Selective one-electron oxidation of duplex DNA oligomers: reaction at thymines†

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The one-electron oxidation of duplex DNA generates a nucleobase radical cation (electron "hole") that migrates long distances by a hopping mechanism. The radical cation reacts irreversibly with H_2O or O_2 to form oxidation products (damaged bases). In normal DNA (containing the four common DNA bases), reaction occurs most frequently at guanine. However, in DNA duplexes that do not contain guanine (*i.e.*, those comprised exclusively of A/T base pairs), we discovered that reaction occurs primarily at thymine and gives products resulting from oxidation of the T-C5 methyl group and from addition to its C5–C6 double bond. This surprising result shows that it is the relative reactivity, not the stability, of a nucleobase radical cation that determines the nature of the products formed from oxidation of DNA. A mechanism for reaction is proposed whereby a thymine radical cation may either lose a proton from its methyl group or H_2O/O_2 may add across its double bond. In the latter case, addition may initiate a tandem reaction that converts both thymines of a TT step to oxidation products.

Introduction

The one-electron oxidation of DNA introduces a radical cation ("hole") into its stacked nucleobases that results in chemical reactions ("damage") that may lead to mutations. In recent years, it has been shown that the reactions of radical cations that damage DNA need not occur at the site of the initial oxidation. In duplex DNA, radical cations may migrate long distances (hundreds of Ångstroms) by a reversible hopping process before being trapped irreversibly by reaction with H_2O or O_2.[1–10] This process can produce a multiplicity of chemical modifications to the DNA that mostly consist of base lesions,[11–13] and it has been implicated in carcinogenesis, other diseases[14–16] and in aging.[17] Apart from its biological relevance, radical cation migration in DNA is of interest because of its potential application to molecular electronic devices.[18–21]

One of the more interesting features of the one-electron oxidation of duplex DNA is that the resulting reaction, which is typically detected as strand cleavage following chemical or enzymatic treatment of the damaged DNA,[12] commonly occurs at G_n (n = 1–3) sites. It had been generally accepted[22] that reaction occurs at guanines primarily because they are the nucleobases having the lowest oxidation potential (E_{ox}).[23,24] Thus, a migrating radical cation pauses briefly at a guanine or a multi-guanine site, and this facilitates trapping by the irreversible reaction with H_2O or O_2.

The migration and reactions of radical cations in duplex DNA oligomers that contain guanine have been studied thoroughly.[1,25–29] However, the consequence of one-electron oxidation of oligomers that do not contain guanine nucleobases had not been examined until the report of our recent experiments.[30] Previously, there were scattered reports indicating that reactions of radical cations in DNA do occur at bases other than guanine.[31] For example, in oligonucleotides that contain both guanine and adenine, the oxidation product 8-oxo-7,8-dihydroadenine (8-oxoAde) is found in low yield in comparison with the guanine oxidation product 8-oxo-7,8-dihydroguanine (8-oxoGua).[32,33] This result is consistent with the idea that relative oxidation potential determines the reaction site for radical cations in DNA because the E_{ox} of adenine is somewhat greater than that of guanine. The pyrimidines, T and C, are much more difficult to oxidize than are the purine bases.[34] Indeed thymine, which has an E_{ox} of *ca.* 2.1 V *vs.* NHE,[35] is the nucleobase that is most difficult to oxidize. However, reactions at thymines are observed when a menadione (2-methyl-1,4-naphthoquinone) group is linked covalently at an internal position of DNA.[36]

We recently reported the results of a preliminary study of the one-electron oxidation of duplex DNA oligomers that do not contain guanines.[30] Surprisingly, reaction occurs primarily at thymines; no reaction could be detected at adenines. This was attributed[37] to the difference in reactivity between adenine and thymine radical cations. Specifically, we found by replacement of T with uracil that the C5-methyl group of thymine is necessary for the oxidation reactions and strand cleavage to occur.

Here we report a detailed examination of the one-electron oxidation of duplex DNA oligomers that do not contain guanines. These studies include assessment of the distance-dependence of radical cation migration in duplexes comprised of only A/T base pairs, the effect of a radical scavenger on the reactions of thymine, and the role played by the thymine C5-methyl group. The products of these reactions were identified by means of

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† Electronic supplementary information (ESI) available: Melting temperature data and circular dichroism data for DNA(1–10); histogram indicating the amount of damage at each TTT step relative to the total damage at all TTT steps for 15 min of irradiation and 20 min of irradiation of DNA(1); analysis of the effect of irradiation time on the reaction and strand cleavage of DNA containing only A/T base pairs. See DOI: 10.1039/b717437c

sophisticated HPLC–MS/MS analysis under conditions of low nucleobase conversion. These experiments indicate that there is a complex mechanism for the reaction of thymine radical cations in duplex DNA that features a competition between proton loss from the C5 methyl group and the addition of H_2O/O_2 across its C5–C6 double bond, with the former process leading to a tandem reaction involving an adjacent nucleobase.[38,39]

Materials and methods

Chemicals were purchased from either Fisher Scientific or Sigma Aldrich (St Louis, MO). T4 polynucleotide kinase (PNK) enzyme and [γ-^{32}P]-ATP were purchased from GE Healthcare. *E. coli* endonuclease III (Endo III) enzyme was purchased from Trevigen Inc. (Gaithersburg, MD). DNA oligomers were synthesized as described elsewhere[39] on an Expedite 8909 DNA synthesizer. Nucleoside phosphoramidites were obtained from Glen Research and were used as received. The extinction coefficients of the oligomers were calculated using a biopolymer properties calculator, and their concentrations were determined from the absorbance at 260 nm. An adenine is substituted for the anthraquinone group in the extinction coefficient calculation. The oligonucleotides were purified by means of reversed phase HPLC on a Hitachi preparative HPLC system using a Dynamax octadecylsilyl silica gel column. Purified oligomers were desalted and characterized by mass spectroscopy. UV melting and cooling curves were recorded on a Cary 1E spectrophotometer equipped with a multicell block, temperature controller, and sample transport accessory. CD spectra were recorded on a JASCO J-720 spectropolarimeter.

Preparation of radiolabeled DNA

The oligomers were radiolabeled at the 5′-end using [γ-^{32}P]-ATP and PNK enzyme. A 5 μL sample of desired single stranded DNA was incubated with 1 μL of [γ -^{32}P]-ATP and 2 μL of PNK enzyme in a total volume of 20 μL at 37 °C for 45 min. After incubation, the DNA sample was suspended in denaturing loading buffer and was purified on a 20% denaturing polyacrylamide gel. The desired DNA band was excised from the gel and eluted with 800 μL of elution buffer (0.5 M NH_4OAc, 10 mM $Mg(OAc)_2$/1.0 mM EDTA/0.1% SDS) at 37 °C for 12 h. The DNA was precipitated from the supernatant by addition of 600 μL of cold ethanol and 2 μL of glycogen solution. The mixture was vortexed, placed on dry ice for *ca.* 60 min, and centrifuged at 13 000 rpm for 45 min. The supernatant was removed, and the residual DNA was washed with 100 μL of 80% ethanol and air-dried. Suitable volumes of water were added for further experimentation.

UVA irradiation and cleavage analysis

Samples for irradiation were prepared by hybridizing a mixture of unlabeled (5.0 μM) and radiolabeled (10 000 cpm) oligonucleotides with complementary AQ-linked DNA in sodium phosphate buffer solution (10 mM) and $MgCl_2$ (2 mM) at pH 7.0. Hybridization was achieved by heating the samples at 90 °C for 10 min, followed by slow cooling to room temperature for 3 h. Samples were irradiated at *ca.* 30 °C in microcentrifuge tubes in a Rayonet Photoreactor (Southern New England Ultraviolet Co., Bransford, CT) equipped with eight 350 nm lamps. To investigate the effect of glutathione, different concentrations (0.05 mM, 0.5 mM, 1.0 mM and 5.0 mM) of glutathione was added to hybridized samples, prior to irradiation. After irradiation, the samples were precipitated once with cold ethanol (100 μL) and 2 μL of glycogen. The precipitated samples were washed twice with 100 μL of 80% ethanol and dried. Then dry DNA oligomers were dissolved in 14 μL of water, and 2 μL of sodium phosphate buffer (100 mM), 2 μL of NaCl (1 M) and 2 μL of Na_2IrCl_6 (100 μM) were added. After 60 min of reaction at 37 °C, 2 μL of HEPES (20 mM) and 2 μL of EDTA (100 mM) were added to quench the reaction. The DNA was precipitated from cold ethanol and dried. For piperidine chemical cleavage analysis, the samples were mixed with 50 μL of piperidine (1 M) and heated at 90 °C for 30 min. After evaporation of the piperidine (Speedvac, high heat) and lyophilization twice with 20 μL of water, the samples were dissolved in denaturing loading dye and subjected to 20% 19 : 1 polyacrylamide gel electrophoresis.

For EcoRIII enzymatic cleavage analysis, the dried samples were mixed with 12 μL of the enzyme and 8 μL of buffer solution, heated first at 37 °C for 2 h and then at 90 °C for 20 min. The samples were then reprecipitated with cold ethanol (100 μL) and 2 μL of glycogen and the precipitated samples were washed with 100 μL of 80% ethanol. After evaporation of the ethanol, the samples were dissolved in denaturing loading dye and subjected to 20% 19:1 polyacrylamide gel electrophoresis. The gels were dried, and the cleavage sites were visualized by autoradiography. Quantification of cleavage bands was performed on a Fuji phosphorimager.

Enzymatic digestion and HPLC–MS/MS analysis

Oligonucleotides, either untreated or exposed to light, were enzymatically digested by incubation (2 h, 37 °C) at pH 6 in the presence of phosphodiesterase II and nuclease P1 (Sigma, St Louis, MO). The pH was then adjusted to 8 by addition of Tris buffer. Treatment (2 h, 37 °C) by phosphodiesterase I and alkaline phosphatase (Sigma, St Louis, MO) yielded normal and oxidized nucleosides. The resulting mixture was separated by high performance liquid chromatography on a Agilent Series 1100 system equipped with an Uptisphere ODB octadecylsilyl silica gel column (2 × 150 mm I.D., 3 μm particle size; Montluçon, France). The mobile phase was a gradient of acetonitrile (0 to 20%) in 2 mM ammonium formate (pH 6.5). Elution of normal nucleosides was monitored on-line by a UV spectrometer set at 280 nm, while oxidized nucleosides were detected by a triple quadrupolar mass spectrometer (API 3000, Sciex/Perkin Elmer, Thornhill, Canada) used in the multiple reaction monitoring mode. Negative electrospray ionization was used for the quantification of the four *cis* and *trans* diastereomers of 5,6-dihydroxy-5,6-dihydrothymidine (ThGly), 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd) and 5-formyl-2′-deoxyuridine (5-FormdUrd). The respective retention times were 3.7, 4.4, 7.9, 8.1, 15.2 and 19.9 min, respectively. 8-Oxo-7,8-dihydro-2′-deoxyadenosine (8-oxodAdo) (retention time 25 min) was detected in the positive ionization mode. Quantification was performed by external calibration.

Determination of quantum yield

The light flux of the Rayonet photoreactor was determined by using sodium 9,10-anthraquinone-2,6-disulfonate (AQDS(2,6)) actinometry at pH 14.[40] The actinometer solutions had an optical density at 330 nm of 0.1 and were degassed by the freeze–pump–thaw technique at high vacuum. The extent of reaction of the actinometer was monitored by UV spectroscopy at various time

intervals. Conversion was kept below 50%, where the extent of reaction was linear with irradiation time. The slope of a plot of extent reaction *vs.* irradiation time yielded a light flux = $(1.3 \pm 0.2) \times 10^{-7}$ Einstein min^{-1}. DNA(4) (Fig. 1) (10 μM in air-saturated phosphate buffer solution) was irradiated in the calibrated Rayonet photoreactor at *ca.* 30 °C. Aliquots were withdrawn and treated with piperidine at various time intervals. After evaporation of the piperidine, the samples were suspended in water. 2′-Deoxycytidine, for use as an internal standard, was added to each sample, and the volume was adjusted to 500 μL. These mixtures were analyzed by reverse phase HPLC on a Hitachi preparative HPLC system using a Dynamax C18 column. The extent of reaction of the DNA cleavage was monitored by the decrease in signal of the TT-containing strand and found to be linear with irradiation time.

DNA(1)	AQ 5′-TTTTAAA TTAAA TTAAA TTAAA TAT ATTT-3′ 3′-AAAATTT$_1$AATTT$_2$AATTT$_3$AATTT$_4$ATATAAA*-5′
DNA(2)	AQ 5′-TTTTAAA TTAAA TTAAA TTAAA CC TATATTT-3′ 3′-AAAATTT$_1$AATTT$_2$AATTT$_3$AATTT$_4$GGATATAAA*-5
DNA(3)	AQ 5′-TTTTAAATTAAATTAAATTAAATAT ATTT-3′ 3′-AAAAUUUAATTTAAUUUAATTTATATAAA*-5′
DNA(4)	AQ 5′-UUUUAAUUAAUUAAUUAAUAUAUUT-3′ 3′-AAAATTAATT AATT AATT ATATAAA*-5′
DNA(5)	AQ 5′-TTTTAA TTAA TTAA TTAA TATA TTT-3′ 3′-AAAATT$_1$AATT$_2$AATT$_3$AATT$_4$ATATAAA*-5′
DNA(6)	AQ 5′-TTTTAATTAA TTAATTAATATATTT-3′ 3′-AAAATUAAUTAATUAAUTATATAAA*-5
DNA(7)	AQ 5′-TTTTAATTAATTAATTAATATATTT-3′ 3′-AAAAUTAATTAATUAAUUATATAAA*-5′
DNA(8)	AQ 5′-TTTTAATTAATTAA TTAATAT ATTT-3′ 3′-AAAAUUAAUUAAUUAAUUAUAUAAA*-5′
DNA(9)	AQ 5′-TTTTAA TATAA TATAA TATAA TAT ATTT-3′ 3′-AAAATT$_1$ATATT$_2$ATATT$_3$ATATT$_4$ATATAAA*-5′
DNA(10)	AQ 5′-TTTTAA TATATAA TATATAA TATATAA TAT ATTT-3′ 3′-AAAATT$_1$ATATATT$_2$ATATATT$_3$ATATATT$_4$ATATAAA*-5′

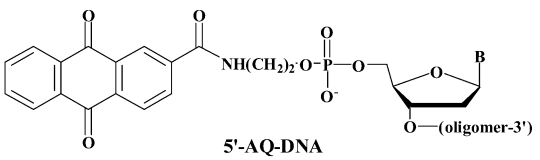


Fig. 1 Structures of DNA oligomers used in the study of long distance charge migration through adenine and thymine containing DNA duplexes. * = [^{32}P]-radiolabel.

Results

Charge migration and reaction in A/T-containing duplex DNA

We prepared the series of DNA oligonucleotides shown in Fig. 1 to probe the results of their one-electron oxidation reactions. Each of the duplexes is fully complementary and exhibits the expected melting and spectroscopic properties.[41] These duplex oligomers contain a covalently linked anthraquinone group (AQ) photosensitizer[14] and a [^{32}P]-radiolabel (indicated by "*" in Fig. 1), to permit analysis of strand cleavage by PAGE and phosphorimagery. Irradiation of the AQ group at 350 nm, where DNA does not absorb significantly, results in its electronic excitation and subsequent rapid intersystem crossing to form the triplet excited state (AQ^{*3}). Significantly, calculations from the Rehm–Weller[42] equation show that AQ^{*3} has sufficient oxidizing power to convert either A or T to their radical cations with the concomitant formation of the AQ radical anion. Although the radical cation is formed initially at the base pair adjacent to the AQ group, it is expected that it will migrate through the oligomer before being trapped by an irreversible reaction. Thus, several of the duplex oligomers examined contain regularly repeating base sequence patterns that allow the distance dependence of the reaction probability to be assessed. For this reason, the irradiation reactions are carried out to low conversion, "single-hit conditions", where, on average, each DNA molecule reacts at best once or not at all.[41] Under these conditions, the amount of strand cleavage observed is directly proportional to the probability of reaction at that site. After irradiation, the samples were either (a) treated with chemical or enzymatic reagents that cause strand cleavage to occur at damaged bases, which enables the identification and quantification of the radical cation reaction sites, or (b) the UVA-irradiated oligomers were treated with nuclease P1, phosphodiesterases and alkaline phosphatase, which digest the oligomer and enable identification of the products formed from the reactions of the nucleobase radical cations.

Radical cation reaction at thymine

The first oligomer we examined is DNA(1), which is comprised exclusively of A/T base pairs. DNA(1) was selected assuming that reaction of the radical cation would occur at adenine, because that base has much lower E_{ox} than thymine. The results of irradiation of DNA(1), its subsequent treatment with piperidine, and then PAGE analysis revealed that significant strand cleavage occurred only at thymine bases, which was an unexpected result. However, the absence of significant strand cleavage at adenine under these conditions does not demonstrate conclusively that reaction of the radical cation does not occur at these bases in DNA(1). This is because the major product expected from the reaction of an adenine radical cation in DNA, namely 8-oxo-7,8-dihydroadenine (8-oxoAde), does not readily result in strand cleavage when treated with piperidine.[43] To resolve this issue, the irradiated samples were treated with Na_2IrCl_6 before their reaction with piperidine. It has been shown that the oxidation of 8-oxoAde by Na_2IrCl_6 gives products that do result in strand cleavage on piperidine treatment.[44] The results of these experiments, shown in Fig. 2, reveal that reaction of the radical cation introduced by UVA irradiation of the covalently linked AQ occurs primarily at thymine, not at adenine; a result confirmed by analysis of the reaction products (see below). More specifically, the thymines closer to the AQ react more often, which reveals that there is a distance dependence to the reaction efficiency. Furthermore, the central and 5′-T of the TTT sequences are more reactive than the 3′-T, which shows that the site of the radical cation reaction is governed by subtle electronic or steric factors. This point will be addressed in greater detail below.

Quenching of the thymine reaction by GG steps

The surprising finding that the one-electron oxidation of DNA(1) results primarily in reaction at thymine was probed further by investigation of DNA(2), which is identical to DNA(1) except that

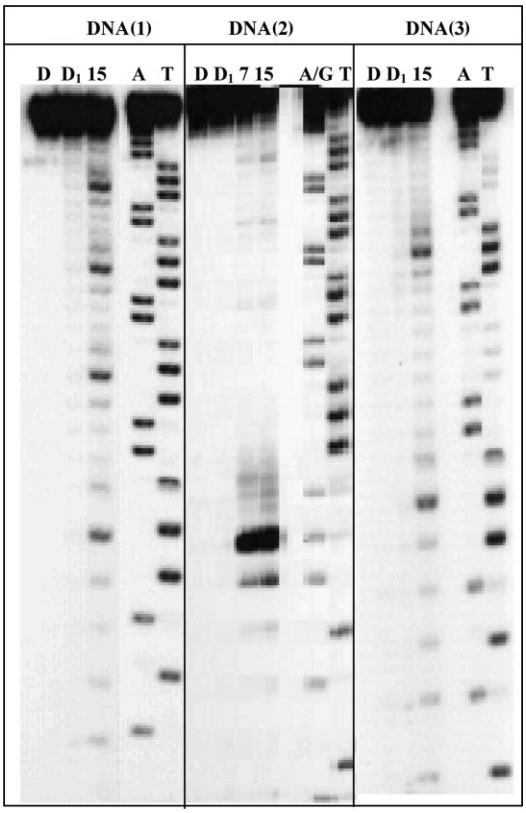


Fig. 2 Autoradiograms of DNA(1–3). D and D1 are control lanes (no UVA irradiation, and UVA irradiation but no piperidine treatment, respectively). The labels above the lanes identify the DNA oligomer and show the time of irradiation in min. Lanes labeled A/G and T are the Maxim–Gilbert sequencing lanes. The figure is a composite formed by editing a larger PAGE gel. All quantitative data were obtained by phosphorimagery of unedited gels.

it contains a single GG step. The GG step of DNA(2) is positioned 22 base pairs (*ca.* 82 Å) from the AQ group. The irradiation of DNA(2) results in quenching of the reaction at the thymines that is seen in DNA(1); instead, most of the observed strand cleavage occurs at the GG step, see Fig. 2. This result indicates that the radical cation introduced by irradiation of the AQ residue migrates reversibly through the DNA duplex and is trapped eventually in an irreversible chemical reaction at the most reactive site.[6] For DNA(1) the most reactive sites are at the thymines, for DNA(2) the most reactive site is the GG step.

Replacement of TTT by UUU

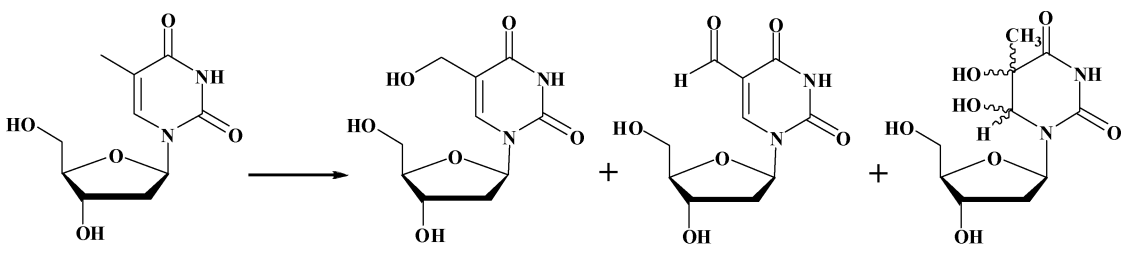
The surprising result that the one-electron oxidation of DNA(1) causes reaction at thymine was probed further by the investigation of DNA(3), which is identical to DNA(1) except that every other TTT sequence is replaced by UUU. Of course, the difference between thymine and uracil is that the latter lacks a methyl group at its C5-position. It has been previously reported that the thymidine radical cation in aqueous solution follows two paths to the formation of the three sets of oxidation products that are shown in Scheme 1.[32,38] Apparently, the thymidine radical cation can lose a proton from its C5-methyl group to form a radical that is trapped by O_2 and eventually generates, through the intermediacy of 5-(hydroperoxymethyl)-2′-deoxyuridine, 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd) and 5-formyl-2′-deoxyuridine (5-FormdUrd). Alternatively, the thymidine radical cation can be attacked in two subsequent steps by H_2O and O_2, which results eventually in formation of the four *cis* and *trans* diastereomers of 5,6-dihydroxy-5,6-dihydrothymidine (*c*- and *t*-ThdGly).

The results of irradiation of DNA(3) are also shown in Fig. 2. Just as in the case of DNA(1), there is no detectable reaction at any nucleobase other than thymine. In particular, there is no significant strand cleavage at the uracils in the UUU segments. This finding suggests that the thymine methyl group plays an important role in the reaction of the radical cation in DNA. We carried out control experiments to confirm this view.

The most common oxidation product of uracil is 5,6-dihydroxy-5,6-dihydrouracil (uracil glycol).[45] Although the reaction rates of $KMnO_4$ with T and U to produce thymine glycol and uracil glycol, respectively, are comparable,[46] we noticed in sequencing lanes that piperidine-induced strand cleavage is less efficient for U than for T. This suggested the possibility that a one-electron oxidation of uracil in DNA could be yielding uracil glycol that is not revealed as strand cleavage by the reaction with Na_2IrCl_6 and piperidine. We explored this possibility by replacing the piperidine treatment of the irradiated DNA by reaction with *E. coli* endonuclease III (Endo III), which is known to cause strand cleavage at uracil glycol.[47] The results are unchanged—the damage pattern revealed by Endo III is the same as the one revealed by piperidine. Clearly, the one electron oxidation of DNA(3) results in the reaction of radical cations at TTT but not at UUU sequences.

Quantum yield of reactions

The significance of our discovery that the reactions of radical cations in DNA comprised of A/T bases occur primarily at



Scheme 1

thymine depends on the quantum yield for these processes. We examined the reaction of DNA(4) to measure the quantum yield for loss of DNA (Φ_{-DNA}). The AQ-linked strand of DNA(4) consists only of A and U nucleobases. For this reason, cleavage of this strand is expected to be negligible. The complementary strand of DNA(4) is comprised of A and T bases. This strand has four TT steps that are separated by AA steps. The one-electron oxidation of DNA with this sequence results in detectable strand cleavage only at the TT steps (see below).

Optically matched samples of DNA(4) and an anthraquinone-2,6-disulfonate actinometer[40] were irradiated at 350 nm for 6 min in a Rayonet photoreactor. The irradiated DNA samples were treated with piperidine, to cause strand cleavage, and then subjected to HPLC analysis to determine the amount of intact A/T strand remaining. The irradiation reaction was carried out to low conversion to minimize errors that would result from the damage to more than one thymine on each DNA molecule. Such over-irradiation would lead to an underestimation of the quantum yield. This experiment indicates that for DNA(4), $\Phi_{-DNA} = (2.1 \pm 0.2)\%$. This value is similar to that obtained for the AQ-sensitized oxidation of DNA containing GG steps,[48] which shows that the reaction of thymine radical cations leading to strand cleavage is not a particularly rare event for DNA oligomers that do not contain guanines.

Quenching of strand cleavage at thymines by glutathione

The reaction of thymine in these oligomers is initiated by the one-electron oxidation of a nucleobase to form a radical cation. The participation of the thymine methyl group in this process is implied by the result obtained when UUU is substituted for TTT. Previous reports indicate that the thymidine radical cation in aqueous solution loses a proton from the methyl group to form a 5-(2′-deoxyuridinyl)methyl radical, which is trapped by molecular oxygen in a subsequent step.[38,49] The radical formed by proton loss from the thymine methyl group may also play a role in its reaction in DNA. We examined the effect of glutathione (GSH), a tripeptide having a cysteine residue that is known to be a free radical scavenger,[50,51] on the strand cleavage that results from irradiation of an AQ-linked oligomer.

As previously noted, the [^{32}P]-labeled strand of DNA(2) contains four TTT sequences separated by AA steps and a GG step following the last TTT sequence. This oligomer was used to differentiate between possible quenching of a precursor to a thymine radical by GSH, which would also quench the reaction at the GG step, and the quenching of a thymine-based radical itself. In the absence of GSH, irradiation of DNA(2) results in some strand cleavage at the TTT sequences, but the major reaction occurs at the GG step; see Fig. 2. Addition of GSH (up to 5 mM) to solutions of DNA(2) before irradiation results in reduction in the amount of strand cleavage detected at the thymines without meaningfully affecting the reaction at the GG step.[52] This result indicates that a thymine radical, not a precursor to the thymine radical that also results in strand cleavage at the GG step, is quenched by GSH.

DNA(5) contains four TT steps in the labeled strand. Its irradiation and subsequent treatment with Na_2IrCl_6 and piperidine results in strand cleavage at each of the TT steps. The addition of GSH to solutions of DNA(2) before irradiation results in a systematic reduction in the amount of strand cleavage. These findings are presented as a histogram of strand cleavage yield in Fig. 3. It should be noted that inhibition of strand cleavage by GSH suggests that whatever product is formed in its reaction with a thymine radical does not result in DNA strand cleavage at that site upon reaction with hot piperidine even after treatment with Na_2IrCl_6. Finally, it should also be noted that control experiments show that GSH does not itself inhibit piperidine-induced strand cleavage at damaged thymines. Significantly, a plot of the reciprocal of total strand cleavage yield at the TT sequences of DNA(5) against GSH concentration is non-linear. This suggests that GSH is quenching more than one radical intermediate that leads, eventually, to strand cleavage at T. These findings support the reaction mechanism for this process, suggested below.

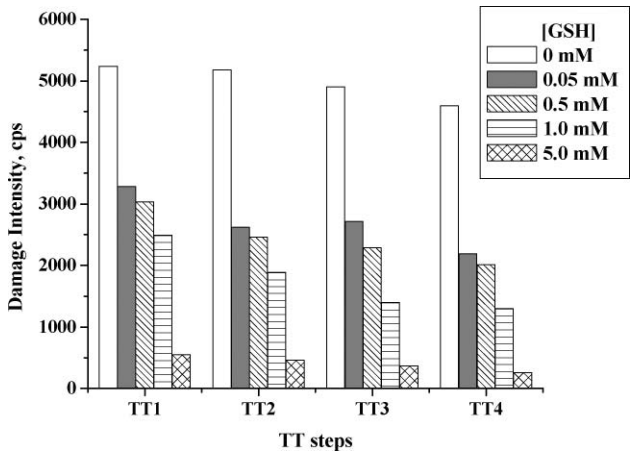


Fig. 3 Histogram showing the effect of glutathione (GSH) on thymine damage of DNA(5). The relative amount of strand cleavage (counts per second, cps) observed by phosphorimagery at the four TT sequences as a function of glutathione concentration, as is indicated.

The complex effect of UT and TU steps

The experimental examination of the one-electron oxidation of DNA leading to strand cleavage at thymine described thus far reveals a process that proceeds through at least two trappable radical intermediates. To further probe the nature and identity of these intermediates, we investigated a set of DNA oligomers, DNA(6) and DNA(7), that contain TT steps with uracils in place of thymines at strategic locations. The pattern of strand cleavage that emerges in these experiments reveals important details about the reactive intermediates and subtle insight into the structural control of the reaction mechanism.

The [^{32}P]-labeled strand of DNA(5) contains four TT steps each separated by two adenine nucleobases. For comparison, DNA(6) is identical with DNA(5) except that the TT steps are replaced by two sets of alternating TU and UT steps, and DNA(7) has, starting from the 3′-end, UT, TT, TU and UU steps in place of TT steps. These oligomers were irradiated, then treated with Na_2IrCl_6 and piperidine, and subjected to PAGE analysis. The results are shown in Fig. 4.

As expected, strand cleavage is detected at each of the four TT steps of DNA(5), and no strand cleavage is observed at the UU step of DNA(7). But, surprisingly, strand cleavage in DNA(6) and DNA(7) is strongly dependent on the order of nucleobases in the

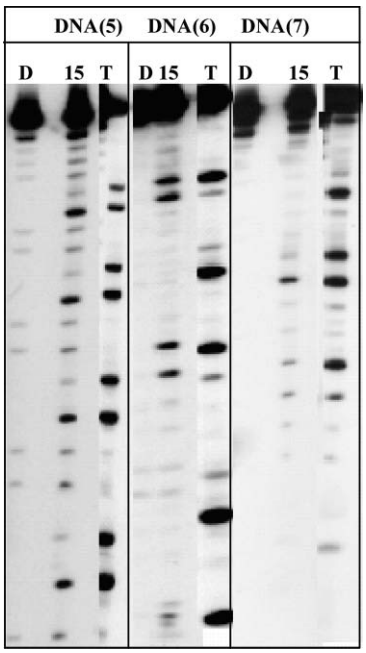


Fig. 4 Autoradiograms of DNA(5–7) showing the complex effect of TU and UT steps. D and 15 represent 0 and 15 min of irradiation, respectively. T indicates the thymidine-sequencing lanes. The figure is a composite formed by editing a larger PAGE gel. All quantitative data were obtained by phosphorimagery of unedited gels.

UT and TU steps. Both the bases are damaged in a 3′-TU-5′ steps but neither reacts in 3′-UT-5′ steps. The same pattern is observed in both oligomers DNA(6) and DNA(7). Evidently, the reactions of the thymine radical cation that lead, eventually, to strand cleavage depend not only on the presence of the thymine methyl groups but also upon the precise placement of those groups. Substitution of a uracil for the T at 5′-side of a TT step has only a modest effect on the relative amount of strand cleavage observed, but replacement of the 3′-T with U nearly completely inhibits reaction at both bases at that step. This finding indicates that the process leading to reaction of thymine radical cation and strand cleavage at TT steps, at least in part, involves two adjacent nucleotides in the DNA duplex—a process that has been referred to as a tandem reaction.[38,53]

Identification of the products formed from the reaction of T radical cation in duplex DNA

A key to understanding the nature of the reactions of thymine radical cation in duplex DNA is to identify the products that are formed in this process. The results reported thus far indicate that at least one of the reaction products (a damaged nucleobase) initiates strand cleavage when treated with piperidine. Since it is known that thymidine glycols and 5-formyl-2′-deoxyuridine are alkali-labile lesions likely to lead to strand cleavage,[53–57] further investigation was required to identify the precise oxidation products that are formed. We carried out a series of HPLC–MS/MS experiments to identify and to quantify the products that result from the one-electron oxidation of duplex DNA oligomers that contain no guanine nucleobases.

The AQ-linked strand of DNA(4) contains only A and U bases, and its complementary strand has four equally spaced TT steps. Experiments with radiolabeled versions of DNA(4) show, as expected, that no strand cleavage occurs in the AQ-linked strand and that the expected strand cleavage, after piperidine treatment, is seen at the TT steps of the complementary strand. Unlabeled DNA(4) was irradiated for various times under the standard conditions; see Fig. 5. Rather than the usual piperidine treatment after irradiation, these samples were treated with nuclease P1, phosphodiesterases II and I and alkaline phosphatase, and the resulting nucleosides were separated by means of HPLC. A typical HPLC profile is shown in Fig. 6. The four product peaks were identified mass spectroscopically by comparison with authentic samples[58] to be *cis*-thymidine glycol (*c*-ThdGly), *trans*-thymidine glycol (*t*-ThdGly), 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd) and 5-formyl-2′-deoxyuridine (5-FormdUrd). Significantly, only a small amount of 8-oxodAdo is detected in these experiments (see Fig. 5). This finding confirms the conclusion drawn from the strand cleavage results that reaction occurs primarily at T in the A/T base pairs.

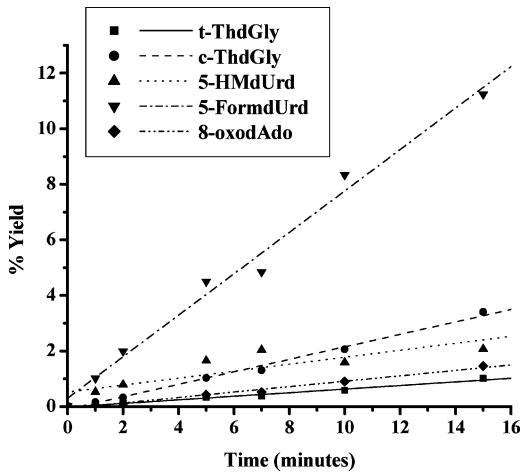


Fig. 5 The yield of different thymidine and 2′-deoxyadenosine oxidation products relative to the amount of unreacted thymidine as a function of the irradiation time.

The products of thymine oxidation resulting from the one-electron oxidation of DNA(4) fall clearly into two sets. The first set, *c*- and *t*-ThdGly, results from the oxidation of the thymine 5,6-double bond, and the two products 5-HMdUrd and 5-FomdUrd, result from reaction at the thymine methyl group. To distinguish between primary and eventual secondary oxidation, the yield of each of these products (relative to thymidine) was determined as a function of the extent of reaction. The results (Fig. 5) show that the product ratios are essentially invariant with time and that the yield of all products extrapolates to zero at zero irradiation time. This finding shows that these oxidized 2′-deoxyribonucleosides are primary thymidine oxidation products, each being formed from reaction originating from either hydration of the thymine radical cation or addition of molecular oxygen to the related deprotonated methyl centered radical. The major product is 5-FormdUrd, which is formed in 63% yield, and the yields of 5-HMdUrd, *cis*-ThdGly and *trans*-ThdGly are 13, 20, and 4%, respectively. The identification of these products and their quantification in relative yields help to identify the mechanism for the one-electron

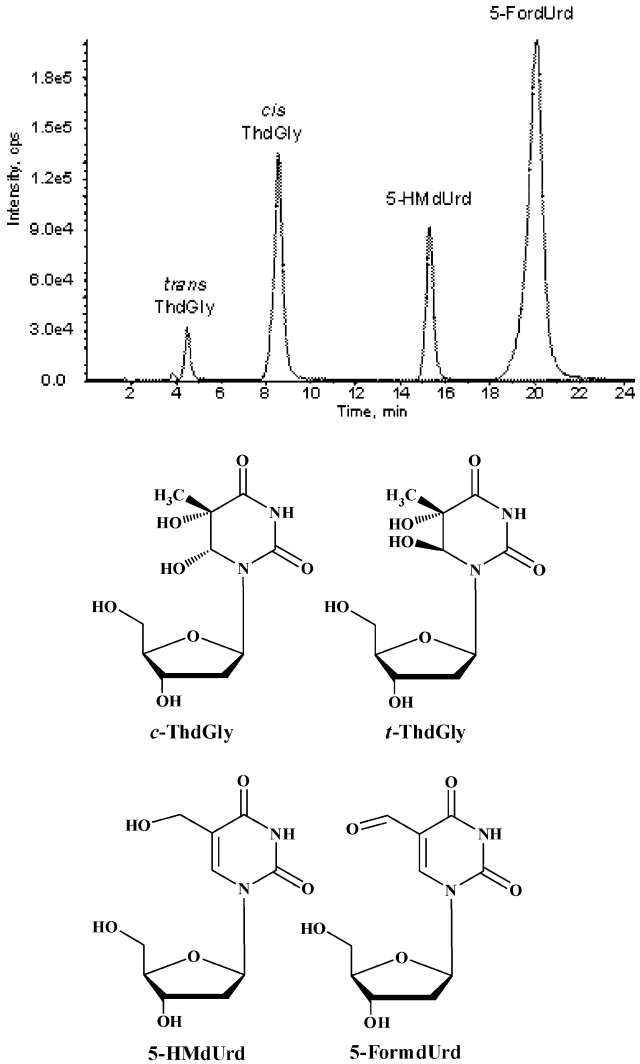


Fig. 6 HPLC trace and structures of the four thymidine oxidation products identified by HPLC–MS/MS analysis.

oxidation of thymine in duplex DNA. This topic is discussed in detail below.

Crosslinking of DNA by reaction of 5-(uracilyl)methyl radical

The formation of 5-FormdUrd and 5-HMdUrd implicates formation of the thymine methyl radical from the initial deprotonation reaction of thymine radical cation in duplex DNA.[49] It is easily seen how this radical could react with O_2 and lead naturally to the formation of 5-HMdUrd and 5-FormdUrd.[59,60] It has been reported that a specially formed thymine methyl radical reacts in DNA with its paired adenine to form a crosslink between the two DNA strands.[61,62] We searched for evidence of crosslinking in the one-electron oxidation of DNA(5) to gain additional support for the intermediacy of the thymine methyl radical in this process.

The covalently linked AQ group of DNA(5), which contains only A/T nucleobases, was irradiated in the usual manner. However, to preserve crosslinks that might be susceptible to cleavage, these samples were analyzed both with and without treatment with Na_2IrCl_6 and piperidine (see Fig. 7). There is no evidence of detectable crosslink formation in the electrophoretic

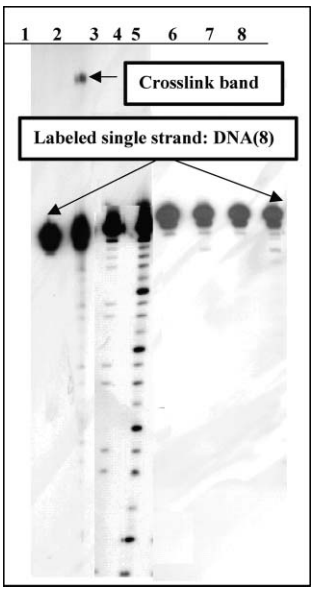


Fig. 7 Autoradiogram showing crosslinking in the UVA-irradiation of DNA(5) (lanes 1–4) and DNA(8) (lanes 5–8). Samples in lanes 1, 2, 5 and 6 were not treated with Na_2IrCl_6 or piperidine, while the samples in lanes 3, 4, 7 and 8 have been treated with those reagents. Lanes 2, 4, 6 and 8 have been irradiated for 20 min and lanes 1, 3, 5 and 7 are the corresponding dark controls. The figure is a composite formed by editing a larger PAGE gel. All quantitative data were obtained by phosphorimagery of unedited gels.

gels of samples irradiated for a few min when the autoradiogram is exposed for the standard amount of time in either samples treated with Na_2IrCl_6 and piperidine or in untreated samples. However, for samples of DNA(5) irradiated for 20 min and with long exposure of the gel to photographic film, weak crosslink bands are detected in the samples untreated with Na_2IrCl_6 and piperidine. Although precise quantification of the crosslink yield is not possible with these experiments, it is clearly very low. No crosslinking is visible with a sequence consisting of only A and U nucleobases on the labeled strand, DNA (8). These observations assist the creation of a proposed mechanism for the reaction of thymine radical cation in DNA.

Distance dependence of thymine damage and strand cleavage

One of the hallmarks of the long-distance oxidation of normal, guanine-containing DNA is that the amount of strand cleavage at a particular guanine is sequence dependent and characteristically decreases exponentially with the distance between the site of initial oxidation (at the AQ group, for example) and a particular guanine.[6,63] This behavior has been interpreted in terms of the phonon-assisted polaron-hopping model.[1,6,63,64]

We investigated three DNA oligomers, DNA(5,9,10), containing a uniform set of TT steps separated by AA, ATA, or ATATA sequences, respectively. These experiments reveal a familiar exponential distance dependence for the reaction of thymine radical cations in duplex DNA. The data from analyses of high resolution PAGE gels for these experiments are shown in the form of semilog plots in Fig. 8. These experiments show that as the distance from the AQ charge injector to the TT increases, the amount of strand cleavage observed at that step decreases. In each case, the semilog plot of strand cleavage yield with distance is linear, but the slope

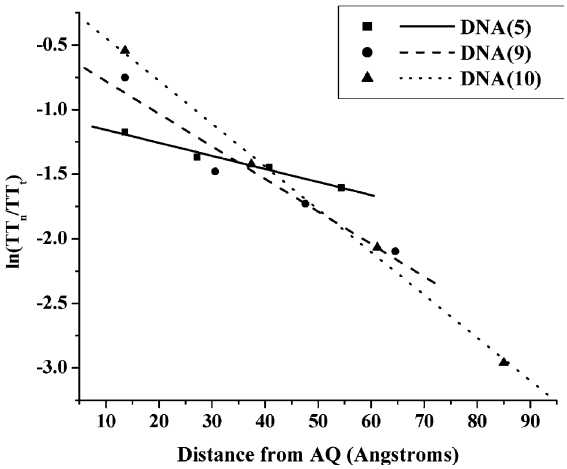


Fig. 8 Semilog plots of strand cleavage at a particular TT step (TT_n) divided by the total strand cleavage observed at all TT steps (TT_t) for DNA(5), DNA(9) and DNA(10) as a function of the distance of each TT step from the covalently-linked AQ (assuming 3.4 Å per base pair).

of the plot is dependent upon the sequence of bases separating the TT steps. The magnitude of the slope in such a plot is linked to the relative rate constants for radical cation hopping (k_{hop}) and irreversible trapping (k_{trap}) by its reaction with H_2O or O_2.[64] These findings reveal that hopping and reaction of the radical cation at thymine exhibit fundamentally the same pattern as the hopping and reaction at guanine-containing sites in duplex DNA.

Discussion

The experimental results reported above reveal surprising and interesting features about the one-electron oxidation of DNA oligomers that do not contain guanine or whose guanine nucleobases are far removed from the site of initial oxidation. First, reaction occurs at thymine despite the fact that it has the highest oxidation potential of the four DNA nucleobases. This observation contradicts the long-held view that one-electron oxidation occurs at guanine because it is the base with the lowest E_{ox}. Clearly, other factors are at play in determining the site of reaction, and these will be considered in detail below. Second, the reaction of the thymine radical cation in DNA leads to a set of products that reveal a complex reaction mechanism involving proton loss from the methyl group of the thymine radical cation, specific water addition across the thymine radical cation 5,6-double bond at C6 and a delicately balanced interplay of distance and steric interactions between adjacent nucleotides. DNA is a complex molecule, and the reactions of the thymine radical cation in DNA reflect this complexity. However, the experiments reported here lead to the proposal of a reaction mechanism that accommodates the product data, the glutathione quenching results, and the complex consequences of uracil substitution for thymine in TT steps. This mechanism has important implications for the oxidative reactions of genomic DNA.[65] Finally, the experiments on the distance dependence of thymine damage underscore the emergent nature of long-distance charge transport in DNA.[6] In certain sequences, runs of A/T base pairs are barriers to radical cation migration. However, in sequences that contain no guanines (which are low-energy radical cation traps), hopping through these A/T sequences occurs very efficiently.

The surprising reactions of thymine radical cation in DNA

DNA can be oxidized by ionizing radiation,[12] photochemically,[11] or chemically with a variety of reagents.[66,67] Independent of the means of introducing a radical cation into DNA, it resides primarily on the nucleobases[7] and it is trapped by reaction with H_2O or O_2[10,68] at guanines,[6] or more often at G_n sites. The reason usually offered to explain this G-selective reaction based upon relative E_{ox} seems obvious, but it is incomplete. It is certainly true that guanines in DNA have the lowest E_{ox} of the four DNA bases, and it seems certain that the E_{ox} of GG and GGG sequences is somewhat lower than that of an "isolated" G (one without a neighboring guanine).[69] It is for these reasons that it has been concluded that radical cations hopping through DNA pause briefly at guanines where they are more "stable" due to the low E_{ox}, and, consequently, it is at guanine that they are irreversibly trapped.

In oligomers containing only A/T base pairs the radical cation must reside primarily on either an adenine or a thymine. The difference in E_{ox} between A and T measured in acetonitrile solution is 0.15 V.[35] The E_{ox} of these nucleobases will certainly be affected by their incorporation into DNA and by changes in solvation, but it seems extraordinarily unlikely that the equilibrium E_{ox} of T will shift to be less than A. Thus, based simply upon the experimentally determined E_{ox} of A and T in solution, the population of the thymine radical cation at equilibrium is expected to be less than 1% of that for the adenine radical cation. Thymine is not the most stable site for the radical cation, but evidently it is the most reactive.

It has been understood for more than 50 years[37] that product yields are not necessarily correlated with the stability of precursor reactive intermediates. This fact is enshrined in the Curtin–Hammett principle, and this principle bears additional discussion in the context of the reactions of thymine radical cations in DNA. The Curtin–Hammett principle states that, for reactions passing rapidly and reversibly through more than one reactive intermediate each leading to a different product, the ratio of products depends on the difference in the free energy of the transition states leading to each product, not specifically on the relative energies of the intermediates.

The Curtin–Hammett principle was formulated to explain the reactivity of molecules that exist in two interconverting forms (conformers), each of which gives a different product.[70] In the current context, the reactive intermediates of the Curtin–Hammett principle can be considered to be the nucleobase radical cations (or radical cations delocalized as a polaron over several bases)[71] that hop rapidly and reversibly from site to site in duplex DNA. The products, of course, are the familiar damaged nucleosides resulting from trapping of the radical cation; among these are 8-oxo-7,8-dihydro-2′-deoxyguanosine, 8-oxodAdo, the thymidine oxidation products identified above, and others.[12,70,72] The key concept of the Curtin–Hammett principle is that the relative abundances of the intermediates (determined by the relative E_{ox} of the nucleobases in the current case) cannot be used to predict the ratio of products formed. The relative yield of products is determined by the difference in free energies of the transition states ($\Delta\Delta G^{\ddagger}$) leading to their formation. In DNA that contains guanine, the energy of the transition state ($\Delta G^{\ddagger}$) for its reaction with H_2O or O_2 evidently is lower than that for the reaction of any other base radical cation. For DNA that contains only A/T base pairs, $\Delta G^{\ddagger}$ for reaction at the thymine radical cation is lower than $\Delta G^{\ddagger}$ for reaction at the

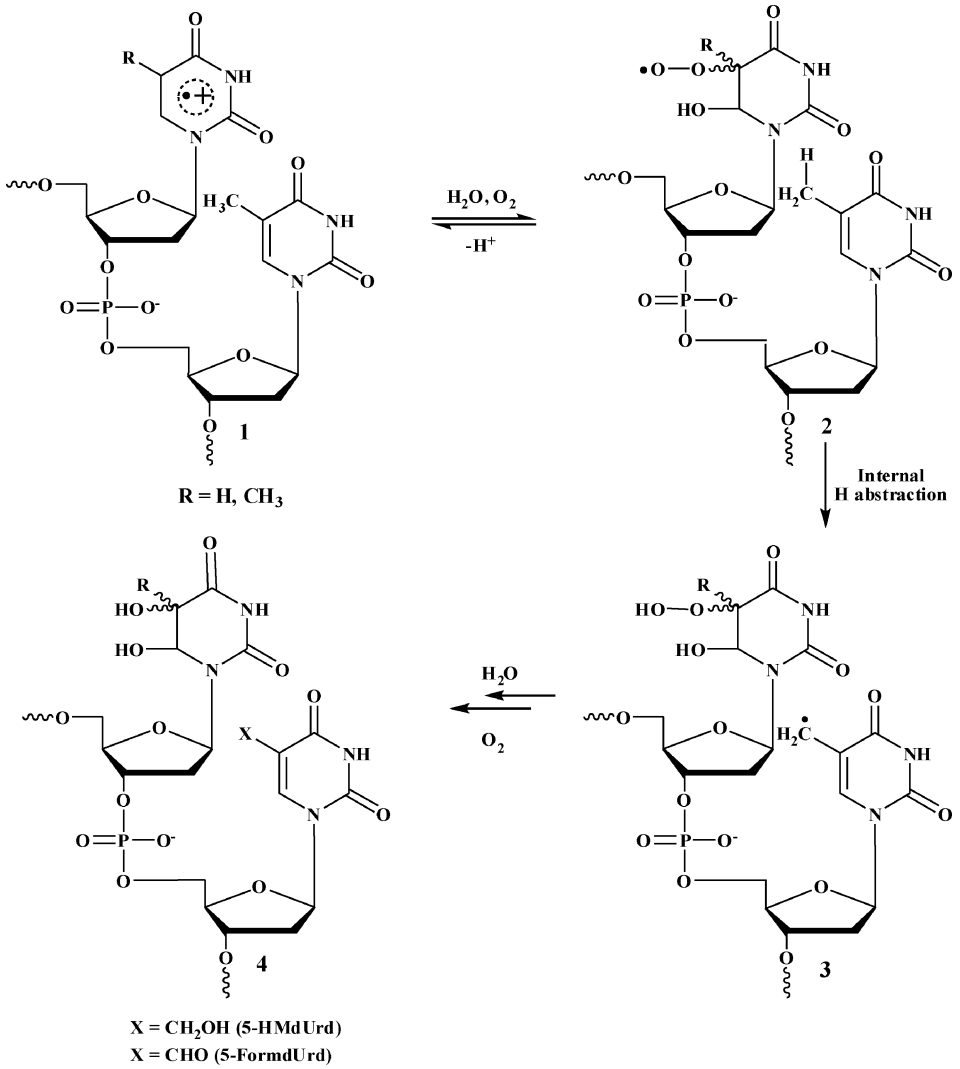


Fig. 9 Possible mechanism for the tandem formation of T-damaged products at the 3′- and 5′-thymines of a TT step starting with the radical cation localized on the 5′-T.

adenine radical cation. Thus despite the fact that A has a much lower E_{ox} than T, very little 8-oxodAdo is formed and nearly all of the products arise from reactions of the thymine radical cation.

At the pH of the experiments reported here, DNA is a polyanion that exists in a continually fluctuating solvation (H_2O) and counter-ion (Na^+) environment. It has been found that the instantaneous positions of the Na^+ ions and solvent molecules have a very large effect on the energy and the localization of radical cations in DNA.[9] The Curtin–Hammett principle explains the composition of product mixtures for reactions that proceed through rapidly equilibrating intermediate conformers. In the current context, it is the hopping of the radical cation (polaron) from one site to the next, driven by motions of H_2O and Na^+ ions, that corresponds to the classical equilibration of conformers. At equilibrium, the fraction of the radical cation that resides on thymines must be very small. But it is not the composition of this equilibrium mixture that determines the product yield, it is $\Delta\Delta G^{\ddagger}$. In the case of reaction of the guanine radical cation with H_2O in DNA, it has been found that the $\Delta G^{\ddagger}$ is controlled by solvation and counter-ion association, and that the product is stabilized by transfer of a proton through water by a Grotthaus[73] mechanism to a nearby phosphate group.[7] It is likely that similar considerations apply to the reaction of thymine radical cation in DNA. That is, there are configurations of solvent and counter-ions that cause the activation free energy for reaction of the thymine radical cation to be lower than that for the reaction of the adenine radical cation. And as a consequence, the vast majority of the products formed come from reaction of the thymine radical cation.

A mechanism for reaction of the thymine radical cations in DNA

The results reported above show that the one-electron oxidation of DNA containing only A/T base pairs proceeds through intermediate free radicals that can be trapped with GSH and give products resulting from reactions at the methyl group and the 5,6-double bond of thymine.[74] Also, for 3′-TT-5′ segments, substitution of U for T at one base can affect reaction at the other, which points to the operation, at least in part, of a tandem reaction mechanism. Based on these findings, we developed a set of plausible mechanistic pathways, which are shown in Fig. 9 and 10. In both cases, the pathways shown commence after the migrating

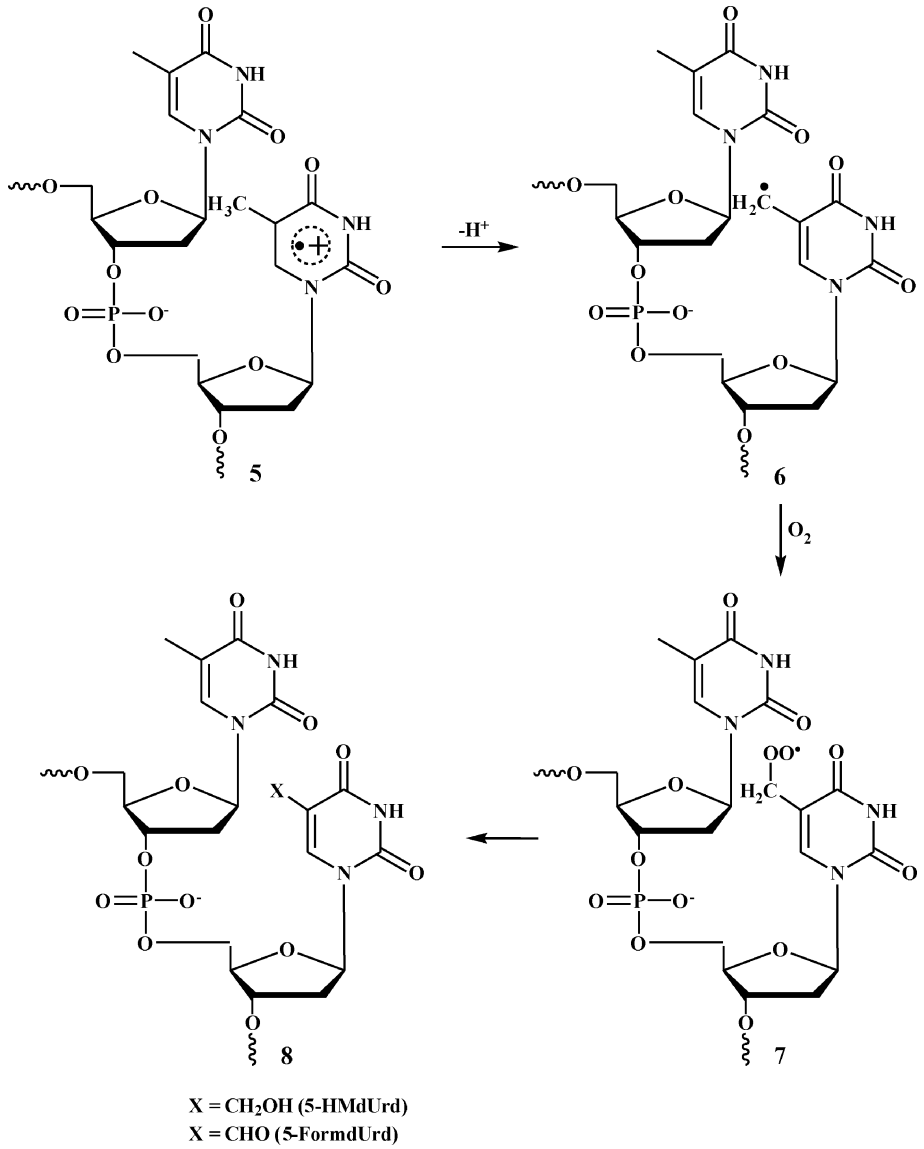


Fig. 10 Possible mechanism for the (tandem?) formation of T-damaged products at the 3′- and 5′-thymines of a TT step starting with the radical cation localized on the 3′-T.

radical cation has been "localized" at a particular thymine by some configuration of solvent molecules and counter-ions.

Consider first the case of a TT step where the radical cation is localized on the 5′-T, as in **1** in Fig. 9 with R = CH_3. A possible reaction of this intermediate is the loss of a proton to form a methyl radical (see **6** in Fig. 10 for a similar structure). Another possibility is that the 5′-T radical cation is trapped by the reversible addition of H_2O (addition of H_2O may be reversible but that of O_2 is not) and O_2 across its 5,6-double bond, which will give an intermediate 6-hydroxy-5,6-dihydrothymidine-5-peroxy radical (**2** in Fig. 9). In this case, our findings suggest that addition across the 5,6-double bond is more likely to lead to strand cleavage than is the loss of a proton from the methyl group of this thymine radical cation. This view is supported by the observation that replacement of the 3′-T by U in TT steps inhibits strand cleavage at both the 3′-U and at the 5′-T. As shown in Fig. 9, the peroxy radical **2** formed by addition across the double bond may abstract a hydrogen atom from the C5-methyl group of the adjacent 3′-T to form a methyl radical, which is shown as **3** in Fig. 9. Of course, this methyl radical can be trapped by O_2, leading eventually to the formation of 5-HMdUrd or 5-FormdUrd. The net result of this sequence of reactions is the conversion of the 5′-T to thymidine glycols and, by a tandem reaction, the 3′-T is converted to the coupled products 5-HMdUrd or 5-FormdUrd. This sequence of reactions accounts for the observation that no strand cleavage is observed at either base when there is a U in place of the 3′-T of a TT step. Clearly, there is no methyl hydrogen atom on the uracil for the 6-hydroxy-5,6-dihydrothymidine-5-peroxy radical (**2**) to abstract. Thus, in 3′-UT-5′ steps, reversibly formed peroxy radical **2** either simply reverts eventually to the radical cation, where it is annihilated to reform thymine, or it gives an undetected product that does not lead to strand cleavage.

To be valid, this sequence of reactions must also account for the observation that strand cleavage occurs at both U and T when a TT step is replaced by 3′-TU-5′. Again, we begin by first considering the case where the radical cation is localized on the 5′-U, (**1** in

Fig. 9 with R = H). Of course, there is no methyl proton to lose and thus the only likely reaction is addition of H_2O/O_2 to form the 6-hydroxy-5,6-dihydrouracil-5-peroxy radical, shown as **2** in Fig. 9 with R = H. The peroxy radical thus formed can abstract a C5-methyl hydrogen atom from the adjacent 3′-T, and this tandem reaction will yield 2′-deoxyuridine glycols from the 5′-U and the coupled products 5-HMdUrd or 5-FormdUrd from the 3′-T. These products are expected to result in strand cleavage upon hot piperidine treatment at both the nucleobases in the 3′-TU-5′ sequence, as is observed experimentally.

The migrating radical cation may also localize on the 3′-T of the TT steps; the sequence of reactions we propose must also fit the experimental observations for this possibility. Just as in the previous case, the 3′-T radical cation, **5** in Fig. 10, may react by losing a proton from its methyl group or by the reversible addition of H_2O/O_2 across the double bond. The latter reaction generates a peroxy radical that is related to **2** of Fig. 9. Molecular modeling studies[75] on B-form DNA show that this peroxy radical generated at the 5′-T is 2.9 Å from the C5-methyl hydrogen of 3′-T; putting it within reaction distance. However, these calculations reveal that for the peroxy radical generated at the 3′-T, the C5-methyl hydrogen of the 5′-T is 4.2 Å away; a distance sufficiently great to render the hydrogen abstraction reaction unlikely. Thus in this view, addition across the double bond of the 3′-T radical cation does not lead to a tandem reaction or to observable strand cleavage. However, the radical formed by loss of a proton from the 3′-T radical cation, **6**, may react with O_2 to form the 5-methylperoxy radical **7** and eventually the coupled products 5-HMdUrd or 5-FormdUrd, as is shown in Fig. 10. This analysis indicates that no strand cleavage at either T of the TT step is expected when the 3′-T is replaced by U and the radical cation is localized at the 3′-position, because formation of the 5-methylperoxy radical is not possible. This is consistent with the experimental observations.

The observation that the central T in 3′-TTT-5′ segments of DNA(2) are damaged most heavily is consistent with the proposed mechanism. The radical cation at the central T is expected to participate in a tandem reaction with the 3′-T to generate piperidine-labile thymine glycol residues. Also, when the radical cation is localized at the 5′-T, it may similarly participate in a tandem reaction generating piperidine-labile 5′-HMdUrd or 5-FormdUrd, again, at the central T. Thus there are two reaction routes that lead to strand cleavage at the central T of TTT segments.

The proposed reaction sequences give two sets of products: glycols arising from water addition to the thymine 5,6-double bond, and 5-HMdUrd or 5-FormdUrd that arise from reaction of molecular oxygen to the 5-(2′-deoxyuridinyl)methyl radical. The reactions outlined in Fig. 9 produce both sets of products in a 1 : 1 ratio by a tandem process. The reactions outlined in Fig. 10 produce only 5-HMdUrd or 5-FormdUrd. Experimentally, we observe that these two sets are formed in a *ca.* 5 : 1 ratio in favor of 5-HMdUrd and 5-FormdUrd. This suggests that proton loss from the thymine radical cation in DNA leads to observable products about four times more frequently than does addition to the 5,6-double bond.

Finally, it has been reported[61,62] that interstrand crosslinking between thymine and its paired adenine is observed when a 5-(2′-deoxyuridinyl) methyl radical (**6** of Fig. 10) is directly generated in DNA by a photochemical reaction. Crosslinking is observed from the one-electron oxidation of DNA oligomers comprised only of A/T bases, which we postulate involves this radical intermediate.

DNA is a complex molecule, and this complexity is reflected in the reactions described here. The mechanisms postulated in Fig. 9 and 10 account for the experimental results, but they are certainly incomplete. For example, the explanation offered for the effect of substituting U for T of TT steps takes account only of the missing methyl group. We have recently shown in the reaction of GG steps[76] that nucleobase substitution at adjacent locations affects local solvation and the controlling steric environment, which control the reaction outcome. These effects may also play a role in the reactions discussed here. We are continuing to test the mechanistic hypothesis offered here.

Long-distance radical cation migration in A/T DNA

The mechanism of long-distance charge migration has been under intensive investigation since the now-discredited claim was made that DNA is a "molecular wire".[71,77] These studies resulted in the development of the phonon-assisted polaron hopping model for long-distance radical cation transport in DNA.[71,78] Briefly, radical cations are localized over a few adjacent bases by small distortions of the DNA structure and by stabilizing changes to the solvation environment.[7] This self-trapped radical cation is referred to as a polaron and the magnitude of the stabilization is referred to as its binding energy.[79] The polaron hops from site to site when thermal motions (phonons) provide sufficient activation energy to overcome their binding. The primary driver for polaron hopping is the motions of the Na^+ counter-ions.[9]

In those cases where the DNA has a regularly repeating pattern of nucleobases, the distance dependence of radical cation reaction efficiency is controlled by two parameters: k_{hop}, and k_{trap}.[64] In these cases, linear semilog plots of reaction efficiency (strand cleavage yield) with distance from the site of initial oxidation are observed, and the slope of that line is determined by k_{ratio}, which is defined as the ratio of k_{hop} to k_{trap}. More generally, the distance dependence of radical cation reactivity in DNA with non-repetitive nucleobase sequences can be understood only by considering the set and sequence of bases in the entire oligonucleotide. In these cases, linear semilog plots are typically not observed and the distance dependence of reactivity emerges by consideration of the interactions of all nucleobases of the DNA oligomer among themselves.[6]

The DNA oligomers examined in this work fall into the first linear distance dependence category. The data, shown in Fig. 8, for DNA(5,9,10) reveal linear semilog plots for these oligomers where four TT steps are separated by AA, ATA and ATATA sequences, respectively. The slopes of the lines for DNA(5,9,10) are -0.009 ± 0.001, -0.02 ± 0.004, and -0.03 ± 0.001 $Å^{-1}$, and the derived values for k_{ratio} are *ca.* 20, 10, and 3, respectively. For each of these oligomers, the rate of hopping is somewhat faster than the rate of the irreversible trapping reactions. This behavior parallels precisely that observed for numerous DNA oligomers that contain regularly spaced guanines, and this fact confirms the central role played by nucleobase radical cations in the reactions at thymines that lead to strand cleavage upon hot piperidine treatment.

Conclusions

The irradiation of AQ-linked DNA oligomers with UVA light absorbed by the anthraquinone leads to the one-electron oxidation of DNA with the concomitant formation of a radical cation that is localized on the nucleobases. The radical cation hops long distances by the phonon-assisted polaron-hopping mechanism and it is trapped irreversibly by reaction with H_2O and O_2 at the most reactive sites. In oligonucleotides that contain guanine, the most reactive sites are the guanines or G_n steps. Coincidentally, guanines (G_n steps) are also the sites having the lowest E_{ox}. In contrast, for DNA oligomers that are comprised only of A/T base pairs, the irreversible trapping reactions occur at T, not at A. Adenine has a much lower E_{ox} than thymine and for that reason the radical cation is more stable at A than at T, but evidently the T radical cation is more reactive than the A radical cation and, as is explained by the Curtin–Hammett principle, the major products observed are those that come from the most reactive site.

The products formed from the reaction of the thymine radical cation in DNA fall into two categories: those resulting from deprotonation of the methyl group, and those resulting from hydration at the 5,6-double bond. The proposed mechanism for formation of these products begins from an orientation of solvent and counter-ions that localizes the radical cation on a particular thymine and enables its reaction. That thymine radical cation in a TT step may either lose a proton from its methyl group or H_2O/O_2 may add across its double bond. In the latter case, this addition may initiate a tandem reaction that converts both thymines of the TT step to oxidation products. However, the major products observed originate with proton loss from the methyl group to form a methyl radical that is subsequently trapped by reaction with O_2.

The findings reported here may have important implications for the oxidative reactions of genomic DNA where there are long stretches of base pairs that contain no or few guanines. In these circumstances, oxidatively induced damage is expected to generate lesions at thymines.

Acknowledgements

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