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Oxidation of DNA: Damage to Nucleobases

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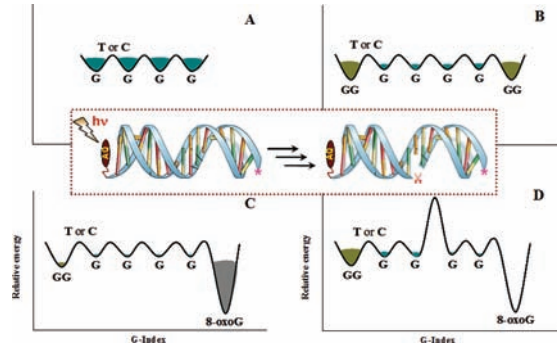
CONSPECTUS

All organisms store the information necessary to maintain life in their DNA. Any process that damages DNA, causing a loss or corruption of that information, jeopardizes the viability of the organism. One-electron oxidation is such a process. In this Account, we address three of the central features of one-electron oxidation of DNA: (i) the migration of the radical cation away from the site of its formation; (ii) the electronic and structural factors that determine the nucleobases at which irreversible reactions most readily occur; (iii) the mechanism of reaction for nucleobase radical cations.



The loss of an electron (ionization) from DNA generates an electron "hole" (a radical cation), located most often on its nucleobases, that migrates reversibly through duplex DNA by hopping until it is trapped in an irreversible chemical reaction. The particular sequence of nucleobases in a DNA oligomer determines both the efficiency of hopping and the specific location and nature of the damaging chemical reaction. In aqueous solution, DNA is a polyanion because of the negative charge carried by its phosphate groups. Counterions to the phosphate groups (typically Na^+) play an important role in facilitating both hopping and the eventual reaction of the radical cation with H_2O. Irreversible reaction of a radical cation with H_2O in duplex DNA occurs preferentially at the most reactive site. In normal DNA, comprising the four common DNA nucleobases G, C, A, and T, reaction occurs most commonly at a guanine, resulting in its conversion primarily to 8-oxo-7,8-dihydroguanine (8-OxoG). Both electronic and steric effects control the outcome of this process. If the DNA oligomer does not contain a suitable guanine, then reaction of the radical cation occurs at the thymine of a TT step, primarily by a tandem process.

The oxidative damage of DNA is a complex process, influenced by charge transport and reactions that are controlled by a combination of enthalpic, entropic, steric, and compositional factors. These processes occur over a broad distribution of energies, times, and spatial scales. The emergence of a complete picture of DNA oxidation will require additional exploration of the structural, kinetic, and dynamic properties of DNA, but this Account offers insight into key elements of this challenge.

Chemical reactions whose outcome alters the DNA of living systems play a major role in processes such as mutagenesis, carcinogenesis, and aging.[1−3] Aerobic organisms are subject to oxidizing conditions resulting from normal metabolism or from exposure to light or ionizing radiation. Under these conditions, DNA is susceptible to loss of an electron from one of its aromatic nucleobases. This one-electron oxidation produces a radical cation (an electron "hole") within the double helix of duplex DNA that results in an irreversible reaction to form a structurally modified ("dam-

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aged") base. Recent reviews have reported on charge transfer (CT),[4−6] photosensitizers and techniques used in the study of CT,[7] and the electrochemical[8] and biological significance of DNA-mediated CT.[5] In this Account, we apply experiment and theory to address three of the central features concerning one-electron oxidation of DNA: (i) migration of the radical cation away from the site of its initial formation; (ii) electronic and structural factors that determine at which nucleobase irreversible reaction occurs; (iii) analysis of the mechanism for reaction of nucleobase radical cations. Oxidative damage of DNA is a complex process influenced by charge transport and by reactions that are controlled by a combination of enthalpic, entropic, steric, and compositional factors. These reactions occur over broad distributions of energies, times, and spatial scales. This Account is a report of our efforts to gain insight into key elements of this challenging problem. Emergence of a complete picture will require additional exploration of the structural, kinetic, and dynamic properties of DNA.

Long-Distance Charge (Hole) Transfer in DNA

In 1962, Eley and Spivey recognized that the stacked aromatic base pairs of duplex DNA could provide a pathway for the efficient movement of charge.[9] Limited notice was taken of this observation until 1993 when Barton and co-workers[10] reported experiments indicating incredibly fast electron transfer over long distances through a so-called "π-way" of stacked bases that was proposed to form DNA into a "molecular wire".[11] The possibility that DNA could serve as an efficient charge conduit sparked experimental and theoretical studies focused on exploring that possibility and its implications to biology and to nanoelectronic technologies where a DNA wire might be exploited as a self-organizing conductor. These studies led to the emergence of three general views of the mechanism of long-distance charge transport in DNA: (i) superexchange whereby charge is transported coherently (in one step) by long-distance tunneling from "donor" to "acceptor" through the intervening "bridging" nucleobases;[12] (ii) an incoherent multistep random walk from donor to acceptor, consisting of short-distance tunneling intervals linked by base sequences that serve as charge "resting" sites;[13,14] (iii) classical hopping,[14−16] where the charge resides on a single base or small number of adjacent bases and thermal fluctuations activate its motion from one base to another.[17,18] After more than a decade of investigation, recent measurements of photosensitized charge transfer by means of femtosecond time scale spectroscopy show conclusively that the dominant mechanism for charge (radical cation) migration in DNA is multi-step hopping.[19−21] Tunneling is ineffective as a mechanism for long-distance charge transport in DNA because of dynamic structural fluctuations.[15,22,23]

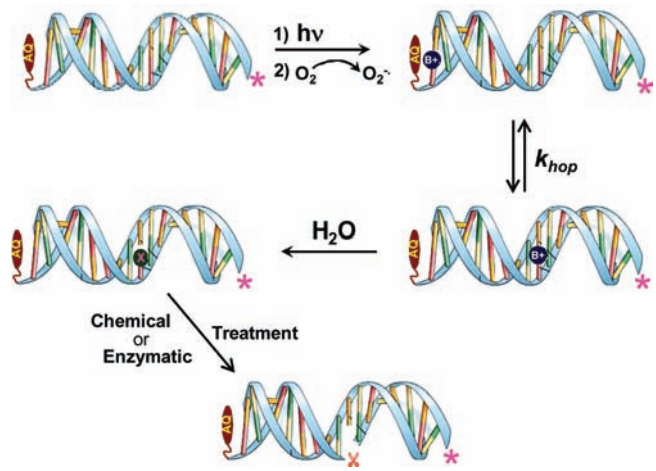

FIGURE 1. A schematic representation of the photooxidation of DNA leading to strand cleavage. In the first step, UV light is absorbed by AQ[15] forming its excited state, which oxidizes an adjacent nucleobase forming the radical cation ($B^{+\bullet}$). In a subsequent step, the concomitantly formed anthraquinone radical anion (not shown) reacts with O_2 to form superoxide ($O_2^{-\bullet}$), and in that process the AQ is regenerated. The $B^{+\bullet}$ may hop reversibly through the duplex DNA (with generic rate constant k_{hop}) until it is trapped in a chemical reaction with H_2O (or another reagent such as $O_2^{-\bullet}$)[25] resulting in a damaged nucleobase that is symbolized generally as "X". Subsequent chemical or enzymatic treatment results in strand cleavage at the site of reaction.

One-Electron Oxidation of DNA

The results of the one-electron oxidation of DNA are essentially independent of the process by which it is oxidized.[24] Experiments in our laboratory rely on photooxidation by a covalently linked anthraquinone derivative (AQ) photosensitizer. The electronically excited AQ is capable of converting any of the four common DNA bases to its radical cation (generically, $B^{+\bullet}$) with the concomitant formation of the anthraquinone radical anion ($AQ^{-\bullet}$). The $AQ^{-\bullet}$ is rapidly consumed by reaction with O_2 to form superoxide ($O_2^{-\bullet}$) and regenerate the AQ, which leaves a $B^{+\bullet}$ with sufficient time to hop and, eventually, to become trapped by an irreversible reaction. The damaged base is revealed by subsequent chemical or enzymatic treatment that causes cleavage of the DNA at the damaged site (see Figure 1). In carefully controlled experiments, the distance dependence of charge transfer is related to the amount of strand cleavage measured at sites remote from the AQ.

The strand cleavage patterns from the one-electron oxidation of DNA oligomers fall into two control regimes: thermodynamic and kinetic. This is revealed in experiments carried

out on duplex DNA oligomers comprised of the regularly repeating base sequences 5′-(AQ)[(A)$_n$(GG)]$_m$(*)-3′ or 5′-(AQ)-[(T)$_n$(GG)]$_m$(*)-3′ paired with their appropriate complementary strands.[26] These oligomers contain *m*GG steps each separated by *n*(A/T) or *n*(T/A) base pairs ($n = 1-8$). The GG steps are highly reactive sites where reaction of a guanine radical cation results in its conversion primarily to 8-oxo-7,8-dihydroguanine (8-OxoG) and other oxidation products.[27] These oligomers have the AQ group linked to the 5′-end and a [^{32}P]-radiolabel (indicated by *) at their 3′-end, which enables analysis by PAGE and radiometry.

One-electron oxidation of the duplex oligomer containing the 5′-[(A)$_1$(GG)]$_6$-3′ sequence results in essentially equivalent reaction at each of its six GG steps. In this case, a semilogarithmic plot of strand cleavage yield versus distance from the AQ has a slope indistinguishable from zero.[28] In contrast, the GG step closest to the AQ of the duplex oligomer containing the 5′-[(T)$_5$(GG)]$_4$-3′ sequence reacts with much higher yield than those that are farther away. In this case, the slope of the semilogarithmic plot is -0.04 ± 0.001 Å^{-1}. The behavior of the other oligomers in this series is similar; each yields a linear semilogarithmic plot with a slope that depends on the number and identity of the base pairs between the GG steps.

If charge migration in DNA occurred by a tunneling mechanism, the slope of these semilogarithmic plots would be a measure of the exponential distance factor β.[29] The hopping model reveals[30] that these slopes are related to the ratio of two generic pseudo-first-order rate constants: one for reversible hopping (k_{hop}) from site-to-site and the other for the irreversible trapping reaction (k_{trap}) that leads to damaged bases. If $k_{hop} \gg k_{trap}$, the slope approaches zero, the outcome of the reaction is under thermodynamic control, and the probability of reaction at every equivalent trapping site in the sequence is the same. If $k_{hop} \leq k_{trap}$, the reaction is under kinetic control and sites closer to the site of initial oxidation react with greater probability. The situation is more complicated for DNA oligomers that do not have a regularly repeating pattern of nucleobases. For these "mixed sequence" oligomers, reactivity depends on the identity and sequence of all the other bases in the oligomer, and the semilogarithmic plots are often not linear and have little meaning.

Radical Cation Traps, Shuttles, and Barriers

In considering qualitative reaction patterns in mixed sequences, it is convenient to consider the nucleobases as falling into one of three broad categories: charge traps, shuttles, and hopping barriers. A trap is a sequence of bases or a single nucleobase where a radical cation will react irreversibly with high probability; a shuttle is a sequence of bases where the charge hops efficiently but there is low likelihood that the radical cation will react; a barrier is a sequence of nucleobases that prohibits or greatly retards charge migration. Critically, the behavior of a set of nucleobases is context dependent; for example, in one circumstance a sequence can act as a trap and in another as a shuttle.

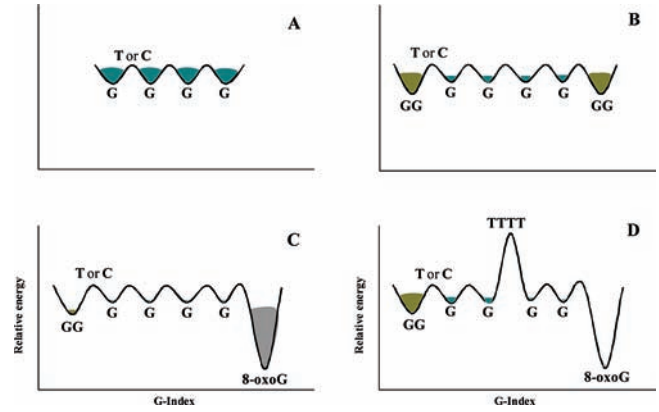

FIGURE 2. A schematic representation of potential energy landscapes for DNA oligomers.[17] A "G" represents an "isolated" guanine, "GG" represents two adjacent guanines, "T" or "C" separate G or GG steps, 8-oxoG stands for 8-oxo-2′-dihydroguanine. The shading represents the relative amount of strand cleavage observed at each site. The *X*-axis ("G-index") is not drawn to scale and represents the position of guanines, GG steps, and 8-oxoG along the oligomer; the intervening barriers may be one base pair or several.

Traps, shuttles, and barriers can be combined to create relative potential energy landscapes that enable the qualitative prediction of reactivity patterns.[17] For example, the 5′-T**G**T-**G**T**G**T**G**T-3′ sequence is a trap in the duplex DNA 5′-(*)AAAT-**G**T**G**T**G**T**G**TAAATT-3′(AQ) (the AQ is linked to the 5′-end of the complementary strand) where oxidation results in equivalent amounts of reaction at each of the four guanines. This is pictured in Figure 2A where each G is depicted as a potential minimum because it is more easily oxidized than are the thymines, which are drawn as maxima, and the amount of strand cleavage is indicated by shading. The character of the 5′-TGTGTGTGT-3′ sequence changes to that of shuttle in the duplex DNA 5′-(*)AAAT**GG**TGTGTGTGT**GG**TAAATT-3′(AQ) (see Figure 2B) where the two flanking GG steps behave as traps because of their significantly lower oxidation potential (E_{ox}) and higher reactivity than that of an "isolated" G. The amount of strand cleavage observed at both GG steps in this oligomer is essentially the same even though one is more than 30 Å farther from the site of initial oxidation (the AQ) than the other. In this oligomer, the 5′-TGTGTGTGT-3′ sequence shuttles the radical cation between the GG steps, and the entire

reaction is under thermodynamic control because hopping is always faster than trapping.

It is possible to transform a GG step from a trap to part of a shuttle. Replacement of the distal (to the AQ) GG step of the previous oligomer by 8-oxoG (symbolized as "**8**") gives the duplex 5′-(*)AAAT**8**TGTGTGTGT**GG**TAAATT-3′(AQ) whose oxidation results in nearly exclusive reaction at the 8-oxoG, see Figure 2C. The 8-oxoG is a deep trap with such high reactivity that once the radical cation arrives at that position it is always consumed. However, reaction at the 8-oxoG can be prevented by the introduction of a kinetic barrier to charge migration. The **TTTT** sequence has this property in the duplex 5′-(*)AAAT**8**TGTG**TTTT**GTGT**GG**TAAATT-3′(AQ). One electron oxidation of this oligomer results in no significant reaction at the 8-oxoG because the radical cation cannot get past the high potential barrier, see Figure 2D. The one-electron oxidation reaction of these two oligomers is under kinetic control because a trapping rate is greater than the rate of hopping. The construction of landscapes comprised of shuttles, traps, and barriers for radical cations in DNA oligomers permits the qualitative prediction of reactivity patterns. The key to the application of this approach is the realization that the character of a particular nucleobase or sequence of bases cannot be determined without consideration of the entire oligonucleotide.

Ion-Gated Charge Hopping

Fundamental insight into the localization of the DNA radical cation (hole) and the mechanism for hopping was obtained by means of quantum mechanical simulations[23] that include the DNA structure (bases, sugars, phosphate groups, and Na^+ counterions) and the solvating H_2O molecules whose inclusion in the theoretical model is necessary to achieve reliable results.[31] The DNA duplex (5′-$G_1A_2G_3G_4$-3′)/(3′-$C_5T_6C_7C_8$-5′) contains the minimum essential features required to simulate charge hopping, a donor base pair (G_1/C_5), a bridge (A_2/T_6) and an acceptor (G_3G_4/C_7C_8). The analysis begins with a classical molecular dynamics (MD) simulation of a seven-base pair duplex oligomer containing the sequence 5′-(AGAGGAG-3′)/(3′-TCTCCTC-5′) and its 12 Na^+ and 840 H_2O molecules. The dynamics of a hydrated 12-base pair duplex DNA oligomer, and in particular the diffusive motion of the Na^+ counterions, obtained from room-temperature MD simulation, is shown in the video clip (accessed from HTML version) where the colored spheres represent the hydrated Na^+. Such simulations generate a Na^+ "visitation map", which gives those locations with a high probability of finding a Na^+.[23] Not surprisingly, the Na^+ are often found near the negatively charged

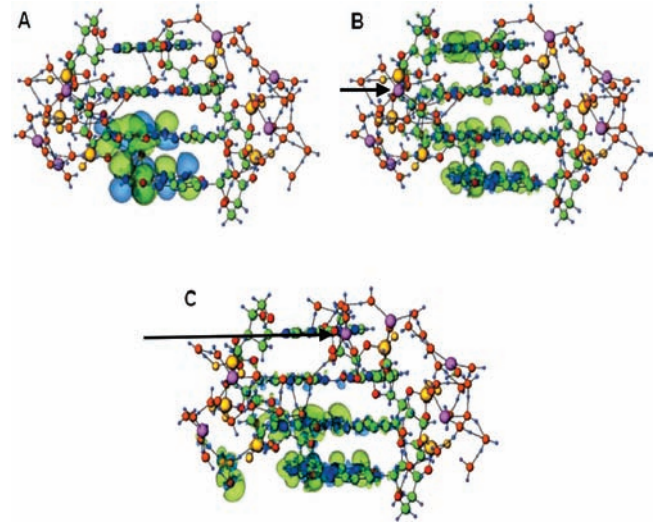

FIGURE 3. Representative structures of the hydrated four-base pair DNA duplex oligomer (5′-$G_1A_2G_3G_4$-3′)/(3′-CTCC-5′) with the nucleobases in numerical order from top to bottom.[23] The six small purple spheres represent the Na^+ counterions. A portion of the network of tightly bound solvating H_2O molecules is also shown with their oxygen atoms represented as orange spheres. Structure A corresponds to the un-ionized (no radical cation) duplex where the Na^+ ions are at positions near the phosphate groups. The blue and green shadings in structure A represent the signs of the isosurfaces of the π-like HOMO, which is located primarily on the G_3G_4 step. Vertical ionization of structure A gives structure B, where the green shading represents the isosurfaces of the total electronic charge differences. The hole density is found principally on guanines G_1, G_3, and G_4. Structure C is similar to structure B except that one Na^+ was moved before ionization from near a phosphate group to the major groove close to N7 of G1 (indicated by the arrows in structures B and C). Inspection of the isosurfaces for structure C shows that the maximum hole density responds to relocation of the Na^+ by moving further away from it, localizing on the G_3G_4 step.

phosphate groups and the N7 atoms of adenine and guanine. Quantum calculations were performed on various configurations of the four-base pair DNA oligomer with particular attention placed on those differing by the transfer of one Na^+ from one high probability location to another.

The highest occupied molecular orbital (HOMO) of the unoxidized DNA with all of the Na^+ located near phosphate groups is found on the G_3G_4 pair, see Figure 3A. Vertical one-electron oxidation (ionization) from this configuration generates a hole that is delocalized over the DNA with highest spatial probabilities found at G_3G_4, less at G_1, and a negligible probability at A_2, see Figure 3B. Structural relaxation of the vertically ionized DNA redistributes the hole density and lowers the ionization potential (IP), thereby creating a self-trapped polaron. The most significant structural changes are not of the DNA itself but of the surrounding medium. In particular, water molecules within 6−8 Å of the DNA realign to maximize their

attractive interaction with the radical cation by orienting their negative ends toward regions with high hole probability density.[32]

The calculations reveal that structural fluctuations, particularly the redistribution of Na^+ counterions and their associated water molecules, are important thermal motions for charge hopping in DNA. Figure 3C is similar to Figure 3B except that one of the hydrated Na^+ associated with a phosphate group of the former has moved to the major groove near N7 of G_1. This change in location of a Na^+ causes a calculated 0.2 eV increase in vertical IP and, most importantly, results in a major redistribution of charge so that it becomes concentrated on the G_3G_4 step. In this ion-gated charge transfer process, only a small fraction of the vast number of thermally accessible Na^+ configurations are effective in enabling hopping, which is usually postulated to occur when a configuration forms that equalizes the energy of the hole on the donor and bridge. The magnitude of k_{hop} is determined by the probability of forming effective configurations; as such, the hopping rate is expected to be much slower than the rate of diffusion of the hydrated Na^+.

Guanines: Sites of High Reactivity for Radical Cations in DNA

It is well-known that oxidation of DNA results in reaction predominantly at guanines.[33] Quite naturally this was attributed to the fact that guanine has the lowest E_{ox} of the nucleobases, and thus it is a "sink" for holes.[27] Similarly, it was found that GG steps are preferred sites for reaction with the 5′-G being especially reactive.[34,35] The relative reactivity of the guanines in a GG step is influenced by the surrounding bases. In particular, the reactivity of the 3′-G is reduced when it is flanked by pyrimidines, which has also been attributed to electronic effects.[36] However, experiments reveal that it is not E_{ox} alone that determines which nucleobase is damaged when DNA is oxidized.

The role that steric effects can play in reactions at GG steps was probed through a combination of experiments and theoretical simulations.[37] Quantum mechanical calculations of the hole spatial distribution in ionized DNA oligomers containing the sequences 5′-XGGX-3′, where X stands for T or uracil (U), show that the radical cation density on the guanines in the sequences TGGT and UGGU are essentially identical. However, the photosensitized oxidation of TGGT gives a ratio of 5′-G to 3′-G reaction equal to 6.1 ± 0.3, while for UGGU this ratio is 3.4 ± 0.2 (this ratio is 1.8 ± 0.1 for AGGA). Clearly, the methyl group of T exerts a significant effect on the reaction of the guanine radical cation with H_2O in these sequences. An important component of that effect was revealed by the MD simulations to be of steric origin.

Water reacts with guanine radical cations primarily by addition at the C8 position resulting, eventually, in the formation of 8-oxoG. Access to C8 occurs through a cone-shaped region extending from the 3′-side of the guanine (the 5′-side is blocked by the adjacent base) oriented along the direction of the developing carbon−oxygen bond. The MD simulations reveal little change in access to the 5′-G of a GG pair for the TGGT and UGGU sequences, while the methyl group of T is found to significantly hinder access of H_2O to the C8 of the 3′-G. Thus, the preference for reaction at the 5′-G of UGGU compared with TGGT appears to be a result of steric inhibition of reaction at the 3′-G by the methyl group on thymine. In general, the relative rate for reaction (k_{trap}) at a particular nucleobase radical cation in DNA is determined by factors that are comprised of both electronic and steric effects.

A Role for Na^+ and Phosphate Groups in the Addition of H_2O to Guanine Radical Cations

Although the addition of H_2O to C8 of the guanine radical cation in duplex DNA is well-known experimentally,[27] computational studies[38,39] did not reveal a low-energy path for this process until the critical roles played by Na^+ and by the negative charge of a nearby phosphate group were explored.[32] This was accomplished by a hybrid quantum mechanical (QM)−classical (molecular mechanics, MM) simulation on a 14 base pair GG-containing DNA sequence complete with the Na^+ and the necessary water molecules. First, an ensemble of configurations (DNA with its associated H_2O molecules and Na^+ counterions), characterized by their high abundance, were selected from a room-temperature MD simulation.[32] Removal of an electron (ionization) from these configurations gives a DNA radical cation, and those configurations of the ionized DNA having the lowest vertical IP were further relaxed. This process yields a small number of highly probable, low-energy configurations; one of these, focused on the reaction site, is shown in Figure 4. In this configuration, a Na^+ is located in the DNA major groove close to N7 of the 5′-G of the GG step and ca. 50% of the calculated hole density is on the 3′-G and 35% is on the 5′-G. Configurations in which the Na^+ occupies different positions yield higher-energy paths for reaction with H_2O.

The steps considered in the addition of H_2O to C8 of a guanine radical cation are depicted in Figure 5. In the first step, Figure 5A, the relaxed radical cation configuration of Figure 4 is shown with the distance between C8 of the 3′-guanine and the oxygen atom of the nearest H_2O molecule (d_{C8-O1}) equal to 2.86 Å and d_{H11-O2} = 1.54 Å.

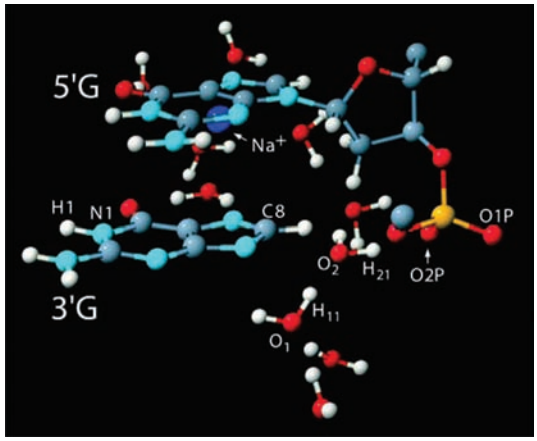

FIGURE 4. The calculated atomic configuration for part of a 14 base pair DNA radical cation focused on the GG step where reaction with H_2O occurs.[32] The reaction site is labeled C8, and there is a Na^+ (dark blue sphere) in the major groove near N7 of the 5′-G. Several of the tightly bound solvating H_2O molecules are shown. The atoms are coded as follows: P, yellow; C, gray; N, light blue; O, red; H, white. This structure was selected from among many high-probability configurations revealed by the MD simulation. The DNA was ionized, and the resulting DNA radical cation and the solvating H_2O molecules were relaxed to give the structures shown here.

The calculations reveal that the reaction proceeds to the transition state (TS), Figure 5B, where 98% of the hole density is on the 3′-G, the d_{C8-O1} distance is reduced to 1.63 Å, and H11 has moved closer to O2, the oxygen atom of an adjacent water molecule ($d_{H11-O2} = 1.13$ Å). In the final step depicted in Figure 5C, the reaction evolves from the TS to form a hydroxylated guanine radical with the concomitant shuttle of a proton through associated water molecules by a Grotthuss-like mechanism.[40] This leads to formation of an asymmetric hydronium ion (H_3O^+) adjacent to the phosphate group, whose Coulombic stabilization underlies the irreversibility of the addition of H_2O to the guanine radical cation. This predicted path is supported experimentally; the reaction yield is reduced when the phosphate group adjacent to the GG step is replaced with a methylphosphonate group, which is electrically neutral.

The results of extensive simulations and their experimental support make it clear that the Na^+ counterions, solvating H_2O molecules, and negative charge of adjacent phosphate groups play critically important roles in defining the reaction of H_2O with a guanine radical cation in duplex DNA. Significantly, related proton-coupled electron transfer reactions are now being found to underlie a number of important processes.[41]

Reaction of the Thymine Radical Cation in DNA

The oxidation of "normal" DNA inevitably results in reaction at guanine, which led us to consider oxidation of an oligomer comprised exclusively of A/T base pairs. It was anticipated that reaction would occur at A because its E_{ox} is lower than that of T; however, the experiments revealed the opposite result, a relatively efficient reaction that occurs almost exclusively at T.[42]

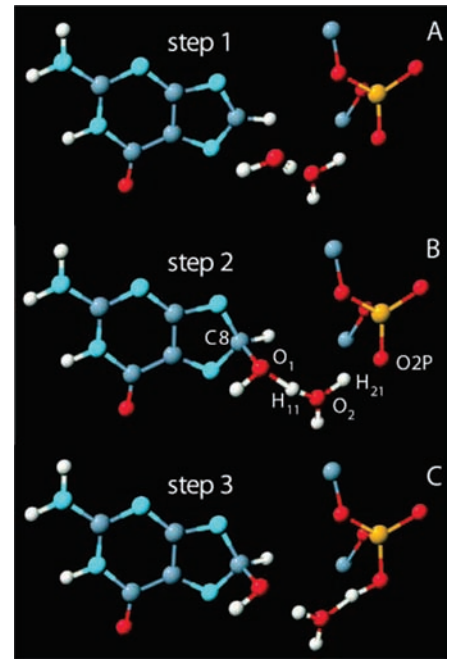

FIGURE 5. "Snapshots" from a QM/MM simulation, taken along the reaction coordinate of the addition of H_2O to C8 of a guanine radical cation embedded in a 14 base-pair duplex DNA.[32] Atoms not directly involved in the reaction are not shown. Step 1 shows the 3′-guanine of the GG step, two H_2O molecules, and an adjacent phosphate group from the representation shown in Figure 4. The transition state is depicted in step 2. It is reached by the approach of a H_2O molecule (labeled O_1) to C8 accompanied by the concomitant elongation of the d_{O1-H11} bond along the axis connecting the oxygen atom to that of a neighboring water molecule (labeled O_2). A Na^+ (see Figure 4) near N7 of the reacting guanine lowers the energy of the transition state compared with other highly probable Na^+ locations. Further evolution along the reaction coordinate leads to step 3 where the formation of a 8-hydroxy-7,8-dihydroguanyl radical[27] is accompanied by generation of H_3O^+. The color scheme is as described in Figure 4.

Irradiation of the duplex sequence (AQ)-3′-AAAA$(TTAA)_4$-ATATAAA*-5′ causes reaction at the thymine bases of the TT steps revealed as distance-dependent strand cleavage when the irradiated samples are treated with hot piperidine. There is no reaction if the thymines of the TT steps are replaced by uracils, which indicates an important role for the C5-methyl group in the reactions of the thymine radical cations. If a GG step is incorporated in a duplex that otherwise is comprised only of T/A base pairs, reaction occurs primarily at the GG sequence even if it is much farther from the site of radical cation injection than the TT steps. That is, when there is a GG step in the oligonucleotide, the $(TTAA)_n$ sequence changes its character from that of a trap to that of a shuttle.

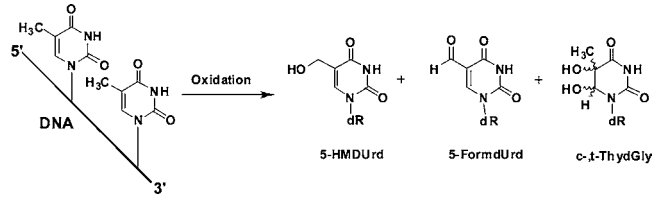

FIGURE 6. The one-electron oxidation of duplex DNA that does not contain a guanine results in the formation of primary products from the tandem reaction of thymines at TT steps: 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd); 5-formyl-2′-deoxyuridine (5-FormdUrd) and *cis* and *trans* diastereomers of 5,6-dihydroxy-5,6-dihydrothymidine (*c*-, *t*-ThdGly).

A thorough analysis of the one-electron oxidation of T in TT steps of duplex DNA reveals the formation of the oxidation products shown in Figure 6.[43] The major products, 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd) and 5-formyl-2′-deoxyuridine (5-FormdUrd), originate with proton loss from the thymine radical cation, trapping of the resulting radical on the methyl group by reaction with O_2, and subsequent reactions of the intermediate peroxyl radical. Formation of the minor products (*cis*- and *trans*-5,6-dihydroxy-5,6-dihydrothymidine (*c*-, *t*-ThdGly)) is initiated by hydration of the thymine radical cation double bond.[44] These primary oxidation products are formed, in part, by a tandem process[45] in which a peroxyl radical reacts with an adjacent thymine resulting in damage to both bases in the TT step.

The 0.15 V difference in E_{ox} between A and T indicates that no more than 1% of the radical cation population resides on T at equilibrium. Obviously then, the observation that reaction occurs at thymine requires that factors other than E_{ox} control the outcome. This is readily understood by application of Curtin−Hammett principle whose key concept is that the relative abundance of the intermediates (determined by the E_{ox} of the nucleobases) 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 guanines, the energy of the transition state for its reaction with H_2O is lower than that for the reaction of any other base radical cation. For DNA that contains only A/T base pairs, the activation free energy for reaction at the thymine radical cation is lower than that for reaction at the adenine radical cation. Thus despite the fact that A has a much lower E_{ox} than T, the products arise predominantly from reactions of the thymine radical cation.

Conclusions

Intensive investigation of the one-electron oxidation of DNA reveals a complex process whereby the resulting charge can migrate long distances by hopping through the double helix until it is trapped irreversibly in a reaction that damages nucleobases. The efficiency of hopping is determined by the specific base sequence with similar sequences playing roles in different oligomers that are determined by the context of the entire oligomer. Molecular relaxation, primarily of solvating H_2O molecules, in the vicinity of the hole trap it as a polaron that hops from one site to another in a process that is gated, in part, by thermal (diffusive) motions of cationic counterions and their associated hydration environment.

Reactions of the radical cations result in damaged nucleobases. The particular site for reaction is determined by kinetic or thermodynamic control, governed by the sequence of nucleobases. Most commonly, reaction occurs at a guanine resulting in the formation primarily of 8-OxoG. The proclivity for reaction at a specific G is determined by both electronic and steric factors and is facilitated by proton transfer to a neighboring phosphate group. If there is no suitable guanine, reaction occurs at TT steps largely by a tandem reaction process that damages both bases.

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FOOTNOTES

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REFERENCES

1 Wallace, S. S. Biological consequences of free radical-damaged DNA bases. *Free Rad. Bio. Med.* **2002**, *33*, 1–14.

2 Franco, R.; Schoneveld, O.; Georgakilas, A. G.; Panayiotidis, M. I. Oxidative stress, DNA methylation and carcinogenesis. *Cancer Lett.* **2008**, *266*, 6–11.

3 Kawanishi, S.; Hiraku, Y.; Oikawa, S. Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Mutat. Res. - Rev. Mut. Res.* **2001**, *488*, 65–76.

4 *Long-Range Charge Transfer in DNA. II.* Schuster, G. B.; Editor Topics in Current Chemistry series 2004, *237*.

5 *Long-Range Charge Transfer in DNA. I.* Schuster, G. B.; Editor Topics in Current Chemistry series 2004, *236*.

6 *Charge Migration in DNA: Perspectives from Physics, Chemistry, and Biology* Chakraborty, T; Editor Springer New York, 2007.

7 Wagenknecht, H. A. Electron transfer processes in DNA: mechanisms, biological relevance and applications in DNA analytics. *Nat. Prod. Rep.* **2006**, *23*, 973–1006.

8 Gorodetsky, A. A.; Buzzeo, M. C.; Barton, J. K. DNA-Mediated Electrochemistry. *Bioconj. Chem.* **2008**, *19*, 2285–2296.

9 Eley, D. D.; Spivey, D. I. Semiconductivity of Organic Substances. Nucleic Acid in Dry State. *Trans. Faraday Soc.* **1962**, *58*, 411–415.

10 Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Ghatlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. Long-Range Photoinduced Electron-Transfer through a DNA Helix. *Science* **1993**, *262*, 1025–1029.

11 Turro, N. J.; Barton, J. K. Paradigms, supermolecules, electron transfer and chemistry at a distance. What's the problem? The science or the paradigm. *J. Biol. Inorg. Chem.* **1998**, *3*, 201–209.

12 Jortner, J.; Bixon, M.; Voityuk, A. A.; Rosch, N. Superexchange mediated charge hopping in DNA. *J. Phys. Chem. A* **2002**, *106*, 7599–7606.

13 Li, X. Q.; Zhang, H. Y.; Yan, Y. J. A superexchange-mediated sequential hopping theory for charge transfer in DNA. *J. Phys. Chem. A* **2001**, *105*, 9563–9567.

14 Giese, B.; Amaudrut, J.; Kohler, A. K.; Spormann, M.; Wessely, S. Direct observation of hole transfer through DNA by hopping between adenine bases and by tunnelling. *Nature* **2001**, *412*, 318–320.

15 Schuster, G. B. Long-range charge transfer in DNA: Transient structural distortions control the distance dependence. *Acc. Chem. Res.* **2000**, *33*, 253–260.

16 Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y. Z.; Schuster, G. B. Long-distance charge transport in duplex DNA: The phonon-assisted polaron-like hopping mechanism. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 8353–8358.

17 Joseph, J.; Schuster, G. B. Emergent functionality of nucleobase radical cations in duplex DNA: Prediction of reactivity using qualitative potential energy landscapes. *J. Am. Chem. Soc.* **2006**, *128*, 6070–6074.

18 Kawai, K.; Kodera, H.; Osakada, Y.; Majima, T. Sequence-independent and rapid long-range charge transfer through DNA. *Nat. Chem.* **2009**, *1*, 156–159.

19 Lewis, F. D.; Zhu, H.; Daublain, P.; Sigmund, K.; Fiebig, T.; Raytchev, M.; Wang, Q.; Shafirovich, V. Getting to guanine: mechanism and dynamics of charge separation and charge recombination in DNA revisited. *Photoch. Photobio. Sci.* **2008**, *7*, 534–539.

20 Daublain, P.; Siegmund, K.; Hariharan, M.; Vura-Weis, J.; Wasielewski, M. R.; Lewis, F. D.; Shafiovich, V.; Wang, Q.; Raytchev, M.; Fiebig, T. Photoinduced charge separation in pyrenedicarboxamide-linked DNA hairpins. *Photoch. Photobio.Sci.* **2008**, *7*, 1501–1508.

21 Triberis, G. P.; Dimakogianni, M. Correlated small polaron hopping transport in 1D disordered systems at high temperatures: a possible charge transport mechanism in DNA. *J. Phys-Condens. Mat.* **2009**, *21*, 035114.

22 Hatcher, E.; Balaeff, A.; Keinan, S.; Venkatramani, R.; Beratan, D. N. PNA versus DNA: Effects of structural fluctuations on electronic structure and hole-transport mechanisms. *J. Am. Chem. Soc.* **2008**, *130*, 11752–11761.

23 Barnett, R. N.; Cleveland, C. L.; Joy, A.; Landman, U.; Schuster, G. B. Charge migration in DNA: Ion-gated transport. *Science* **2001**, *294*, 567–571.

24 Lee, Y. A.; Durandin, A.; Dedon, P. C.; Geacintov, N. E.; Sharirovich, V. Oxidation of guanine in G, GG, and GGG sequence contexts by aromatic pyrenyl radical cations and carbonate radical anions: Relationship between kinetics and distribution of alkali-labile lesions. *J. Phys. Chem. B* **2008**, *112*, 1834–1844.

25 Yun, B. H.; Lee, Y. A.; Kim, S. K.; Kuzmin, V.; Kolbanovskiy, A.; Dedon, P. C.; Geacintov, N. E.; Shafirovich, V. Photosensitized oxidative DNA damage: From hole injection to chemical product formation and strand cleavage. *J. Am. Chem. Soc.* **2007**, *129*, 9321–9332.

26 Liu, C. S.; Hernandez, R.; Schuster, G. B. Mechanism for radical cation transport in duplex DNA oligonucleotides. *J. Am. Chem. Soc.* **2004**, *126*, 2877–2884.

27 Cadet, J.; Douki, T.; Ravanat, J. L. Oxidatively generated damage to the guanine moiety of DNA: Mechanistic aspects and formation in cells. *Acc. Chem. Res.* **2008**, *41*, 1075–1083.

28 Meaningful slopes are obtained only from experiments carried to low conversion where each DNA molecule reacts once or not at all; that is, single-hit conditions.

29 Marcus, R. A. Chemical + Electrochemical Electron-Transfer Theory. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155–196.

30 The analysis of these data was carried out in collaboration with Professor Rigoberto Hernandez, School of Chemistry and Biochemistry, Georgia Institute of Technology.

31 Barnett, R. N.; Cleveland, C. L.; Landman, U.; Boone, E.; Kanvah, S.; Schuster, G. B. Effect of base sequence and hydration on the electronic and hole transport properties of duplex DNA: Theory and experiment. *J. Phys. Chem. A* **2003**, *107*, 3525–3537.

32 Barnett, R. N.; Bongiorno, A.; Cleveland, C. L.; Joy, A.; Landman, U.; Schuster, G. B. Oxidative damage to DNA: Counterion-assisted addition of water to ionized DNA. *J. Am. Chem. Soc.* **2006**, *128*, 10795–10800.

33 Berg, H. Redox Processes during Photodynamic Damage of DNA 0.2. New Model for Electron Exchange and Strand Breaking. *Bioelectrochem. Bioenerget.* **1978**, *5*, 347–356.

34 Ito, K.; Inoue, S.; Yamamoto, K.; Kawanishi, S. 8-Hydroxydeoxyguanosine Formation at the 5′ Site of 5′-GG-3′ Sequences in Double-Stranded DNA by UV-Radiation with Riboflavin. *J. Biol. Chem.* **1993**, *268*, 13221–13227.

35 Prat, F.; Houk, K. N.; Foote, C. S. Effect of guanine stacking on the oxidation of 8-oxoguanine in B-DNA. *J. Am. Chem. Soc.* **1998**, *120*, 845–846.

36 Senthilkumar, K.; Grozema, F. C.; Guerra, C. F.; Bickelhaupt, F. M.; Siebbeles, L. D. A. Mapping the sites for selective oxidation of guanines in DNA. *J. Am. Chem. Soc.* **2003**, *125*, 13658–13659.

37 Cleveland, C. L.; Barnett, R. N.; Bongiorno, A.; Joseph, J.; Liu, C.; Schuster, G. B.; Landman, U. Steric Effects on Water Accessability Control Sequence-Selectivity of Radical Cation Reactions in DNA. *J. Am. Chem. Soc.* **2007**, *129*, 8408–8409.

38 Reynisson, H.; Steenken, S. DFT calculations on the electrophilic reaction with water of the guanine and adenine radical cations. A model for the situation in DNA. *Phys. Chem. Chem. Phys.* **2002**, *4*, 527–532.

39 Gervasio, F. L.; Laio, A.; Iannuzzi, M.; Parrinello, M. Influence of DNA structure on the reactivity of the guanine radical cation. *Chem.—Eur. J.* **2004**, *10*, 4846–4852.

40 de Grotthuss, C. J. T. Memoir on the decomposition of water and of the bodies that it holds in solution by means of galvanic electricity. *BBA - Bioenergetics* **2006**, *1757*, 871–875.

41 Irebo, T.; Reece, S. Y.; Sjodin, M.; Nocera, D. G.; Hammarstrom, L. Proton-coupled electron transfer of tyrosine oxidation: Buffer dependence and parallel mechanisms. *J. Am. Chem. Soc.* **2007**, *129*, 15462–15464.

42 Ghosh, A.; Joy, A.; Schuster, G. B.; Douki, T.; Cadet, J. Selective one-electron oxidation of duplex DNA oligomers: reaction at thymines. *Org. Biomol. Chem* **2008**, *6*, 916–928.

43 This work was carried out in collaboration with Professor Jean Cadet and Dr. T. Douki of CEA/Grenoble, France.

44 Cadet, J.; Douki, T.; Gasparutto, D.; Ravanat, J. L. Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat. Res.* **2003**, *531*, 5–23.

45 Hong, I. S.; Carter, K. N.; Sato, K.; Greenberg, M. M. Characterization and mechanism of formation of tandem lesions in DNA by a nucleobase peroxyl radical. *J. Am. Chem. Soc.* **2007**, *129*, 4089–4098.