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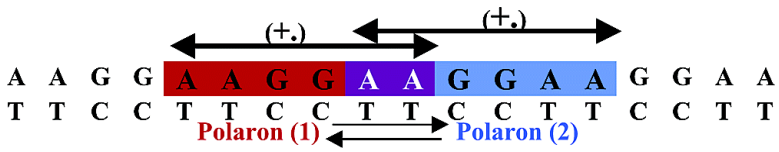

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Base Sequence Effects in Radical Cation Migration in Duplex DNA: Support for the Polaron-Like Hopping Model

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Abstract: A series of anthraquinone-linked (AQ) duplex DNA oligomers were prepared and investigated. Irradiation of the AQ injects a radical cation into the DNA. The radical cation migrates through the DNA and reacts selectively at GG steps, which leads to strand cleavage after treatment with piperidine. The oligomers investigated in this work were selected to assess the effect on long-distance charge transport of placing a T base (or bases) in a strand of repeating purine bases. With notable exceptions, the amount of strand scission decreases with the distance between the AQ and the GG step. The results are consistent only with models for long-distance transport, such as thermally activated polaron-like hopping, that incorporate radical cation delocalization over two or more adjacent bases.

Damage to DNA by its one-electron oxidation has been associated with as many as half of all human cancers.[1] Because guanine has the lowest oxidation potential of the four common DNA bases, its radical cation (formed by loss of an electron) is ususally involved in oxidative reactions. In duplex DNA, guanine radical cations react with water to form mainly 7,8-dihydro-8-oxoguanine (8-OxoG).[2,3] Unrepaired 8-OxoG lesions cause G→T transversions in prokaryotic and eukariotic cells, which often leads to harmful outcomes. The development of a detailed understanding of the mechanism for migration of a radical cation from the site of initial DNA oxidation to a remote, reactive guanine is an important step in the process that will lead to a deep understanding of DNA damage and its repair.

DNA is a helical polyanion built by the union of two linear polymeric strands that are composed of sugars (deoxyribose) linked by phosphates. Each sugar contains an aromatic base (G, C, A, or T) bound to C1′ of the sugar. The two strands are normally complementary so that when they combine to form a duplex, each base on one strand forms a Watson−Crick hydrogen bond with its counterpart on the opposite strand. At normal physiological pH (ca. 7.4), the phosphates of the backbone polymer are fully ionized, so there must be a positively charged counterion to neutralize each phosphate anion. These counterions play an important, if underappreciated, role in modulating the electronic and structural properties of DNA.[4,5]

High-resolution X-ray crystallography of DNA reveals exquisite details about its structure. In B-form DNA, the medium most commonly used for the study of long-distance radical cation transport in solution,[6] the average distance from one base pair to the next is 3.4 Å, and each base pair is rotated around the long axis of the helix by about 36° with respect to its adjacent base pairs.[7] The regular order of stacked bases revealed by this structure led naturally to the proposal that DNA would support long-distance, coherent electron transport.[8] This exciting possibility was revived and supported by measurements of apparently rapid photoinduced charge transfer over more than 40 Å between metallointercalators tethered to opposing 5′-termini of a 15 base pair DNA duplex, which prompted the suggestion that DNA is a "molecular wire".[9,10] However, careful kinetic measurements on related systems showed the invalidity of wire-type behavior.[11] Recently, Sen and co-workers[12] showed that the appearance of rapid, long-distance charge transfer for DNA linked to metallointercalators could be an artifact caused by the formation of aggregates. Currently, there are no data consistent with the existence of a coherent electron transfer process in DNA over a distance greater than one or two base pairs.[13]

The crystallographic structure of DNA is not a good model for consideration of the possibility that it behaves like a molecular wire in solution because this representation does not reveal the extent of instantaneous disorder inherent in this assembly. DNA is a dynamic molecule with motions of its constituent atoms, corresponding counterions, and solvating water molecules that occur on time scales that range from femtoseconds to milliseconds or more. This is revealed clearly

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by consideration of careful molecular dynamics simulations.[14] It is apparent from analysis of these simulations that duplex DNA in solution has the standard B-form structure on average, but at any instant, over long distances (more than two or three base pairs) the DNA is somewhat disordered. Disorder cannot be tolerated in a coherent, single-step charge transfer process because it greatly reduces the electronic interaction that couples one base pair to the next.[15] Consequently, DNA in solution cannot be a molecular wire and this mechanistic possibility must be discarded.

Nonetheless, radical cations introduced into duplex DNA by one-electron oxidation do migrate long distances before being trapped at $(G)_n$ steps ($n = 2, 3$) by reaction with water or oxygen.[6,16−18] The mechanism of this long-distance radical cation migration in duplex DNA is an area of intense experimental and theoretical investigation. The currently considered mechanistic possibilities for this process fall into two categories: (i) an incoherent random-walk of multiple, short tunneling steps;[19,20] (ii) a polaron-like hopping process where dynamical structural distortions generate delocalized, self-trapped radical cations of finite spatial extent that are transported by thermal activation.[4,6,21−23] The effect of DNA base sequence on the efficiency of charge transport can provide compelling information for assessment of these mechanisms. However, previous investigations of sequence effects have resulted in apparently ambiguous findings.[13,24−27] In the work described here, we report that the effect of base sequence is a collective phenomenon that results from patterns of interactions between the base pairs of the duplex.[28]

Materials and Methods

[γ-^{32}P]ATP, [α-^{32}P]ATP radioactive isotopes and terminal dinucleotide transferase (TdT) were purchased from Amersham Bioscience. T4 polynucleotide kinase (T4PNK) was purchased from New England Biolab and stored at −20 °C. Unmodified DNA oligomers and anthraquinone-containing complementary oligomers were synthesized as described elsewhere on an Applied Biosystems DNA synthesizer.[3,33] The extinction coefficients of the oligomers were calculated using an online biopolymer calculator, and their concentrations in solution were determined from the absorbance at 260 nm, where the anthraquinone was replaced with adenine in the extinction coefficient determination. Reverse-phase HPLC was performed on a Hitachi system using a Dynamax C18 column. Atmospheric-pressure electron impact ionization mass spectra of the oligomers were obtained by MALDI-TOF mass spectrometry. UV melting and cooling curves were recorded on a Cary 1 E spectrophotometer equipped with a multicell block, temperature controller, and sample transport accessory. CD spectra were recorded in a JASCO J-720 spectropolarimeter.

Cleavage Analysis by Radiolabeling and Polyacrylamide Gel Electrophoresis (PAGE). DNA oligonucleotides were radiolabeled according to standard procedures either at the 5′-end or 3′- end with [γ-^{32}P]ATP and bacterial T4PNK for 5′-labeling or with [α-^{32}P]ATP and TdT for 3′-labeling. Radiolabeled DNA was purified by 20% PAGE at 400 V. Samples for irradiation were prepared by hybridizing a mixture of unlabeled (5.0 μM) and radiolabeled (10 000 cpm) oligonucleotides with AQ or non-AQ complementary strands (5.0 μM) in 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 Company, 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), lyopholization twice with water (20 μL), and dissolution in dye solution (0.25% w/v bromophenol blue, 0.25% w/v xylene cyanol, 30% v/v formamide in water), the samples (3000 cpm) were electrophoresed on a 20% 19:1 acrylamide:bisacrylamide gel containing urea (7 M) at 70 W for 90 min. The gels were dried, and the cleavage sites were visualized by autoradiography. Quantification of cleavage bands was performed on a phosphorimager.

Results

We prepared a series of DNA oligomers that are covalently linked to an anthraquinone group (AQ) at a 5′-end; see Figure 1. Irradiation of these DNA conjugates forms a base radical cation that can migrate through the duplex until it is trapped at a GG step by irreversible reaction. The site of reaction is identified by treatment of the irradiated samples with piperidine (which cleaves the DNA at damaged sites), gel electrophoresis, and autoradiography. Because these experiments are performed under single-hit (i.e., low conversion) conditions where, on average, each DNA duplex reacts once or not at all, the distance dependence of the cleavage yields reflects the ratio of rates of radical cation migration and trapping. If migration is much slower than trapping, then most reaction will occur at GG steps near the AQ; however, if migration is considerably faster than trapping, all GG steps will react with approximately the same efficiency. We assume that the rate of the trapping reaction is the same at each GG step, which permits the extraction of relative radical cation migration rates from the reactivity data.[6]

DNA(1) through DNA(10) contain two AAGGAAGGAA segments that surround a variable region that contains four base pairs. In DNA(1) through DNA(5), the AQ is linked at the 5′-end of the GG-containing strand, and this strand is labeled with ^{32}P at its 3′-terminus. For DNA(6) through DNA(10), the GG steps are in the complementary strand, and it is 5′-labeled. It is important to note that DNA(1), (6), (12), and (13) contain only purines in the GG-containing strand, which has been suggested to facilitate charge migration.[29]

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DNA	
(1-5)	5'AQ- A A G G$_1$ A A G G$_2$ A A X$_1$X$_2$X$_3$X$_4$ A A G G$_3$ A A G G$_4$ A A A A* -3' 3'- T T C C T T C C T T Y$_1$Y$_2$Y$_3$Y$_4$ T T C C T T C C T T T T^1 -5'
(6-10)	5'AQ- T T C C T T C C T T Y$_4$Y$_3$Y$_2$Y$_1$ T T C C T T C C T T T T^1 -3' 3'- A A G G$_1$ A A G G$_2$ A A X$_4$X$_3$X$_2$X$_1$ A A G G$_3$ A A G G$_4$ A A A A* -5'
11	5'AQ- A T GG$_1$ A T GG$_2$ A T GG$_3$ A T GG$_4$ A T GG$_5$ A T GG$_6$ A T A T* -3' 3'- T A C C T A C C T A C C T A C C T A C C T A C C T A T A -5'
12	5'AQ- A A GG$_1$ A A GG$_2$ A A GG$_3$ A A GG$_4$ A A GG$_5$ A A GG$_6$ A A A A* -3' 3'- T T C C T T C C T T C C T T C C T T C C T T C C T T T T -5'
13	5'AQ- T T C C T T C C T T C C T T C C T T C C T T C C T T T T -3' 3'- A A GG$_1$ A A GG$_2$ A A GG$_3$ A A GG$_4$ A A GG$_5$ A A GG$_6$ A A A A* -5'
14	5'AQ- A T A G G$_1$ A T A GG$_2$ A T A GG$_3$ A T A GG$_4$ A T A T* -3' T A T C C T A T C C T A T C C T A T C C T A T A -5'
15	5'AQ- A T T A G G$_1$ A T T A GG$_2$ A T T A GG$_3$ A T T A GG$_4$ A T A T* -3' T A A T C C T A A T C C T A A T C C T A A T C C T A T A -5'

[1]Only DNA (1,6) contain the $(A/T)_4$ sequence (shaded region at end opposite AQ), the others in this group contain an $(A/T)_2$ sequence to avoid symmetry. * = ^{32}P label.

DNA(1-5)	$X_1X_2X_3X_4/Y_1Y_2Y_3Y_4$	DNA(6-10)	$Y_4Y_3Y_2Y_1/X_4X_3X_2X_1$
(1)	$A_1A_2A_3A_4/T_1T_2T_3T_4$	(6)	$T_4T_3T_2T_1/A_4A_3A_2A_1$
(2)	$A_1T_2A_3T_4/T_1A_2T_3A_4$	(7)	$T_4A_3T_2A_1/A_4T_3A_2T_1$
(3)	$T_1A_2T_3A_4/A_1T_2A_3T_4$	(8)	$A_4T_3A_2T_1/T_4A_3T_2A_1$
(4)	$T_1A_2A_3A_4/A_1T_2T_3T_4$	(9)	$A_4T_3T_2T_1/T_4A_3A_2A_1$
(5)	$A_1T_2A_3A_4/T_1A_2T_3T_4$	(10)	$T_4A_3T_2T_1/A_4T_3A_2A_1$

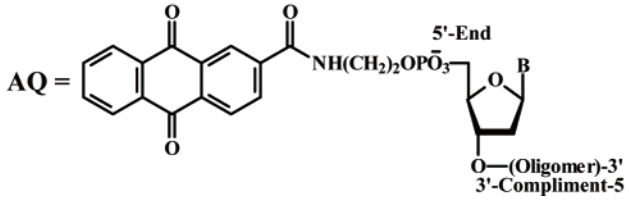

Figure 1. Structures of DNA oligomers studied in this work.

Table 1. Relative Clevage Yield at Each GG step

DNA	GG_1/GG_1	GG_2/GG_1	GG_3/GG_1	GG_4/GG_1	GG_5/GG_1	GG_6/GG_1
1	1.00	1.27	0.37	0.37		
2	1.00	1.24	0.06	0.05		
3	1.00	1.25	0.10	0.08		
4	1.00	1.10	0.08	0.08		
5	1.00	1.50	0.13	0.10		
6	1.00	1.10	0.30	0.27		
7	1.00	1.17	0.08	0.07		
8	1.00	0.95	0.04	0.09		
9	1.00	1.31	0.10	0.10		
10	1.00	0.94	0.03	0.03		
11	1.00	0.63	0.53	0.49	0.48	0.46
12	1.00	0.98	0.95	0.95	0.94	0.92
13	1.00	0.99	0.96	0.95	0.94	0.92
14	1.00	0.33	0.15	0.11		
15	1.00	0.20	0.06	0.04		

DNA(1) and DNA(6) contain an $(A/T)_8$ segment between the $(GG)_2$ and $(GG)_3$ steps. Consistent with previous findings,[24,27] radical cation migration through this segment occurs with only modest attenuation of reaction between $(GG)_2$ and $(GG)_3$ (these data are summarized in Table 1, and copies of the gels are included in the Supporting Information accompanying this paper). DNA(5) and DNA(10) are formed by "switching" a single (A/T) base pair in the $(A)_8$ segment of DNA(1) and DNA-(6) so that the sequence between $(GG)_2$ and $(GG)_3$ becomes $(A)_3(T)(A)_4$. In stark contrast to the behavior of the $(A)_8$ segment of DNA(1) and DNA(6), the $(A)_3(T)(A)_4$ segment of DNA(5) and DNA(10) provides a considerable barrier for radical cation migration. In contrast to the linear distance relationship usually observed between the log of the amount of reaction at a GG step and its distance from the site of radical cation injection, DNA(1) and DNA(6) and DNA(5) and DNA(10) show a "stepped" behavior, and the step is far larger for DNA(5) and DNA(10) than it is for DNA(1) and DNA(6); see Figure 2. Analysis of the results from investigation of DNA(1) through DNA(10) shows that this effect is general: Switching any one of several base pairs in the $(A)_8$ region of DNA(1) or DNA(6) causes formation of a significant barrier to migration from $(GG)_2$ to $(GG)_3$. This is a surprising result because there are several examples where the presence of one or more T bases on a GG-containing strand has only a modest affect on radical cation migration.[12,21,25−27,30−35]

Figure 3 shows plots of the distance dependence of strand cleavage at GG steps for DNA(11) through DNA(15). These data reveal a striking effect of specific base sequence. For DNA-(12) and DNA(13), where the GG steps are embedded in an $(AAGG)_6$ strand, there is no significant attenuation of reaction efficiency between $(GG)_1$ and $(GG)_6$, which is a distance of ca. 70 Å. This behavior indicates that charge migration in this duplex is far faster than irreversible trapping of the radical cation. In contrast, for DNA(11), where the GG steps are contained in an $(ATGG)_6$ sequence, the efficiency of strand cleavage falls off exponentially with a distance dependence (slope of the line in Figure 3) of -0.005 ± 0.001 Å^{-1}. Significantly, although a single T is a sufficient barrier to stop

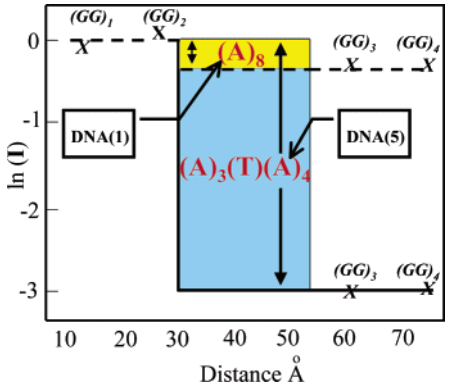

Figure 2. Illustration of the "stepped" distance dependence of strand cleavage at GG steps for DNA(1) and DNA(5) after irradiation and treatment with piperidine. The size of the "yellow" step is the natural log of the reduction in the amount of strand cleavage detected at $(GG)_2$ and $(GG)_3$ of DNA(1), which contains an $(A)_8$ sequence between these GG steps. The sum of "yellow" and "blue" steps is the natural log of the reduction in the amount of strand cleavage detected at $(GG)_2$ and $(GG)_3$ of DNA(5), which contains an $(A)_3(T)(A)_4$ sequence between these GG steps. Distance to a GG step is calculated by assuming 3.4 Å per base pair.

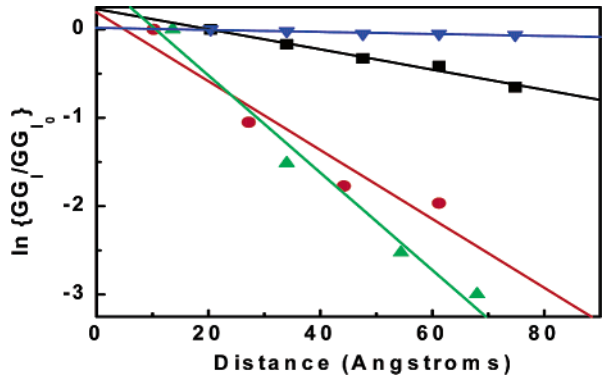

Figure 3. Plots of the distance dependence of the efficiency of strand cleavage at remote GG steps. Strand cleavage is normalized to the amount of reaction at GG_1 (except for DNA(11), for which GG_2 is used as the standard because of the unique placement of GG_1). Distance to a GG step is calculated by assuming 3.4 Å per base pair. The lines are least-squares fits of the data: blue triangles, DNA(12); black squares, DNA(11); red circles, DNA(14); and green triangles, DNA(15).

efficient radical cation migration through the variable segment of DNA(1) through DNA(10), five T bases do not prevent measurable strand cleavage at $(GG)_6$ in DNA(11). Comparable results are obtained for DNA(14) and DNA(15), where the GG steps are incorporated in $(ATAGG)_4$ and $(ATTAGG)_4$ segments and the slopes are -0.04 ± 0.007 and -0.06 ± 0.01 Å^{-1}, respectively. These findings show that the effect of a T (or multiple T bases) on radical cation migration cannot be understood without consideration of the complete sequence of bases in the duplex.

Discussion

Numerous experimental observations have now demonstrated undeniably that a radical cation introduced at one location in duplex DNA can migrate 200 Å, or more, and cause a reaction leading to strand cleavage at a remote guanine.[21,27,34,36,37] Consideration of the dynamical nature of DNA in solution led us to propose that this long-distance migration was the result of a radical cation hopping process.[38] In this view, the radical cation is trapped in a shallow minimum localized on a single base, or delocalized over several bases, and some process causes it to move from one location to the next until it is finally trapped irreversibly by reaction with H_2O. In one variant of the charge-hopping model, called "hole-resting site", the radical cation is localized on individual guanines and tunnels through bridges composed of A/T and T/A bases, within a strand or from strand-to-strand, until it is trapped at a guanine.[19,39] Although this was considered to be a general process when it was first proposed, now it is viewed to be valid only for bridges of three or fewer bases.[40] In a second possibility, the polaron-like hopping model, a structural distortion of the DNA stabilizes and delocalizes the radical cation over several bases. Migration of the charge occurs by thermal motions of the DNA and its environment (primarily the Na^+ counterions) that cause bases to be added to or removed from the polaron.[6,21,41] The key differences between these representations is that in the hole resting site model, the radical cation is confined to guanines and migrates by quantum mechanical tunneling through orbitals of the bridging A/T bases without ever residing on the bridging bases. In the polaron-like hopping representation, the radical cation resides briefly on the bases of the bridge and its migration over this barrier occurs as a consequence of thermal activation.

We arbitrarily divide the barrier to radical cation migration into two categories in order to consider the effect of base sequence in the DNA oligomers studied here. A "high" barrier to radical cation migration is one that slows the rate of transport so that the rate of reaction of the radical cation with water occurs significantly faster than hopping. In contrast, a "low" barrier to charge migration is one that permits the radical cation to migrate from DNA reactive site to reactive site considerably faster than it is trapped by reaction with water. Figure 4 presents a model that suggests the emergence of base sequence effects consistent with our experimental findings and adds further support to the polaron-hopping mechanistic proposal.

It is undoubtedly true that delocalization of a radical cation will stabilize it by lowering its energy. It is also well known that the ionization potentials of the purines (G and A) are considerably lower than for the pyrimidines (T and C). Each of the oligomers in the series DNA(2) through DNA(5) and DNA-(7) through DNA(10) contains one strand with two AAG-GAAGGAA (all-purine) segments. In contrast, the repeating all-purine segment of DNA(11) is GGA, and these segments are isolated by thymines at each end. It is reasonable that delocalization of a radical cation will be greater in the long all-purine segments of the DNA oligomers that contain the uninterrupted AAGGAAGGAA sequence than for DNA(11), in which GGA segments that are "terminated" by thymines. The thymines that separate the all-purine segments create barriers to radical cation migration. The height of barrier formed by the thymine depends on the sequence of bases preceding and following it.

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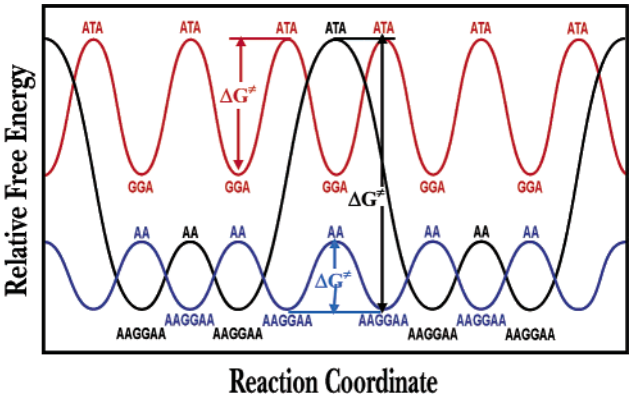

Figure 4. Reaction coordinate diagram illustrating the emergence of sequence effects in long-distance charge transport in duplex DNA. The black curve represents DNA(4), which exhibits behavior qualitatively similar to DNA(2) through DNA(5) and DNA(7) through DNA(10). In these cases, the radical cation is delocalized and stabilized in polarons, identified arbitrarily here as AAGGAA sequences in the AAGGAAGGAA segments surrounding the ATA in the variable region. This delocalization of the radical cation stabilizes it and results in a high barrier ($\Delta G^{\ddagger}$) at the ATA sequence; trapping of the radical cation by water occurs much faster than this barrier can be crossed. For DNA(12) and DNA(13), the blue curve, the same AAGGAA polaron is identified and there are no thymines that create a high barrier for hopping from one polaron to the next, which occurs faster than trapping by water. The red curve represents DNA(11), an intermediate case where the polaron is assumed to be the GGA sequence, which is less delocalized and therefore higher in energy than AAGGAA. Consequently, the barrier introduced by the ATA sequence is lower than for DNA(4) and the rate of crossing this barrier is comparable with reaction of the radical cation with water.

As illustrated in Figure 4, the barrier to radical cation migration imposed by a thymine in an ATA sequence is lower for DNA(11) than it is for oligomers containing AAGGAAGGAA segments [DNA(2) through DNA(5) and DNA(7) through DNA(10)] because the GGA polaron identified for DNA(11) is smaller (less delocalized) and therefore of higher energy than that formed in the longer all-purine segments. Migration of the polaron over the low barrier in the case of DNA(11) is competitive with trapping by water (slope of the line in Figure 3 is -0.005 ± 0.001 Å^{-1}). For DNA(2) through DNA(5) and DNA(7) through DNA(10), the barrier created by one or two thymine bases is sufficiently high that the migration of the radical cation is much slower than its reaction with water and the stepped behavior of Figure 2 is observed.

The results from investigation of DNA(12) through DNA(15) are consistent with this model and provide further support for it. DNA(12) and DNA(13) each contain a strand with an $(AAGG)_6$ sequence. Consequently, there are six GG steps embedded in all-purine segments that have no thymines to create barriers to charge migration. In these cases, within experimental error, there is no distance dependence to the reaction of the radical cation (the slope for these oligomers in Figure 3 is indistinguishable from zero). In other words, the reaction of the radical cation with water at each GG step in DNA(12) and DNA(13) is equally probable, which indicates that migration is far faster than is trapping with water.

DNA(14) and DNA(15) contain strands with AGGA segments separated by T and TT steps, respectively. The all-purine segments in these cases are longer than those in DNA(11), and this results in greater stabilization of the polaron and a concomitant higher barrier to charge migration; see Figure 4. The relative barrier heights are reflected in the slopes of the lines in Figure 3: for DNA(14), when one T forms the barrier, the slope is -0.04 ± 0.007 Å^{-1}, and for DNA(15), when a TT step forms the barrier, the slope is -0.06 ± 0.01 Å^{-1}. If a radical cation in DNA is localized on only one base,[42] then the barrier to crossing the thymine barriers would not depend on the sequence of neighboring bases, which is clearly inconsistent with the findings reported here.

Inclusion of charge delocalization is the distinguishing feature of the polaron-like hopping model.[6,21] Delocalization is excluded in the short tunneling steps between "hole resting sites" model, which is built by assuming that charge localized on G bases does not reside on "bridges" composed of A and T bases.[19,20,40] The limit of charge delocalization is not revealed by these experiments. A recent quantum calculation of vertically ionized d(GAGG)/d(CTCC) in aqueous NaCl solution shows that the radical cation is delocalized over all three G bases of this duplex.[4] The experiments we report here demonstrate that the delocalization of a radical cation over at least a portion of the all-purine strands of duplex DNA is necessary to accommodate findings from the sequence dependence of radical cation migration efficiencies. For DNA(12) and DNA(13), which show essentially no distance dependence, it could be that this delocalization occurs over all 24 bases of the $(AAGG)_6$ strand, a possibility that seems unlikely. However, it is clear that models for long-distance radical cation transport in duplex DNA that require localization of charge on one base are inconsistent with these results.

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Supporting Information Available: Autoradiograms of PAGE results (15) used in the analysis of strand cleavage for DNA(1) through DNA(15). This material is available free of charge via the Internet at http://pubs.acs.org.

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