms of DNA duplexes, T_m and CD spectral characterization data for the oligonucleotides and are available. See DOI: 10.1039/b922881k



Scheme 1 Primary products formed from the one-electron oxidation of thymine and guanine in duplex DNA.





AQ

Fig. 1 Structures of DNA oligomers investigated.

Results

The DNA oligomers we investigated are shown in Fig. 1. Each DNA duplex contains an AQ photosensitizer[20] linked covalently to a 5′ terminus of the DNA oligomer and is radiolabeled with [^{32}P] (indicated by a * in Fig. 1) that is used in the analysis of strand cleavage by PAGE and autoradiography. The melting temperatures (T_m) of these duplexes are consistent with expected values ranging from 50 °C to 64 °C, and their CD spectra indicate that their global structure is that of standard B-form DNA. The first-derivative melting curves and CD spectral data are given in the supporting information.

In one series investigated, DNA(**1**)-DNA(**5**), the duplex oligomers contain a sequence of three TT steps, DNA(**1**), or a GG step and three TT steps, [DNA(**2**)- DNA(**5**)], separated by a variable segment of A/T and T/A nucleobases. Also in this series, the distance to the GG step from the TT steps is increased systematically. The GG step is a site of high reactivity for reaction of the radical cation in duplex DNA.[21] For instance, in DNA(**2**) the GG step is placed *ca*. 6.8 Å from the last TT step of the sequence. Similarly the GG steps in DNA(**3**), DNA(**4**) and DNA(**5**) are 27 Å, 48 Å and 48 Å from TT_3 step, respectively. A variable segment, referred to as the "bridge", is between the last TT step of this series and the GG step and is comprised of a series of A/T nucleobases. In one case, DNA(**5**), the bridge contains a segment of 11-contiguous thymine bases on the labeled strand. The second series of DNA oligomers, DNA(**6**) and DNA(**7**), have four GG steps, three of these are at positions equivalent position to the TT steps in DNA(**1–4**) and the fourth GG step is separated by variable bridges similar to those of DNA(**4**) and DNA(**5**). In the last series, DNA(**8**) has a single guanine nucleobase instead of the GG step *ca*. 27 Å beyond last TT step in the oligomer, and DNA (**9**), unlike the other oligomers, has a GG step between the AQ and the first TT step in the duplex oligomer.

The duplex DNA oligomers (5 μM) were dissolved in pH 7.0 buffer solutions containing sodium phosphate (10 mM) and $MgCl_2$ (2 mM) and irradiated at 350 nm where the AQ absorbs and the DNA nucleobases are essentially transparent. The electronically excited state of the AQ formed by absorption of light is a strong oxidant that converts an adjacent base to its radical cation with concomitant formation of the AQ radical anion. The AQ is regenerated rapidly by reaction of the radical anion with O_2 (forming superoxide). This process, photosensitized electron transfer followed by rapid reaction of the AQ radical anion, results in the "injection" of a radical cation into the nucleobases of the DNA duplex at the nucleobase pair adjacent to the AQ group.[22]

Radiolabeled samples of duplexes DNA(**1**)-DNA(**5**) were irradiated to low conversion where each oligomer reacts once or not at all (so called single hit conditions) and then treated with piperidine for 30 min at 90 °C to reveal the site of damaged nucleobases as strand cleavage.[1] The results are shown as a histogram in Fig. 2. (Autoradiograms of experimental PAGE are given in the ESI†).

As expected, for DNA(**1**) distance dependent strand cleavage is observed at the TT steps, and for DNA(**2**) strand cleavage occurs almost exclusively at the nearby GG step. The results of irradiation of DNA oligomers (**3**), (**4**), and (**5**) give similar results with predominant strand cleavage occurring at the increasingly remote GG step. In DNA(**3**) and (**4**), the GG step is 27 Å and 48 Å from the last TT step, respectively, and it is the predominant site for strand cleavage. Interestingly, the GG step in DNA(**5**), which has 11-contiguous thymines in the bridge, displays similar reactivity to the GG step in DNA(**4**) that contains a mixed sequence A/T bridge of similar length. A similar result is seen for DNA(**8**), where

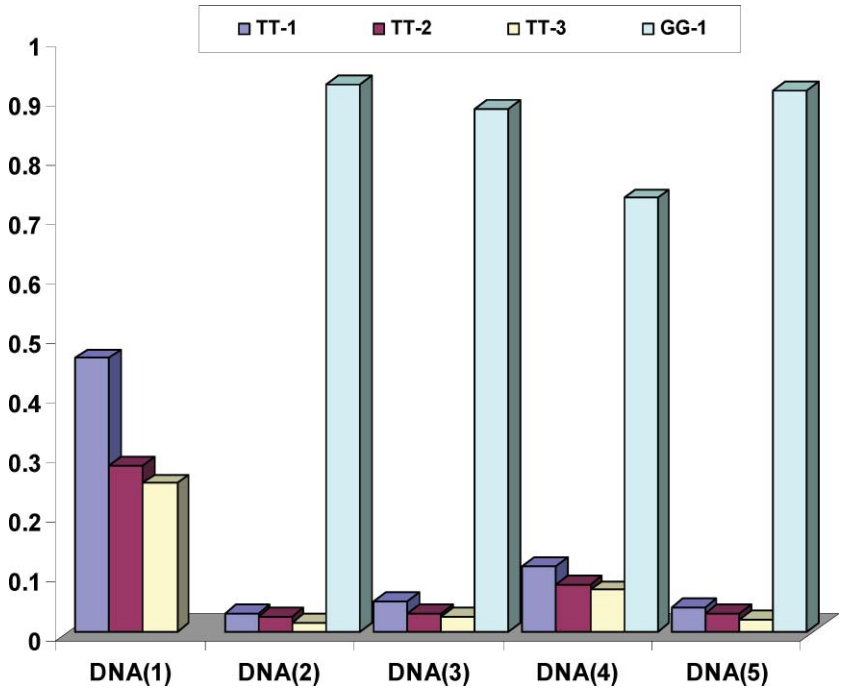


Fig. 2 Strand cleavage ratios of DNA duplexes (**1**)–(**5**). The corresponding PAGE autoradiography gels are given in the supporting information.†

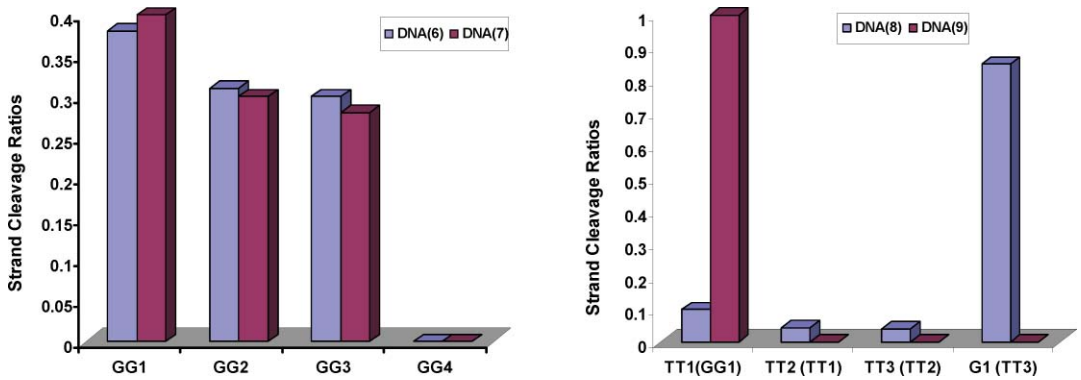


Fig. 3 Histograms depicting the strand cleavage ratios for DNA duplexes (**6**)–(**9**). DNA(**9**) has no detectable damage at the TT steps. The corresponding autoradiograms are given in the supporting information.†

strand cleavage occurs predominantly at the remote single guanine nucleobase.

In order to probe further the effect of the bridge on the reactions of radical cations in DNA, we studied DNA(**6**) and DNA(**7**). These oligomers contain GG steps in place of the TT steps of DNA(**4**) and DNA(**5**) and a fourth GG step separated by a variable segment containing a mixed A/T bridge or a contiguous T bridge. Irradiation and piperidine treatment cause strand cleavage. In contrast to DNA(**2**)-DNA(**5**), where the majority of the strand cleavage is observed at the distant GG step, in DNA(**6**) and DNA(**7**) strand cleavage occurs predominantly at the GG steps closer to AQ and there is essentially no reaction observed at the distant GG step. In addition, exclusive reaction is observed at the GG step closer to the AQ in DNA(**9**) with no detectable reaction at the following TT steps. These findings indicate that the barrier to radical cation hopping created by the bridging nucleobases is dependent on the context of the entire duplex oligomer.[23] The results are shown as a histogram in Fig. 3.

Discussion

UV irradiation of these duplex DNA oligomers at 350 nm, where the AQ photosensitizer absorbs light, injects a radical cation that migrates through the duplex by hopping.[24] The radical cation is trapped irreversibly by reaction with H_2O or O_2 to create a "damaged" DNA nucleobase that is revealed as strand cleavage by subsequent piperidine treatment. Typically, reaction of the radical cation occurs at guanine, which has the lowest-oxidation potential of the common nucleobases.[25,26] In contrast, we previously found that the one-electron oxidation of DNA(**1**), which contains no guanines, results in distance-dependent strand cleavage at the thymines of each of its three TT steps.[19] In contrast, little strand cleavage is observed at the TT steps of DNA(**2**), which contains a remote GG step. Similarly, predominant reaction is observed at the GG step in DNA(**3**–**5**) where bridges of varying composition separate it from the site of radical cation injection (the base pair adjacent to AQ). Even a bridge of eleven contiguous T/A

base pairs, DNA(**5**), does not meaningfully inhibit radical cation reaction at the remote GG step. The amount of reaction at specific TT steps increases slightly with increasing bridge length, but this result is barely significant statistically. For comparison, in DNA(**6**) and DNA(**7**), where GG steps replace the TT steps of DNA(**4**–**5**), a distant dependent reaction occurs at the intervening guanines, and little reaction occurs at the remote GG step.

The site and amount of reaction of a radical cation at a particular nucleobase in a duplex DNA oligomer is dependent on the ratio of rate constants for hopping (k_{hop}) and the irreversible trapping reaction (k_{trap}) of the radical cation.[26] Previous studies of mixed sequence DNA indicate that A/T base pairs can act as kinetic barriers to radical cation hopping.[23,27] The findings reported here show that the role of an A/T bridge is context dependent. For all of the oligonucleotides examined, radical cation reaction at guanine nucleobases is faster than reaction at the TT steps. Even the single guanine in DNA(**8**) "protects" the preceding thymines from reaction. Specifically, the bridging A/T base sequences in DNA(**4**) and in DNA(**5**) do not inhibit reaction at the remote GG step. And for DNA(**9**), strand cleavage is observed essentially exclusively at the proximal (to the AQ) GG step, with no significant reaction at the more distal TT steps. However for DNA(**6**) and DNA(**7**), where the TT steps before the bridge are replaced by GG steps, the bridging A/T base sequences that in DNA(**4**) and DNA(**5**) present no barrier to hopping are effective in essentially completely inhibiting reaction at the remote GG step. This finding confirms that the entire sequence of DNA bases in an oligomer must be considered when evaluating the role a particular sequence plays in the hopping or reaction of oxidized DNA.

We have advanced the concept of qualitative potential energy landscapes to account for patterns of radical cation reaction in duplex DNA.[23] These landscapes are comprised of three elements: "traps" are nucleobases where irreversible reaction of the radical cation occurs; "barriers" are sequences of nucleobases that inhibit radical cation hopping; and "shuttles" are nucleobase sequences through which radical cations may hop but undergo little reaction. This proposal was advanced to account for patterns of reaction among oligonucleotides containing guanines. The results reported here show that these qualitative concepts also can be used to reliably account for the patterns of reaction observed in oligonucleotides that do not contain guanine.

The results show that the specific sequence of nucleobases determines the distance dependence and the efficiency of radical cation migration in a predictable manner. In particular, a sequence of A/T base pairs can behave either as a trap, shuttle or barrier, depending on context. Thus for the oligomers examined in this work, the presence of even a single remote guanine nucleobase can act as a trap for the radical cation and in this case sequences of A/T bases play the role of shuttle. However, when guanines are absent from the oligonucleotides, these A/T nucleobase sequences have the character of a trap, and reaction occurs primarily at TT steps. And, quite strikingly, the same A/T sequences take on the character of barriers when they occur between GG steps.

The results reported here suggest that one electron oxidation of duplex DNA will result in reaction at thymine only in sequences that do not contain guanine, or perhaps in complex DNA constructs that contain conformations or structures that inhibit radical cation hopping such as was found to be the case for some DNA condensates.[28]

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