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Mechanism for Radical Cation Transport in Duplex DNA Oligonucleotides

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Abstract: We investigated the photoinduced one-electron oxidation of a series of DNA oligomers having a covalently linked anthraquinone group (AQ) and containing $[(A)_nGG]_m$ or $[(T)_nGG]_m$ segments. These oligomers have *m* GG steps, where $m = 4$ or 6, separated by $(A)_n$ or $(T)_n$ segments, where $n = 1-7$ for the $(A)_n$ set and 1−5 for the $(T)_n$ set. Irradiation with UV light that is absorbed by the AQ causes injection of a radical cation into the DNA. The radical cation migrates through the DNA, causing chemical reaction, primarily at GG steps, that leads to strand cleavage after piperidine treatment. The uniform, systematic structure of the DNA oligonucleotides investigated permits the numerical solution of a kinetic scheme that models these reactions. This analysis yields two rate constants, k_{hop}, for hopping of the radical cation from one site to adjacent sites, and k_{trap}, for irreversible reaction of the radical cation with H_2O or O_2. Analysis of these findings indicates that radical cation hopping in these duplex DNA oligomers is a process that occurs on a microsecond time scale. The value of k_{hop} depends on the number of base pairs in the $(A)_n$ and $(T)_n$ segments in a systematic way. We interpret these results in terms of a thermally activated adiabatic mechanism for radical cation hopping that we identify as phonon-assisted polaron hopping.

The current intensive investigation of long-distance charge transport in duplex DNA is motivated in part by its relevance to two important subjects. First, some mutations in living systems may be a result of charges moving through DNA and causing chemical reactions ("damage") at some of its bases.[1−3] Investigation of charge transport and the concomitant reactions of the DNA bases is significant because enhanced understanding may yield insight into damage prevention or repair.[4] Second, a growing community envisions use of DNA as a self-organizing conductor in "molecular electronics" applications.[5] As part of the examination for this role, the conductivity of "dry" (or dehydrated) samples of DNA has been studied in the solid state under a range of conditions with broadly varying results.[6] From these experiments, it has been reported that DNA can behave as a proximity-induced superconductor,[7] a conducting metal,[8,9] a semiconductor,[10−12] or an insulator.[13,14] It is reasonable to suspect that this wide range of reported properties is a consequence of difficulties in assigning precise structures to samples formed from DNA under the unnatural conditions required by these experiments.

On the other hand, recent examinations of long-distance charge transport through DNA oligonucleotides dissolved in aqueous buffer solutions have led to a convergence of mechanistic views. Experiments reported from several laboratories show that a radical cation ("hole") introduced into a duplex DNA oligomer in solution, by any of several means, will migrate long distances before being irreversibly trapped by reaction with water or molecular oxygen.[15−22] An understanding of the mechanism for radical cation transport has been intensively pursued both using experimental observation and by the development and application of theory.

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It is now generally accepted that radical cations move long distances through DNA by a series of short hops, a process suggested some time ago.[23] At present, there are two proposals under consideration concerning the nature of these hops. In one, termed the hole-resting site model,[20] radical cations are localized on individual guanines, because these have the lowest oxidation potential of the four DNA bases,[24] and tunnel from guanine to guanine (either on the same strand or, with some kinetic penalty, on the complementary strand) through "bridges" composed of A/T or T/A base pairs. Experimental evidence[25,26] restricts this model to cases where no more than three A/T base pairs separate guanines; for longer bridges, some other hopping mechanism is thought to operate.[27−29] Recent time-resolved spectroscopic measurements[30−32] have yielded results suggesting that tunneling may not be the rate-determining step for radical cation migration even over distances of one or two base pairs. The fastest possible isoenergetic hop (i.e., from G to G through a single A in the sequence GAG) has been modeled and assigned a rate constant by Lewis, Wasielewski, and co-workers[30] that is 2 orders of magnitude less than the value expected for tunneling by theory.[33,34] Similarly, Lewis reports that radical cation transport through a T in the sequence GTG is slower than the transport through an A; however, these measurements are at the limit of the spectroscopic method's reliability and are prone to significant error.[35] Clearly, the time required for radical cation transport in GAG (and especially GTG) is far greater than that for which large-amplitude structural motions of DNA[36] very strongly modulate base-to-base electronic coupling.[37,38] Consequently, it is unlikely that calculated tunneling rate constants will be reliable (even over short distances) that take as a starting point the assignment of DNA bases to their fixed nuclear positions obtained by X-ray crystallography of B-form DNA[39] or do not include rarely occurring distorted conformations that are far from the canonical structure when the electronic coupling between bases is estimated.[37] Even if more complete tunneling rate calculations were performed, it is unclear that they would fully describe the results, and thus, it is necessary to consider processes other than tunneling to be the rate-determining step for radical cation migration in DNA.

The second mechanism under current consideration for long-distance radical cation migration in DNA is called phonon-assisted polaron hopping.[40] In this view, the radical cation is self-stabilized in a distortion of the DNA and its nearby environment (water molecules and counterions to the phosphate anions of the backbone)[41] that spreads the charge over several bases (the polaron). Thermal activation (phonons) causes the polaron to hop from one site to a neighboring site. The number of hops required is determined by the extent of charge delocalization, and the time scale for these hops is set by the height of the activation barrier. The latter, in turn, is determined by the nature and number of bases that comprise the polaron and the composition of the bases that form barriers between extended polaron sites.[42] Thus, the rate-determining step for charge migration by phonon-assisted polaron hopping is the modulation of the path-dependent free energy by the collective motion of the DNA and its environment (with time scales ranging from femtoseconds to milliseconds).[36] Tunneling does not play a role.

We report herein an examination of base sequence effects on long-distance radical cation migration in DNA. We prepared a series of DNA conjugates containing an anthraquinone (AQ) group linked to a 5′-terminus of DNA duplexes having a regular, repeating pattern, either $[(A)_nGG]_m$ or $[(T)_nGG]_m$; see Figure 1. Irradiation of the AQ injects a radical cation into the DNA that migrates long distances before it is trapped at the GG steps. The pattern of reactivity that results is detected as strand cleavage by means of polyacrylamide gel electrophoresis after treatment of the irradiated DNA samples with piperidine. The regularity in the structures of the DNA constructs investigated here allows development of a straightforward kinetic model that provides estimates of the hopping times for radical cations in these sequences that range from 0.1 to 15 μs. These findings help to define the mechanism for radical cation transport in duplex DNA.

Materials and Methods

DNA oligomers were synthesized as described elsewhere on an Applied Biosystems DNA synthesizer[17] and purified by reversed-phase HPLC with a Hitachi system using a Dynamax C18 column. All DNA samples were analyzed by mass spectrometry and by UV melting and cooling curves. Copies of the mass spectra and a table of the melting temperature data are available as Supporting Information. Radiolabeled samples were prepared as previously described.[42]

Samples for irradiation were prepared by hybridizing a mixture of unlabeled (5.0 μM) and radiolabeled (10000 cpm) oligonucleotides with the non-AQ complementary strands (5.0 μM) in 10 mM Na_3PO_4 buffer (pH 7.0). Hybridization was achieved by heating the samples at 90 °C for 10 min, followed by slow cooling to room temperature overnight. Samples were irradiated at 350 nm at ca. 30 °C in microcentrifuge tubes in a Rayonet photoreactor (Southern New England Ultraviolet Co., Barnsford, CT). After irradiation, the samples were precipitated once with cold ethanol (100 μL) in the presence of glycogen (2 μL, 20 mg/mL), washed with 80% ethanol (2 × 100 μL), dried (Speedvac, low heat), and treated with piperidine (100 μL, 1 M solution) at 90 °C for 30 min. After evaporation of the piperidine (Speedvac, medium heat), coevaporation twice with water (20 μL), and dissolution in dye solution, the samples (3000 cpm) were electrophoresed on a 20% 19:1

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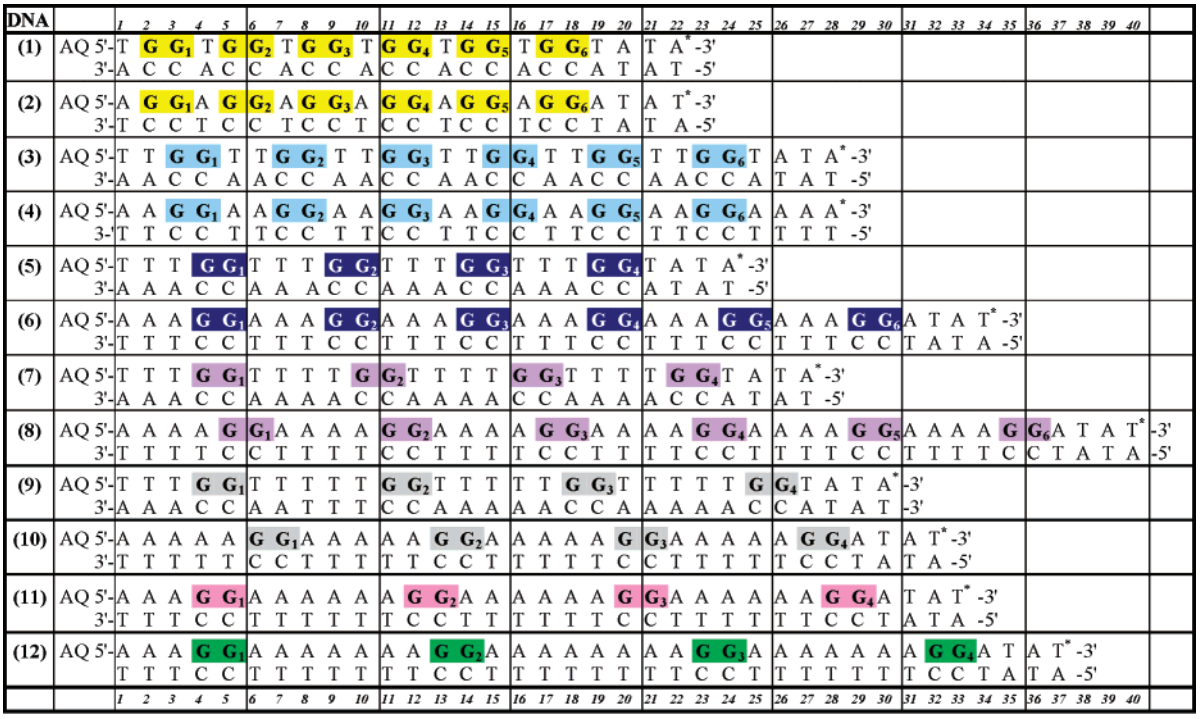

Figure 1. Structures of DNA constructs used in this work.

acrylamide/bisacrylamide gel containing urea (7 M) at 70 W for ca. 90 min. The gels were dried, and the cleavage sites were visualized by autoradiography. Quantification of cleavage bands was performed on a Fuji phosphorimager.

Results

(1) Experimental Determination of the Distance Dependence of Radical Cation Reaction at GG Steps. Figure 1 shows the series of DNA constructs that we prepared to examine the effect of base sequence and sequence length on radical cation transport in DNA. In these experiments, the extent of reaction is controlled so that, on average, each DNA oligomer reacts once or not at all (single-hit conditions). This was demonstrated (see the Supporting Information) by showing that, within experimental error, the results obtained are independent of irradiation time at the relevant extent of reaction. Under these circumstances, the observed pattern of cleavage reveals the relative rates for charge migration (k_{hop}) and for irreversible trapping reaction (k_{trap}) of the radical cation.[40] A typical result (gel) is shown in Figure 2 for DNA(1); similar gels for the other DNA constructs examined in this work are available (see the Supporting Information). The experimental results from the quantitative analysis of the effect of distance on reaction efficiency at GG steps for DNA(1) through DNA(12) are compiled in Table 1.

There are obvious patterns in the data that depend on the relative magnitudes of k_{hop} and k_{trap}. If k_{trap} is much greater than k_{hop}, then only GG steps that are close to the AQ react. In contrast, if k_{trap} is much less than k_{hop}, then all GG steps in the oligomer react to an approximately equal extent. In cases where the rates for trapping and radical cation migration are not vastly different, the amount of reaction at the GG steps falls off with its distance from the AQ. These experiments provide the data to calculate a unitless parameter, k_{ratio}, which is the ratio of k_{hop} to k_{trap}; see below.

There is a barely discernible distance dependence for the radical cation reaction in DNA(1) where the base sequence $(GGT)_6$ generates six GG steps with each separated by a single T. Figure 3 shows that the amount of reaction observed at the GG steps of DNA(1) versus the distance from the AQ is apparently linear in a semilog plot with a slope of -0.003 ± 0.002 Å^{-1}. Thus, in this case, k_{hop} does not completely dominate k_{trap}. Similar plots for the other DNA oligomers examined are shown in Figure 3 for the GG$(T)_n$GG series and in Figure 4 for the GG$(A)_n$GG series of oligomers.

In comparison with the results for DNA(1), there is no experimentally measurable distance dependence for reaction of the six GG steps in DNA(2). In this case, a single A separates each GG step in the base sequence $(AGG)_6$. The slope of the apparent line in the semilog plot for DNA(2) is experimentally indistinguishable from zero (-0.002 ± 0.002 Å^{-1}). Consequently, isoenergetic charge migration from GG to GG through a single A base occurs much faster than the irreversible trapping of the radical cation.

Table 1.

DNA (B)$_n$[a]	GG$_1$/∑GG$_m$[b]	GG$_2$/∑GG$_m$	GG$_3$/∑GG$_m$	GG$_4$/∑GG$_m$	GG$_5$/∑GG$_m$	GG$_6$/∑GG$_m$	slope (Å^{-1})	k_{ratio}
1 (T)$_1$	0.16	0.19	0.17	0.17	0.17	0.14	−0.003 ± 0.002	100
2 (A)$_1$	0.16	0.20	0.15	0.17	0.16	0.16	−0.002 ± 0.002	>200
3 (T)$_2$	0.32	0.23	0.19	0.11	0.09	0.06	−0.02 ± 0.001	10
4 (A)$_2$	0.17	0.17	0.17	0.17	0.16	0.16	−0.001 ± 0.0003	>300
5 (T)$_3$	0.49	0.26	0.16	0.09			−0.03 ± 0.001	3
6 (A)$_3$	0.26	0.20	0.17	0.13	0.12	0.12	−0.009 ± 0.001	20
7 (T)$_4$	0.58	0.27	0.10	0.05			−0.04 ± 0.002	2
8 (A)$_4$	0.54	0.23	0.10	0.06	0.04	0.03	−0.03 ± 0.003	3
9 (T)$_5$	0.69	0.22	0.05	0.04			−0.04 ± 0.008	1
10 (A)$_5$	0.63	0.26	0.08	0.03			−0.04 ± 0.001	1
11 (A)$_6$	0.67	0.20	0.08	0.05			−0.04 ± 0.002	1
12 (A)$_7$	0.73	0.18	0.06	0.03			−0.03 ± 0.004	1

[a] B stands for the base separating the GG steps, and *n* gives the number of bases. [b] These values are the ratios of the amount of strand cleavage (i.e., "counts" from the phosphorimager) observed for the 5′-G at the indicated GG step normalized by dividing each by the total of all counts obtained for the 5′-G's at each of the *m* GG steps. For DNA(1) and DNA(2) slightly more strand cleavage is observed at GG$_2$ than at GG$_1$. This may be due to back electron transfer from the GG step closest to the AQ. All of the data are included in the analysis.

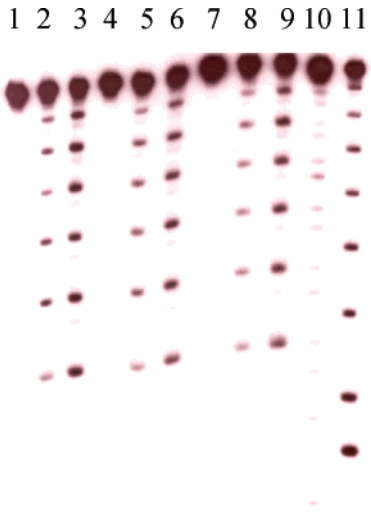

Figure 2. Autoradiogram of the reaction of DNA(1) (repeated three times) showing strand cleavage following irradiation (350 nm) and piperidine treatment. The samples were prepared by hybridizing unlabeled (5 μM) and 10000 cpm ^{32}P-labeled oligonucleotides with the non-AQ complementary strand in 10 mM sodium phosphate buffer (pH 7.0). Cleavage products were separated on a 20% denaturing polyacrylamide gel. Lanes 1, 4, and 7 are for 0 min of irradiation. Lanes 2, 5, and 8 are for 10 min of irradiation. Lanes 3, 6, and 9 are for 20 min of irradiation. The results are independent of irradiation time, which is an indication of single-hit conditions. Lanes 10 and 11 are for Maxim−Gilbert sequencing.

Comparison of the results from DNA(1) and DNA(2) reveals an apparent acceleration of the charge transport rate by changing the T base that separates GG steps in DNA(1) to A in DNA(2). Of course, the sequence GGTGG of DNA(1) contains an A in its complementary strand opposite the T. These findings indicate that an additional barrier to charge migration is imposed when the "bridging" A (a purine base with a relatively low oxidation potential)[43] is moved from the GG-containing strand to its complement. The effect of switching the bridge from A/T to T/A has been investigated previously by theory[44] and experimentally,[30,45] yielding results that depend very strongly on the sequence of other bases surrounding the switched base pair.[42]

More significant results are obtained from the examination of DNA(3) through DNA(12). For DNA(3), the sequence (TTGG)$_6$ generates six GG steps separated by TT sequences.

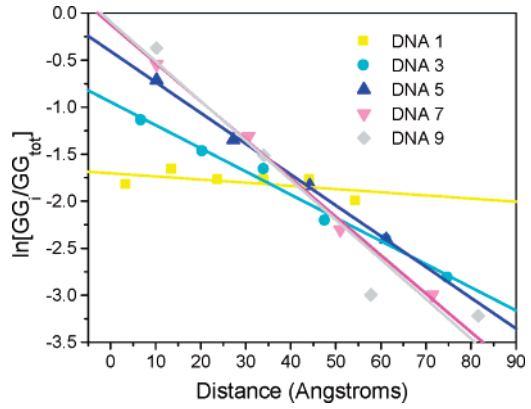

Figure 3. Semilog plots of the amount of strand cleavage at the GG steps of the indicated oligomers (see Figure 1) as a function of distance from the 5′-linked AQ. Strand cleavage is normalized to the total amount of reaction at all GG steps. The distance to a GG step is calculated by assuming 3.4 Å per base pair. The lines are least-squares fits of the data.

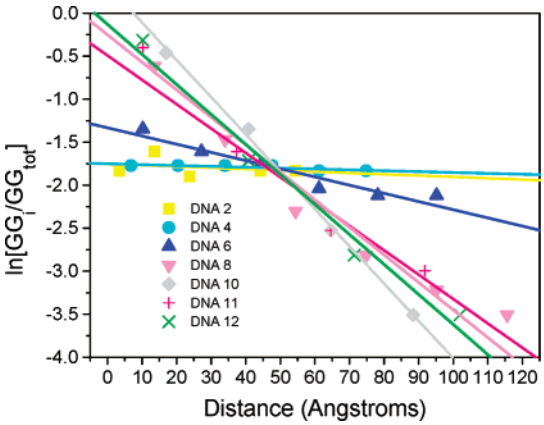

Figure 4. Semilog plots of the amount of strand cleavage at the GG steps of the indicated oligomers (see Figure 1) as a function of distance from the 5′-linked AQ. Strand cleavage is normalized to the total amount of reaction at all GG steps. The distance to a GG step is calculated by assuming 3.4 Å per base pair. The lines are least-squares fits of the data.

The slope of the line in the semilog distance plot in this case is −0.02 ± 0.001 Å^{-1} (Figure 3), which indicates that the trapping reaction is not overwhelmed by the rate of radical cation migration. Consequently, approximately 32% of the total reaction of DNA(3) occurs at GG$_1$, and 6% occurs at GG$_6$. In contrast, as has been reported previously,[42] for DNA(4) in which an (A)$_2$ sequence separates each of the six GG steps, the amount

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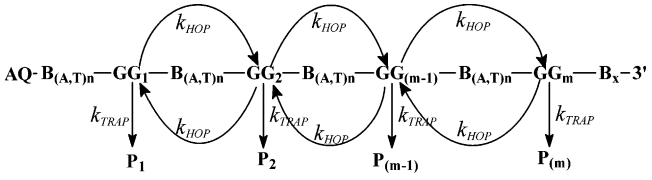
Scheme 1. Kinetic Model for Radical Cation Hopping



$$-\frac{d[GG_1]}{dt} = (k_{HOP} + k_{TRAP})[GG_1] - k_{HOP}[GG_2]$$

$$-\frac{d[GG_2]}{dt} = (2k_{HOP} + k_{TRAP})[GG_2] - k_{HOP}([GG_1] + [GG_3])$$

$$\vdots$$

$$-\frac{d[GG_{(m-1)}]}{dt} = (2k_{HOP} + k_{TRAP})[GG_{(m-1)}] - k_{HOP}([GG_{(m-2)}] + [GG_{(m)}])$$

$$-\frac{d[GG_m]}{dt} = (k_{HOP} + k_{TRAP})[GG_m] - k_{HOP}[GG_{(m-1)}]$$

of reaction is distributed essentially equally (ca. 17%) among all of the GG steps and the slope (-0.001 ± 0.0003 Å^{-1}) of the semilog plot (Figure 4) is within experimental error of zero. This comparison reveals more clearly the effect of replacing T bases separating GG steps with A bases than do the results from DNA(1) and DNA(2).

DNA(5), DNA(7), and DNA(9) have $(T)_3$, $(T)_4$, and $(T)_5$ sequences separating each GG step, respectively, and DNA-(6,8,10,11,12) have $(A)_n$ sequences separating the GG steps where $n = 3-7$, respectively. In each of these cases, the irreversible trapping reaction competes kinetically with radical cation migration, as indicated by obtaining nonzero slopes from the semilog plots of reaction versus distance (Table 1 and Figures 3 and 4). The slope of the line (-0.009 ± 0.001 Å^{-1}) showing the distance dependence for reaction of DNA(6), which has an $(A)_3$ spacer sequence, is less than the slopes for DNA-(8,10,11,12), which have an essentially constant value of -0.035 ± 0.002 Å^{-1}. This change of the distance dependence for radical cation migration in $(A)_n$ sequences for $n > 3$ has been reported previously[25] and was attributed to a switch in mechanism from tunneling when $n < 3$ to a polaron hopping mechanism when $n > 3$.[46] Similarly, for the $(T)_n$ series, the slope of the semilog plot changes ca. 10-fold from -0.003 to -0.03 Å^{-1} as n goes from 1 to 3, and then remains essentially constant at -0.04 Å^{-1} when $n > 3$.

(2) Kinetic Modeling of the Distance Dependence of Reaction Efficiency for DNA(1) through DNA(12). Scheme 1 shows a simple kinetic model for the migration of a radical cation through duplex DNA. The DNA assemblies are constructed by arranging bases in the order $[(B_{a,t})_nGG]_m$, where $(B_{a,t})_n$ stands for $(A)_n$ or $(T)_n$ sequences that separate the GG steps in a set of m (GG) steps, which is the arrangement of the constructs shown in Figure 1. The irreversible trapping reaction of radical cations at the GG_1 through GG_m steps leads to formation of products P_1 through P_m that are detected as strand cleavage. There are three key assumptions embedded in this scheme. First, radical cations are restricted to locations that contain GG steps. That is, once on GG_1 the radical cation can hop only to or toward GG_2. Similarly, the radical cation at GG_m cannot hop onto bases in the "trailing" $(B)_x$ sequence. Second, it is assumed that k_{hop} is independent of direction in the DNA. That is, the hopping rate constant from GG to GG across an $(A)_n$ or $(T)_n$ sequence is the same from 5′ to 3′ as it is from 3′ to 5′. There is experimental evidence to support this assumption. We recently reported that the amount of strand cleavage at GG steps that bracket an eight-base-pair (A/T) segment is about the same whether the radical cation is introduced at the 5′-end or 3′-end.[42] If there were a large dependence of k_{hop} on hopping direction, this would have caused a significant difference in the amount of reaction at these GG steps. The third assumption in this kinetic model is that k_{trap} is the same for the sequence AGGA as it is for the sequence TGGT. Similar assumptions have generally been made for the analysis of relative reactivity data for radical cations in DNA,[21,47-49] and we have tested it by comparing the relative reactivity of radical cations at GG steps in AGGA, TGGA, AGGT, and TGGT sequences, where only small differences are detected.[50]

The set of differential equations in Scheme 1 can be analyzed generally for k_{ratio} ($=k_{hop}/k_{trap}$), the dimensionless parameter whose value is expected to vary with n in the $[(B_{a,t})_nGG]_m$ constructs. It is important to note that the overall lifetime of the radical cation ($1/k_{trap}$) is the same in all constructs and that this value is determined solely by the rate of the irreversible trapping reaction.

Results of the simulation of the time-dependent radical cation population among the m GG steps for values of $k_{ratio} = 1$, 10, and 100 are displayed in Figure 5. These simulations encompass ca. 80% of the radical cation lifetime, that is, ca. 2.5 times the value of $1/k_{trap}$. When k_{ratio} is set to 1 (Figure 5a), the rate of the irreversible trapping reaction is comparable to the rate of hopping, and the radical cation is consumed before a significant population arrives at the distant GG sites. In contrast, when $k_{ratio} = 100$ or greater (Figure 5c), the rate of hopping is much faster than trapping and the radical cation eventually populates all of the m GG steps approximately equally. The intermediate case ($k_{ratio} = 10$, Figure 5b) shows that the radical cation will occupy all GG steps but that the population distribution will vary with the distance for most of the lifetime of the radical cation.

The reaction sequence shown in Scheme 1 can also be used to simulate the relative yields of strand cleavage products P_1 through P_m. The number of base pairs between the GG step influences the value of k_{ratio}, and this affects the expected distribution of products. Also, because the DNA constructs we examined are of finite length and the radical cations are "injected" entirely at GG_1, the dependence of product yield on distance for some values of k_{ratio} will be nonexponential because GG_1 and GG_m uniquely occupy terminal positions. For the other GG steps, the efficiency of product formation is proportional to $k_{trap}/(2k_{hop} + k_{trap})$, but for the terminal GG steps, because hopping can occur in only one direction, this fraction is $k_{trap}/(k_{hop} + k_{trap})$. When k_{ratio} is a very large value, $k_{hop} \gg k_{trap}$, the distribution of radical cations among the GG steps is determined primarily by its thermodynamic stability on each of the GG steps. In this regime, because all of the GG steps in the $[(B_{a,t})_nGG]_m$ constructs are essentially identical and are expected to have the same ionization potential, the radical cation will distribute uniformly among the m GG steps before any

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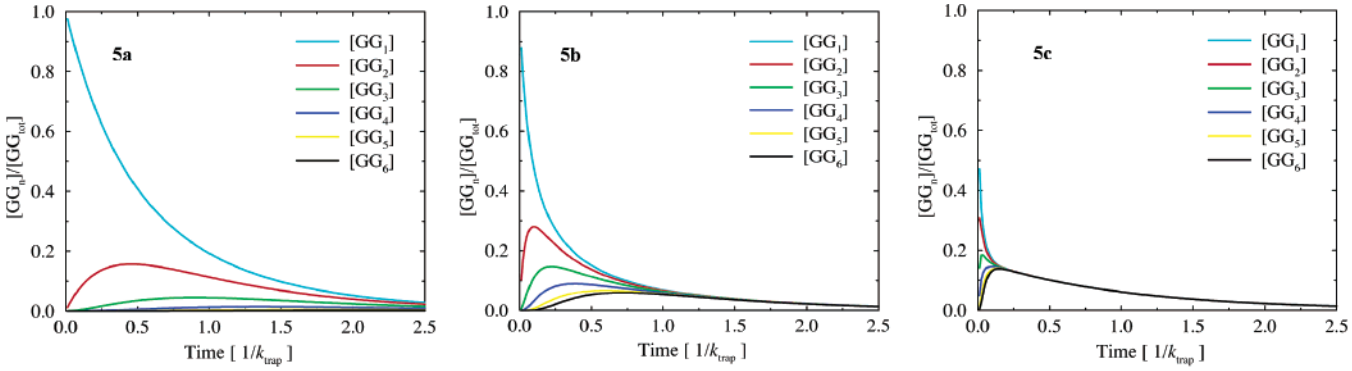


Figure 5. (a−c) Computed time-dependent relative populations at a given GG step (*m*) in a strand with six steps for three different values of k_{ratio}. (a)−(c) correspond to values of this ratio equal to 1, 10, and 100, respectively. The dimensionless units of time are in units of $1/k_{trap}$.

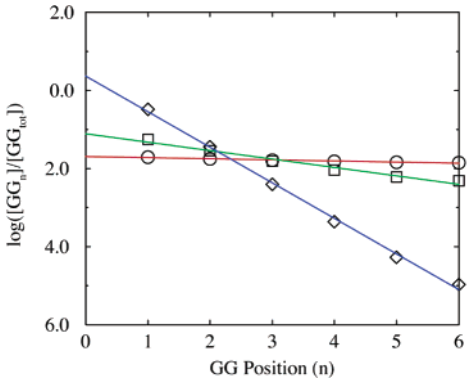
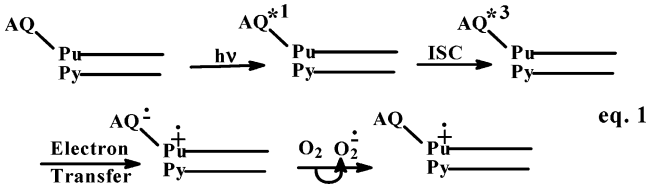


Figure 6. Logarithm of the computed final relative populations of reaction at a given GG step (*m*) in oligomers with six steps displayed for three different values of k_{ratio}. The circles, squares, and triangles correspond to values of this ratio equal to 100, 10, and 1, respectively. The straight lines are least-squares fits to the corresponding points. The lack of perfect agreement is a manifestation of subtle nonlinear effects due to end effects as discussed in the text.

significant trapping has occurred, and the slope of the semilog plot of reaction yield versus distance will be near zero. Alternatively, when $k_{hop} \ll k_{trap}$, the likelihood that the radical cation will populate GG_m is slight, and the semilog plot will be approximately linear. However, for cases where $k_{hop} \approx k_{trap}$, in principle, the semilog plot will be nonlinear because of the unique circumstances of GG_1 and GG_m. This nonlinearity may not be detectable experimentally. If it is, it can be treated explicitly, or it may be avoided by excluding P_1 and P_m from the data analysis. Figure 6 shows the simulated yields of products P_1 through P_6 for the cases where $k_{ratio} = 1$, 10, and 100. The extent of deviation from linear behavior due to the end effects is apparent for the case when $k_{ratio} = 1$; however, it is considerably less than the error typical for experimental determination of strand cleavage efficiency. The results of similar simulations based upon the experimental results reported above give the estimates of k_{ratio} for these DNA constructs that are reported in Table 1.

Discussion

(1) Generation of a Radical Cation in DNA without an Internal Radical Anion for Annihilation. Irradiation of AQ with UV light gives its singlet excited state, and this is followed by rapid intersystem crossing (ISC) to yield the triplet state (AQ^{*3}).[51] When the AQ is covalently linked to DNA, the AQ^{*3} rapidly removes an electron from the adjacent purine base (Pu), forming its radical cation (see eq 1).



The anthraquinone radical anion ($AQ^{\bullet-}$) formed in this reaction loses an electron to molecular oxygen (which forms superoxide) and thus leaves the base radical cation in the DNA with no local "partner" for charge annihilation.[21,52] This is a critical point[42,53] and an important distinction between the experiments described here and the recently reported time-resolved spectroscopic measurements of charge transport in DNA.[30] In the spectroscopic experiments, the electron acceptor is the singlet excited state of a covalently linked stilbene derivative that forms a base radical cation and the stilbene radical anion having overall singlet multiplicity. Annihilation (charge recombination) of this radical ion pair to form ground-state products occurs rapidly because it is exothermic and not restricted by spin conservation rules. The rate of this annihilation reaction controls the lifetime of the base radical cation. For the cases studied,[30] in which a guanine is separated from the stilbene by one or two A/T base pairs, the lifetime of the first-formed guanine radical cation is estimated to be 90 ps and 2 ns, respectively. Any process for radical cation hopping that requires significantly more time than the charge recombination reaction will not compete successfully and will not be observed in these experiments.

In contrast, because the $AQ^{\bullet-}$ formed from irradiation of the covalently linked AQ is consumed by reaction with O_2, there is no radical anion within the DNA duplex containing the base radical cation. Consequently, the radical cation lifetime is determined by the rate of the irreversible trapping reaction (k_{trap}).[54] This is a relatively slow reaction,[55] which results in a long lifetime for the base radical cation, and this permits the

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Table 2. Estimates of k_{hop}

DNA	bridge	k_{hop}[a] (s^{-1})	DNA	bridge	k_{hop}[a] (s^{-1})
1	$(T)_1$	$\sim 6 \times 10^6$	2	$(A)_1$	$< 1 \times 10^7$ [b]
3	$(T)_2$	6×10^5	4	$(A)_2$	
5	$(T)_3$	2×10^5	6	$(A)_3$	1×10^6
7	$(T)_4$	1×10^5	8	$(A)_4$	1×10^5
9	$(T)_5$	6×10^4	10	$(A)_5$	6×10^4
			11	$(A)_6$	6×10^4
			12	$(A)_7$	6×10^4

[a] The values for k_{hop} reported here were obtained from our measurement of k_{ratio} and Giese's estimate[55] of k_{trap}. [b] This value is derived from the data of Lewis and co-workers[30] and is based on the assumptions that are described in the text.

long-distance migrations (hundreds of angstroms) to occur that cannot be observed in the time-resolved spectroscopic experiments.

(2) Values of k_{ratio} for Radical Cation Migration in DNA. Values for k_{ratio} (Table 1) were determined through least-squares fits varying the model parameters of the data on the distance dependence of strand cleavage efficiency for DNA(1−12) by numerical simulation using Mathematica of the kinetic model shown in Scheme 1. In the $(A)_n$ series, values of k_{ratio} range from ca. 1 for DNA oligonucleotides in which $(A)_n$ ($n = 5$, 6, or 7) segments separate GG steps to >300 for DNA(2) and DNA(4) where the separating segments are (A) and $(A)_2$, respectively. Of course, a value for $k_{ratio} > 200$ simply means that it is too large to be determined by the current method. As expected, k_{ratio} decreases with the number of A bases separating the GG steps. Clearly, the value of k_{ratio} is related to the number of A bases between the GG steps by a complex rule. For example, increasing the number of separating A bases from 2 to 3 results in a decrease of k_{ratio} by a factor of about 10, whereas increasing the separating A bases from 4 to 5 results in at most a 2-fold decrease in k_{ratio}. This finding, which is similar to the observations of Giese and co-workers,[25] provides insight into the details of the mechanism for radical cation migration.

The value of k_{ratio} is also dependent on the number of bases separating the GG steps in the $(T)_n$ series. It appears that k_{ratio} is somewhat smaller for this series than for the $(A)_n$ series at an equivalent value for n when n is small (1, 2, or 3). However, when $n > 3$, the $(A)_n$ and the $(T)_n$ series have the same values of k_{ratio}, as far as we have determined. This reveals that the "kinetic penalty" incurred when the radical cation crosses from the T-containing strand to its complement to remain primarily on low oxidation potential purines (A and G) is relatively modest. When $n < 3$, the rate-determining step is the crossover, but when $n > 4$, migration through the bridge becomes rate-determining.

(3) Assigning Values to k_{hop}. Our findings (k_{ratio}), in combination with recent kinetic measurements,[30] and the estimate of the rate constant for irreversible trapping of base radical cations (k_{trap}) in DNA,[55] permit approximation of the rates of radical cation migration (k_{hop}) through the DNA base sequences examined here.

There have been no reports of the direct determination of k_{trap}. However, Giese and Spichty[55] deduced that its value is 6×10^4 s^{-1} at pH 7 by analysis of kinetic data in combination with product yields. For the DNA sequences examined here, estimates of k_{hop} can be readily generated by using this value for k_{trap} in combination with the k_{ratio} values reported in Table 1. These estimates are presented in Table 2.

A value for k_{hop} has been obtained by Lewis and co-workers[30] using time-resolved absorption spectroscopy and kinetic modeling. These experiments give 10^7 s^{-1} for the magnitude of the isothermal rate constant for radical cation hopping from G to G across a barrier formed by a single A (i.e., k_{GAG}, a specific instance of the general rate constant k_{hop}). The sequence of bases in DNA(2) is analogous to that studied by Lewis and co-workers except that in that case GG steps are separated by a single A in the sequence GGAGG. Since the radical cation will be slightly more stable at a GG step than at a single G (this stabilization was estimated by Lewis using the same spectroscopic and modeling procedure to be −0.052 eV), the hopping rate constant in GGAGG may be somewhat less than 10^7 s^{-1}. Consequently, we have assigned $k_{GGAGG} < 10^7$ s^{-1} for DNA(2) in Table 2. Clearly, caution is required in the comparison of this rate constant to the others listed in Table 2 because the others are converted from k_{ratio} on the basis of the value of k_{trap} deduced by Giese. However, the inescapable conclusion from these data is that radical cation migration in DNA is a slow process. The fastest hop takes 0.1 μs, and the slowest we have determined, $(A)_n$ or $(T)_n$, $n \geq 4$, has a lifetime of about 15 μs. This is a much longer time scale than is required for large-amplitude motions of the DNA and its water and counterion environment,[36] which we have suggested to be the processes that control the dynamics of radical cation migration.[38,40,41]

(4) Mechanism of Radical Cation Migration in DNA. The apparently linear semilog plots for radical cation migration in DNA(1−12) suggest that the distance dependence follows an exponential rule, $Ae^{\gamma r}$, where γ is the slope of the line and r is distance. Related behavior has been observed by others and was interpreted at first to indicate coherent, rapid, long-distance charge transfer (wirelike DNA).[56] However, subsequent experiments revealed that other mechanisms must be considered.[19]

In one mechanistic proposal, two regimes for radical cation transport operate over different distances separating adjacent G bases. In this view, when $n \leq 3$ for $(A)_n$ bridges, the radical cation jumps nonadiabatically from guanine to guanine through virtual orbitals of the bridge without ever actually residing on the bridging bases.[28] When $n \geq 4$, the charge migration process changes so that radical cations of the A bases of the bridge participate as real chemical intermediates. In support of this view, recent calculations show that tunneling may be limited by a strongly distance dependent solvent reorganization energy (λ_s).[57] As is clear from inspection of the data in Table 2, the experimental results from investigation of DNA(1−12) reveal a transition when $n > 4$.

We proposed the phonon-assisted polaron hopping mechanism to explain the observation that $\gamma = -0.02$ $Å^{-1}$ for mixed sequence DNA.[40] In this proposal, the radical cation creates a shallow minimum for itself by distorting the DNA structure, which delocalizes it over some number of adjacent base pairs (a polaron). The radical cation then hops adiabatically from minimum to minimum by a thermally activated (i.e., phonon-assisted) process as a result of the motions of the DNA and its solvent and counterion environment.[41] In this view, the radical cation exists as a real chemical intermediate on the A bases of the bridge separating GG steps independent of the length of

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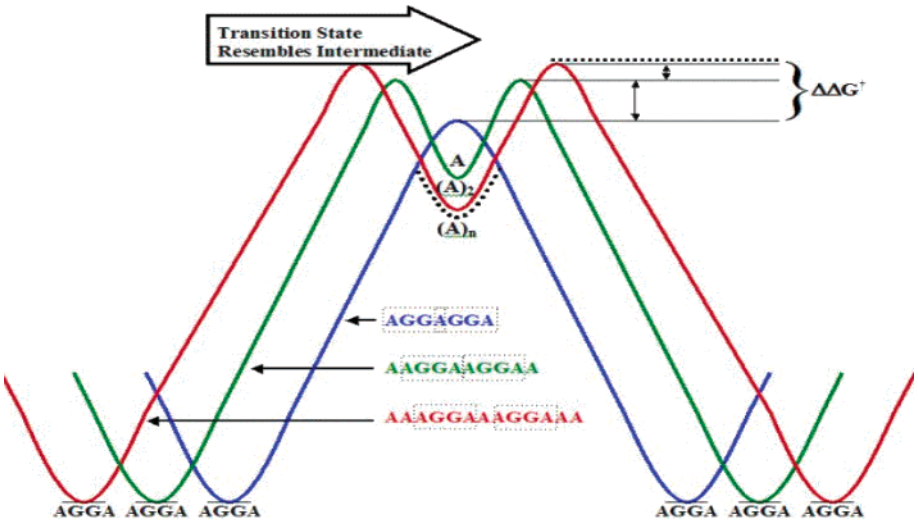

Figure 7. A schematic of the free energy surface encountered by the polaron along the symmetric hopping path. In the bridgeless case of AGGAGGA, there is presumably a barrier at the center of the reaction path. With the introduction of the bridge in the AGG$(A)_n$GGA sequences, the central structure becomes an intermediate. The new putative transition-state structures now lie between the initial reactant structure and the intermediate. The energies of these transition structures may increase with bridge length because they become increasingly distant from the low-energy minima and increasingly "intermediate-like".

the bridge. This mechanism is generally accepted as operational when the radical cation migration is across a bridge of more than three (A/T) base pairs.[30,58,59] The experiments reported here were designed to assess the possibility that this mechanism is also sufficient to explain radical cation hopping over shorter distances.

Transition-state theory[60] provides a basis for explaining the effect of bridge length on the rate of radical cation transport without invoking tunneling or a change in mechanism. Figure 7 is a schematic representation of the reaction path where two GG steps are separated by an $(A)_n$ bridge. We arbitrarily assign the polaron to encompass the AGGA sequence. This means that the radical cation is delocalized over these four bases, but it does not mean that it is spread equally over all of them. The hopping process then moves the radical cation density smoothly from one AGGA sequence to an adjacent one. For DNA(2), which is composed of repeating (AGG) sequences, there are no "additional" bases separating these polarons and thus there is no intermediate. However, for the other sequences examined, where as many as six A bases separate the AGGA polarons, formation of an intermediate bridge radical cation is possible. The relative energy of the transition state is related to that of the intermediate by the Hammond postulate.[61] As the number of bases in the bridge increases, the relative free energy of activation ($\Delta\Delta G^{\ddagger}$) for hopping increases because the transition state resembles increasingly the intermediate. If we further assume that $(A)_4$ is the limit to the extent of delocalization of the radical cation (there must be some limit), then longer bridges will contain more than one intermediate and $\Delta G^{\ddagger}$ for the rate-determining step will no longer change. In other words, when the number of bases in the bridge is greater than 4, we presume that the radical cation is not delocalized on the entire bridge, and this causes the value of k_{hop} to become approximately constant at ca. 6×10^4 s^{-1}. This relatively simple classical model of the mechanism for radical cation migration in DNA is consistent with the data and does not require a change in mechanism for bridges of different lengths.

Conclusions

We examined long-distance radical cation migration in a series of DNA oligomers containing regularly repeating base pair sequences. The amount of oxidative reaction (strand cleavage) detected at GG steps in these oligomers and the observed distance dependence of reaction can be explained by a simple kinetic scheme involving only two rate constants. This model provides estimates of the ratio of the rates for radical cation hopping and radical cation reaction with water. By assuming that the reaction rate with water is constant, values for the hopping rate constant can be assigned. Hopping from one GG step to an adjacent GG step is a relatively slow process. The fastest hop has a half-life of about 0.1 μs, and the slowest hop takes about 15 μs. The hopping rate depends on the number and kind of bases that separate the GG steps. It is fastest when the separating bases on the GG-containing strand are A and somewhat slower when they are T but reaches a limit when there are four or more bases separating the GG steps. We propose a single mechanism, phonon-assisted polaron hopping, to accommodate these results and find no need to postulate a change of mechanism from tunneling to hopping.

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Supporting Information Available: Experimental figures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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