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Sequence Dependence of Charge Transport through DNA Domains

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Abstract: Here we examine the photooxidation of two kinetically fast electron hole traps, N_4-cyclopropylcytosine (^{CP}C) and N_2-cyclopropylamine-guanosine (^{CP}G), incorporated in DNA duplexes of various sequence using different photooxidants. DNA oxidation studies are carried out either with noncovalently bound $[Ru(phen)(dppz)(bpy')]^{3+}$ (dppz = dipyridophenazine) and $[Rh(phi)_2(bpy)]^{3+}$ (phi = phenanthrenequinone diimine) or with anthraquinone tethered to DNA. Because the cyclopropylamine-substituted bases decompose rapidly upon oxidation, their efficiency of decomposition provides a measure of relative hole localization. Consistent with a higher oxidation potential for ^{CP}C versus ^{CP}G in DNA, ^{CP}C decomposes with photooxidation by $[Rh(phi)_2(bpy)]^{3+}$, while ^{CP}G undergoes ring-opening both with photoexcited $[Rh(phi)_2(bpy)]^{3+}$ and with $[Ru(phen)(dppz)(bpy')]^{3+}$. Anthraquinone-modified DNA assemblies of identical base composition but different base sequence are also probed. Single and double base substitutions within adenine tracts modulate ^{CP}C decomposition. In fact, the entire sequence within the DNA assembly is seen to govern ^{CP}C oxidation, not simply the bases intervening between ^{CP}C and the tethered photooxidant. These data are reconciled in the context of a mechanistic model of conformationally gated charge transport through delocalized DNA domains. Photooxidations of anthraquinone-modified DNA assemblies containing both ^{CP}C and ^{CP}G, but with varied distances separating the modified bases, point to a domain size of at least three bases. Our model for DNA charge transport is distinguished from polaron models. In our model, delocalized domains within the base pair stack form transiently based upon sequence-dependent DNA structure and dynamics. Given these results, DNA charge transport is indeed remarkably sensitive to DNA sequence and structure.

Introduction

Charge transport (CT) through double-helical DNA to promote oxidative damage from a distance has been demonstrated through biochemical and spectroscopic assays in many DNA assemblies containing different pendant photooxidants.[1−6] The DNA base pair stack mediates charge transport over at least 200 Å,[7,8] and the reaction is remarkably sensitive to the dynamic structure and stacking within the DNA duplex.[2,9] Given this exquisite sensitivity to stacking, DNA-mediated CT chemistry uniquely provides a means to detect anomalies in the base pair stack. Thus, DNA CT has provided the basis for novel electrochemical sensors for mutational analysis,[10] and indeed, DNA CT may play a role in the detection of mismatches and lesions within the cell.[11]

While DNA charge transport is now well accepted based upon experiment, mechanistic descriptions of how charge migration through DNA proceeds are still not well established. Physical measurements have been used to characterize DNA as a wide band gap semiconductor,[12−14] but in many of these studies, the integrity of the base pair stack has been unclear. Mechanistic descriptions have focused on one-step superexchange-mediated tunneling or incoherent multistep hopping of localized charge (generally holes).[4,15−17] The fundamental difference between

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these two mechanisms is whether the charge actually occupies the DNA bridge.[18] For CT through donor−bridge−acceptor systems, occupancy of the bridge depends on the energetic barrier for charge injection from the donor to the bridge.[19] More recently, models of incoherent CT have been considered as involving a mixture of localized charge hopping among low-energy sites, guanines and sometimes adenines, and tunneling through higher energy pyrimidine bases.[20] These mechanisms do not provide a rationale, however, for the sensitivity of CT to DNA stacking.

We have found that DNA CT is gated by the dynamical motions of the DNA bases[21] and have described DNA CT as conformationally gated hopping through transient, well-stacked DNA domains.[22] Dynamical motion is, in fact, necessary to promote CT through the base stack; experiments at 77 K show no detectable CT between bases.[23]

Most recently, we have obtained experimental evidence in support of partial delocalization using cyclopropylamine-substituted bases as fast hole traps to monitor radical occupation on the DNA bridge.[24] Despite a significant difference in oxidation potential between purines and pyrimidines, within the base pair stack we observe comparable efficiency of oxidation of N_4-cyclopropylamine-cytosine (^{CP}C) and a neighboring N_2-cyclopropylamine-guanosine (^{CP}G). This comparable reactivity points to orbital mixing between these neighboring bases in the stack. Delocalization clearly does not occur over the entire duplex, however. Spectroscopic investigations with 2-aminopurine of base−base CT as a function of bridge length and temperature show evidence for a domain size of about four base pairs.[22] It should be noted that a partially delocalized model for DNA CT was also recently supported by calculations of a variable-range hopping model, in which it was found that delocalized bridge states are required to account for experimental yields of DNA hole transport.[25]

Thus, we have considered that the extent of delocalization within the DNA duplex depends on the sequence-dependent dynamics of DNA. We distinguish our model of conformationally gated delocalized domains from polaron models[3,26,27] for charge transport, since polarons are expected to form as a result of structural distortions *in response* to the migrating charge.[26] We consider instead that it is the sequence-dependent motions of DNA that lead to delocalized domains that form and break up transiently, facilitating and limiting CT.

Here we look in more detail at the energetics and sequence dependence of CT using cyclopropylamine-substituted bases as fast kinetic traps to probe hole occupation. These cyclopropylamine-modified bases allow us to examine how domains vary as a function of base sequence, position, and length, and perhaps they will also allow us to distinguish dynamical domains from polarons. Certainly these data allow us to characterize features of the rich sequence-dependent dynamical structure of DNA and its consequences with respect to DNA CT.

Experimental Section

Synthesis and Characterization of the Oligonucleotides and Photooxidants. (a) Oligonucleotides Containing ^{CP}C and ^{CP}G. All DNA oligonucleotides containing ^{CP}C and ^{CP}G were synthesized with the terminal dimethoxytrityl group intact on an Applied Biosystems 394 DNA synthesizer, using 4-thio-uracil and 2-fluoro-inosine phosphoramidites as the ^{CP}C and ^{CP}G precursors, respectively.[24,28] The DNA resin was reacted with 1 M diaza(1,3)bicyclo[5.4.0]undecane (DBU) in acetonitrile in order to remove the protecting groups on the precursor bases. The DNA oligonucleotides were incubated for 16 h in 6 M aqueous cyclopropylamine at 60 °C and simultaneously deprotected and cleaved from the resin. The cleaved DNA strands were dried in vacuo and resuspended in buffer prior to purification by HPLC. Following the first purification, the dimethoxytrityl group was removed with 80% acetic acid and the strands were repurified by HPLC. MALDI-TOF mass spectrometry was used to characterize the strands.

(b) Anthraquinone-Tethered Oligonucleotides. An anthraquinone derivative [anthraquinone-2-carboxylic acid (2-hydroxyethyl) amide, AQ] was synthesized and converted into its respective phosphoramidite.[29] The AQ phosphoramidite was incorporated onto the 5′ end of the DNA oligonucleotides employing a 15 min coupling time. The DNA was deprotected, cleaved from the resin overnight at 60 °C in ammonium hydroxide, and dried in vacuo. The resulting oligonucleotides were purified once by HPLC and characterized by MALDI-TOF mass spectrometry.

(c) DNA Duplexes. The DNA oligonucleotides were suspended in a buffer containing 50 mM NaCl and 20 mM sodium phosphate, pH 7.0, and quantified by UV−visible spectroscopy. DNA duplexes were annealed by combining equal moles of the desired DNA complements in the buffer and heating at 90 °C for 5 min, followed by cooling to ambient temperature over 2 h. Melting temperatures were determined for all substituted DNA duplexes prepared. Cyclopropylamine substitution on the DNA bases leads to changes in melting temperature of <2 °C, indicating that substitution causes little destabilization of the duplex.[24]

(d) Metal Photooxidants. The metal complexes $[Ru(phen)(dppz)(bpy')]Cl_2$ (phen = 1,10-phenanthroline, dppz = dipyrido[3,2-a:2′,3′-c]phenazine, bpy′ = 4-(4′-methyl-2,2′-bipyridyl) valerate) and $[Rh(phi)_2(bpy)]Cl_3$ (phi = phenanthrenequinone diimine) were synthesized as previously described[30] and characterized by ESI mass spectrometry and ^{1}H NMR.

Photooxidations. For ruthenium oxidations, aliquots (30 μL) contained 5 μM DNA duplex, 5 μM $[Ru(phen)(dppz)(bpy')]^{2+}$, and 50 μM $[Ru(NH_3)_6]Cl_3$. Anaerobic samples were prepared by utilizing the freeze−pump−thaw method in airtight cuvettes under Ar. Flash-quench-generated oxidation was accomplished by irradiation for 0−30 min using a Liconix He:Cd laser (~12 mW) at 442 nm. For rhodium oxidations, aliquots contained 5 μM DNA duplex and 5 μM $[Rh(phi)_2(bpy)]^{3+}$. Rhodium samples were irradiated for 0−10 min at 365 nm,

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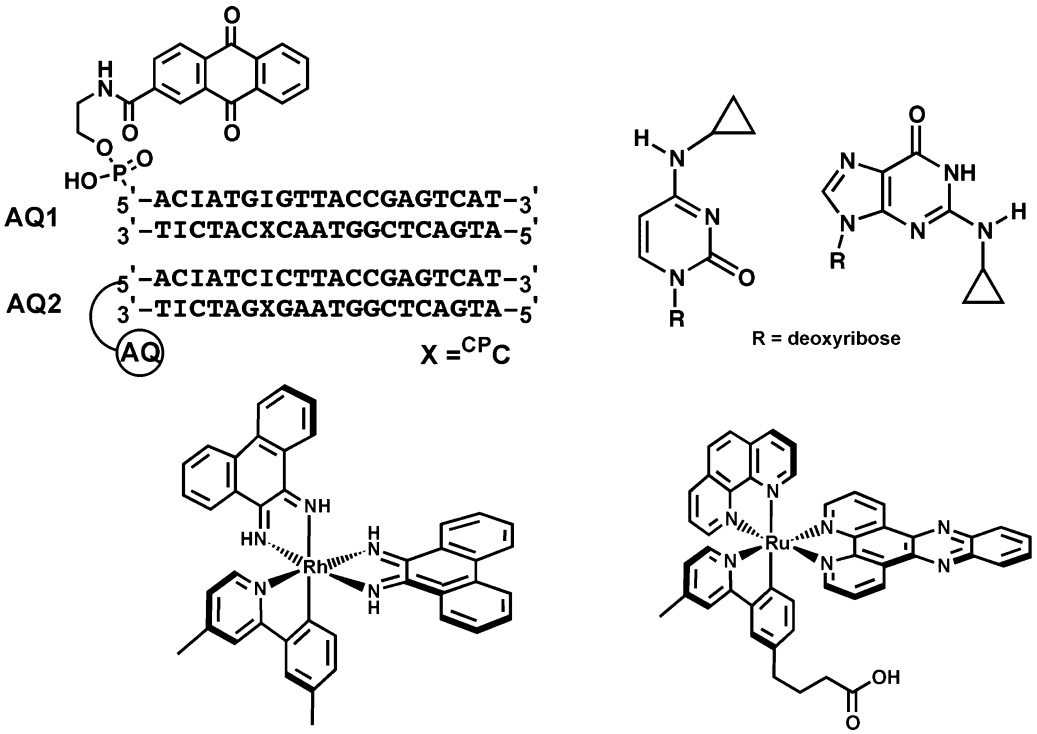

Figure 1. Shown are some DNA assemblies used to explore DNA CT as well as photooxidants and modified bases as hole traps. In **AQ1** and **AQ2**, an anthraquinone derivative is covalently tethered through the phosphate backbone to the 5′ end of the complements to ^{CP}C-containing DNA strands. The sequences of the DNA assemblies are shown either with the structure of the photooxidant in **AQ1** or schematically in **AQ2**. The structures of ^{CP}C, ^{CP}G, $[Ru(phen)(dppz)(bpy')]^{2+}$, and $[Rh(phi)_2(bpy)]^{3+}$ are also shown.

using a 1000 W Hg/Xe lamp equipped with a 320 nm long pass filter and a monochromator. For anthraquinone−DNA oxidations, aliquots of 10 or 15 μM AQ-tethered duplexes were irradiated at 350 nm for 0−10 min, using the same apparatus as for the rhodium oxidations.

HPLC Analysis of Base Products. Following irradiation, the samples were digested by phosphodiesterase I and alkaline phosphatase at 37 °C for 4.5−24 h to generate individual nucleosides. Reverse-phase HPLC (Chemcobond 5-ODS-H, 4.6 mm × 100 mm) was applied to analyze the oxidation of ^{CP}C and ^{CP}G nucleosides. Oxidation yields of ^{CP}C and ^{CP}G were determined by the peak area from HPLC analysis normalized to that of thymidine. All photooxidations were carried out at least three times, and the results were averaged.

Results

Design of DNA Assemblies. A range of DNA assemblies was prepared that contain tethered AQ on one strand and either or both ^{CP}G and ^{CP}C on the other strand. In these assemblies, it is considered that AQ stacks at the end of the helix. Thus the photooxidant, AQ, is spatially well separated from the hole traps, ^{CP}G and ^{CP}C. To limit competition with hole trapping at guanine sites and therefore to provide the largest possible window through which to monitor decomposition of ^{CP}C, inosines, rather than guanosines, are utilized at many sites in the duplexes, particularly base-paired to ^{CP}C. Figure 1 shows two representative assemblies. We also examined photooxidation of DNA assemblies containing ^{CP}C and ^{CP}G using the noncovalent intercalators, $[Ru(phen)(bpy')(dppz)]^{3+}$ and $[Rh(phi)_2(bpy)]^{3+}$.

Photooxidation of ^{CP}C in 5′-C^{CP}CC-3′ and 5′-G^{CP}CG-3′. To probe the effects of the flanking bases in a DNA duplex, two assemblies, **AQ1** and **AQ2**, were designed (Figure 1). **AQ1** and **AQ2** are similar in sequence, except that in **AQ1** a 5′-C^{CP}-CC-3′ segment is placed six base pairs away from the AQ-tethered end, while in **AQ2** a 5′-G^{CP}CG-3′ segment is at the corresponding position. ^{CP}C decomposes efficiently after 10 min of irradiation in both assemblies: 92% in **AQ1** and 96% in **AQ2**. Moreover, as shown in Figure 2, ^{CP}C in **AQ1** and **AQ2** have similar but not identical decomposition profiles over time. When the two flanking bases are guanines, ^{CP}C decomposes slightly more efficiently as compared to the case when ^{CP}C is flanked by two cytosines. Thus, flanking bases have a small but significant effect on ^{CP}C decomposition.

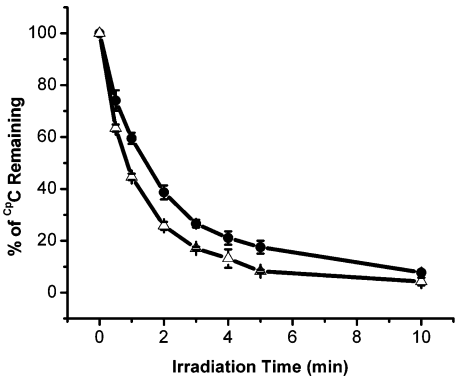

Figure 2. Plot of the % of ^{CP}C remaining in **AQ1** (●) and **AQ2** (△) as a function of irradiation time. Standard errors based upon three trials are shown. ^{CP}C is flanked by cytosines in **AQ1** and guanines in **AQ2**, while the remainder of the sequence is identical in both assemblies.

Variation in Sequences across Strands. To probe variations between the complementary strands, we utilized four assemblies, **AQ3** through **AQ6**, as shown in Table 1. All four assemblies are identical with respect to base content and position but are different with respect to the sequence of their single strands. In all cases, ^{CP}C is located on the complement to the AQ-tethered strand, nine bases away, and is base-paired to inosine (I). ^{CP}C−I is flanked by four AT base pairs on either side. In **AQ3**, the

Table 1. Effect of A_n and T_n Tracts on ^{CP}C Decomposition in DNA Duplexes

DNA #	Sequence[a,b]	% Decomposition of ^{CP}C[c,d]
AQ3	5′-AQ- ACIATTTT ITTTTCCAGTCAT -3′ 3′- TICTAAAA^{CP}CAAAAGGTCAGTA -5′	88
AQ4	5′-AQ- ACIAAAAA IAAAACCAGTCAT -3′ 3′- TICTTTTT^{CP}CTTTTGGTCAGTA -5′	13
AQ5	5′-AQ- ACIATTTT IAAAACCAGTCAT -3′ 3′- TICTAAAA^{CP}CTTTTGGTCAGTA -5′	92
AQ6	5′-AQ- ACIAAAAA ITTTTCCAGTCAT -3′ 3′- TICTTTTT^{CP}CAAAAGGTCAGTA -5′	71

[a] AQ refers to the anthraquinone derivative as described in the Experimental Section. [b] Melting temperatures for duplexes are 51 ± 1 °C. [c] Following 5 min of irradiation. Duplexes (5 μM) were irradiated in 50 mM NaCl and 20 mM sodium phosphate buffer. Details are in the Experimental Section. [d] Data are averaged over at least three data sets. Deviations are less than 5%.

four-base segments surrounding ^{CP}C are all adenines, whereas in **AQ4** they are all thymines. **AQ5** has ^{CP}C flanked by one adenine segment, A_4, and one thymine segment, T_4, while A_4 and T_4 are reversed in **AQ6**.

AQ3 and **AQ5**, both containing an A_4 tract in front of ^{CP}C, have similar decomposition efficiencies of 88% and 92%, respectively. However, both assemblies show greater reactivity on ^{CP}C than in **AQ4** and **AQ6**, both of which contain a proximal T_4. In fact, **AQ4** has both a proximal and a distal T_4 segment, and the ^{CP}C decomposition is significantly diminished to only 13%, one-seventh that in **AQ5**. Replacing the distal T_4 with A_4 in **AQ6** results in a 5-fold increase in ^{CP}C decomposition. It is therefore apparent that the full sequence of DNA, both before and after the kinetic trap, affects the efficiency of hole trapping.

Reactivity in Adenine Tracts. The effects of repetitive adenines are monitored using two sets of assemblies, as shown in Table 2. The first type of perturbation we examined is a single base substitution within the A-tract. In **AQ7**, ^{CP}C is placed after the sixth base in an eight base-pair adenine tract. The structural coherence of the adenine tract is then disrupted by replacing the fourth adenine with either a T, G, or C in **AQ8**, **AQ9**, and **AQ10**, respectively. As is evident, trapping reactivity is mildly affected by a single base interruption in adenine stacking. Following irradiation, **AQ8**, **AQ9**, and **AQ10** show somewhat less decomposition of ^{CP}C than **AQ7**, in which the A-tract remains intact. Interestingly, the base substitution with the most pronounced effect in diminishing the decomposition of ^{CP}C is guanine in **AQ9**; **AQ9** shows only 72% decomposition of ^{CP}C after 5 min of irradiation, as compared with 91% for **AQ7**. This result may reflect the lower oxidation potential of guanine. Upon irradiation, **AQ8** and **AQ10** with T and C substitutions, respectively, show similar efficiencies of ^{CP}C decomposition (81% and 78%).

In addition to a single base substitution, the coherence of the six-adenine tract preceding ^{CP}C can be disrupted by decreasing the number of adenine doublets in the A-tract. In **AQ11**, **AQ12**, and **AQ13**, the six adenines preceding ^{CP}C are modified to contain either two (**AQ11**), one (**AQ12**), or no (**AQ13**) adenine doublets. As shown in Table 2, **AQ7**, with three adenine doublets, yields more decomposition of ^{CP}C than **AQ11**, **AQ12**, and **AQ13**, which contain two, one, or no adenine doublets in the proximal A-tract. When no adenine doublets are present,

Table 2. Effect of Coherence of the A-Tract on ^{CP}C Decomposition in DNA Duplex

DNA #	Sequence[a,b]	% Decomposition of ^{CP}C[c,d]
AQ7	5′-AQ- ACIATTTTTT ITTCCAGTCAT -3′ 3′- TICTAAAAAA^{CP}CAAGGTCAGTA -5′	91
AQ8	5′-AQ- ACIATTTATT ITTCCAGTCAT -3′ 3′- TICTAAATAA^{CP}CAAGGTCAGTA -5′	81
AQ9	5′-AQ- ACIATTTCTT ITTCCAGTCAT -3′ 3′- TICTAAAGAA^{CP}CAAGGTCAGTA -5′	72
AQ10	5′-AQ- ACIATTTGTT ITTCCAGTCAT -3′ 3′- TICTAAACAA^{CP}CAAGGTCAGTA -5′	78
AQ11	5′-AQ- ACIATTAATT ITTCCAGTCAT -3′ 3′- TICTAATTAA^{CP}CAAGGTCAGTA -5′	85
AQ12	5′-AQ- ACIATATATT ITTCCAGTCAT -3′ 3′- TICTATATAA^{CP}CAAGGTCAGTA -5′	71
AQ13	5′-AQ- ACIATATATA ITTCCAGTCAT -3′ 3′- TICTATATAT^{CP}CAAGGTCAGTA -5′	41

[a] AQ refers to the anthraquinone derivative as described in the Experimental Section. [b] Melting temperatures for duplexes are 50 ± 2 °C. [c] Following 5 min of irradiation. Duplexes (5 μM) were irradiated in 50 mM NaCl and 20 mM sodium phosphate buffer. Details are in the Experimental Section. [d] Data are averaged over at least three data sets. Deviations are less than 5%.

decomposition of ^{CP}C is significantly reduced to 41% in **AQ13**, compared with 91% in **AQ7**. When one or two adenine doublets are present, decomposition is mildly reduced (85% for **AQ11** and 71% for **AQ12**).

Competition between ^{CP}C and ^{CP}G. To investigate the domain size in an adenine tract, sequences **AQC0**−**AQC5** were designed. All assemblies contain a seven base-pair adenine tract and have one ^{CP}C and ^{CP}G separated by various numbers of adenines (0−5) within the tract. Although the position of ^{CP}C remains fixed after the second adenine in the tract, ^{CP}G is placed at distances further along the tract, as shown in Table 3.

The decomposition of ^{CP}C in all six assemblies shows similar efficiencies, ranging from 77% to 89%. A small increase in decomposition of ^{CP}C occurs when ^{CP}G is moved two or more adenines away. In contrast, ^{CP}G decomposition can be divided into two distinct regions, the efficient region seen with **AQC0** and **AQC1** assemblies versus the inefficient region found with **AQC2**, **AQC3**, **AQC4**, and **AQC5**, as shown in Figure 3. ^{CP}G is most efficiently decomposed in **AQC0**, where ^{CP}G is neighboring ^{CP}C. A 10% decrease in decomposition of ^{CP}G is observed in **AQC1**, in which ^{CP}G is moved one adenine base away from ^{CP}C. In **AQC2**−**AQC5**, in which ^{CP}G is at least two adenines away from ^{CP}C, the decomposition of CPG is dramatically diminished to about 50%, regardless of the position of ^{CP}G. A similar but less pronounced trend is observed when these experiments are repeated using the **AQG*n*** series, which have the same sequence as the corresponding **AQC*n***, but with the positions of ^{CP}G and ^{CP}C switched (data not shown). It should be noted in Table 3 that the decomposition ratio of the two traps is inverted after more than one adenine is inserted between ^{CP}G and ^{CP}C. When ^{CP}G is next to ^{CP}C in **AQC0**, there is more decomposition of ^{CP}G than ^{CP}C. In **AQC1**, decomposition profiles of ^{CP}C and ^{CP}G overlap. Decomposition of ^{CP}C becomes more pronounced than on ^{CP}G in **AQC2**−**AQC5**, in which ^{CP}G is two or more adenines away from ^{CP}C.

Table 3. Sequence and Base Decomposition in Assemblies Containing ^{CP}C and ^{CP}G

DNA #	Sequence[a,b]	% Decomposition of ^{CP}C and ^{CP}G[c]
AQC0	5′-AQ- ACIATT I CTTTTTACCGAGTCAT -3′ 3′- TICTAA^{CP}C^{CP}GAAAAATGGCTCAGTA -5′	79[d]/88[e]
AQC1	5′-AQ- ACIATT IT CTTTTACCGAGTCAT -3′ 3′- TICTAA^{CP}CA^{CP}GAAAATGGCTCAGTA -5′	77/78
AQC2	5′-AQ- ACIATT ITT CTTTACCGAGTCAT -3′ 3′- TICTAA^{CP}CAA^{CP}GAAATGGCTCAGTA -5′	88/50
AQC3	5′-AQ- ACIATT ITTT CTTACCGAGTCAT -3′ 3′- TICTAA^{CP}CAAA^{CP}GAATGGCTCAGTA -5′	83/43
AQC4	5′-AQ- ACIATT ITTTT CTACCGAGTCAT -3′ 3′- TICTAA^{CP}CAAAA^{CP}GATGGCTCAGTA -5′	89/48
AQC5	5′-AQ- ACIATT ITTTTT CACCGAGTCAT -3′ 3′- TICTAA^{CP}CAAAAA^{CP}GTGGCTCAGTA -5′	88/46

[a] AQ refers to the anthraquinone derivative as described in the Experimental Section. [b] Melting temperatures for duplexes are 55 ± 2 °C. [c] Data are averaged over at least three data sets. Deviations are less than 5%. [d] Decomposition of ^{CP}C after 5 min of irradiation. Duplexes (10 μM) were irradiated in 50 mM NaCl and 20 mM sodium phosphate buffer. Details are in the Experimental Section. [e] Decomposition of ^{CP}G after 5 min of irradiation. Duplexes (10 μM) were irradiated in 50 mM NaCl and 20 mM sodium phosphate buffer. Details are in the Experimental Section.

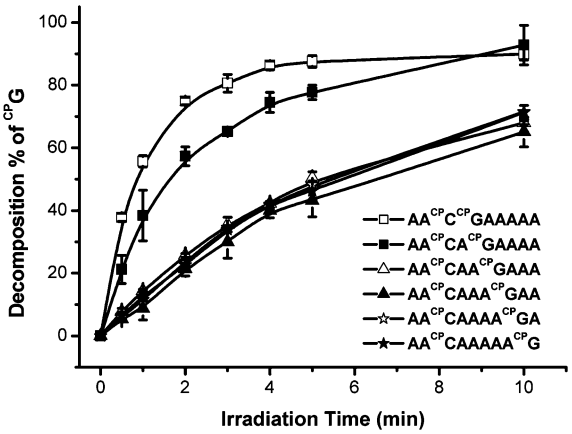

Figure 3. Plot of the % decomposition of ^{CP}G in **AQC*n*** assemblies as a function of irradiation time. In **AQC*n*** assemblies, ^{CP}G is placed *n* adenines away from ^{CP}C, while the position of ^{CP}C is fixed. The data with standard errors are shown as **AQC0** (□), **AQC1** (■), **AQC2** (△), **AQC3** (▲), **AQC4** (☆), and **AQC5** (★).

Decomposition of ^{CP}C and ^{CP}G in DNA Assemblies by Noncovalently Bound Oxidants. To vary the energy of the photooxidant relative to that of the isolated bases, a ground-state ruthenium photooxidant with known potential was used to probe the DNA duplex ring-opening reaction. Both the rhodium and anthraquinone photooxidants have excited-state potentials higher than those of the individual bases,[31,32] whereas ruthenium(III) has a potential sufficient to oxidize only guanine.[33] As shown in Table 4, sequences containing either ^{CP}C(**C-1**) or ^{CP}G (**G-2**) in roughly the same position within the oligonucleotide were irradiated at 442 nm in the presence of the $[Ru(phen)(dppz)(bpy')]^{2+}$ complex and $[Ru(NH_3)_6]^{3+}$ quencher in order to initiate oxidation via the flash-quench technique.[34]

First we consider oxidative decomposition by $[Ru(phen)(dppz)(bpy')]^{3+}$. Since singlet oxygen is generated upon photolysis of the ruthenium complex in the absence of quencher,[35,36] and singlet oxygen can potentially contribute to ring opening, all ruthenium samples were irradiated under anaerobic conditions. When oxygen is eliminated from the system, damage patterns solely resulting from charge-transfer events are revealed.[37] Table 4 shows that considerable ring opening occurs only with quencher in **G-2**, which contains ^{CP}G, while the ^{CP}C in **C-1** remains essentially intact. When no quencher is added to the irradiated samples, a small amount of ring opening occurs in **G-2** but not **C-1**. ^{CP}C, incorporated in DNA, shows little reaction with $[Ru(phen)(dppz)(bpy')]^{2+}$ in the presence or absence of quencher. In contrast, ^{CP}G decomposes completely within 30 min of irradiation in the presence of $[Ru(phen)(dppz)(bpy')]^{2+}$ and quencher and to a small extent if the quencher is excluded.

For comparison, we also examined base decomposition using noncovalent $[Rh(phi)_2(bpy)]^{3+}$ as the photooxidant, since the rhodium complex is a far more potent photooxidant. As shown in Table 4, ^{CP}G in **G-2** decomposes completely after 10 min of irradiation. Decomposition of ^{CP}C in **C-1** is also significant, 58%, as compared to the case of ruthenium. Although the decomposition of ^{CP}C is less pronounced than that of ^{CP}G, both of the cyclopropylamine bases are oxidized by $[Rh(phi)_2(bpy)]^{3+}$.

Discussion

Application of Fast Hole Traps as a Measure of Hole Delocalization. The experiments described here provide a sensitive assay for radical occupation in the DNA bridge during the course of charge transport. Most mechanistic studies of long-range oxidative DNA damage have utilized guanine damage as a reporter of the efficiency of charge transport.[1−5] However, these studies actually measure the yield of a mixture of irreversible guanine oxidation products several steps removed from the guanine radical. In fact, the guanine radical lifetime itself is quite long (milliseconds),[34] and on that time scale other reactions, including back electron transfers,[38] may proceed. Here instead we utilize a kinetically fast hole trap, cyclopropylamine-substituted cytosine and guanosine. Although the kinetics of ring-opening upon oxidation within DNA have not yet been measured, model studies suggest the ring-opening time to be on the time scale of 10^{-11} s.[39,40] It is because of this fast time scale for ring-opening that the cyclopropylamine-substituted bases can provide a snapshot of radical occupation during the

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(37) In the presence of oxygen, ^{CP}C and ^{CP}G ring opening is observed with and without $[Ru(NH_3)_6]^{3+}$ quencher, although the effect is more pronounced in the case of ^{CP}G. As ruthenium is a known sensitizer for singlet oxygen, this effect can be eliminated if the samples are irradiated under argon.
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Table 4. Percent ^{CP}C and ^{CP}G Decomposition with Noncovalently Bound $[Ru(phen)(dppz)(bpy')]^{2+}$ and $[Rh(phi)_2(bpy)]^{3+}$

	Sequence[a]	Photooxidant		% Decomposition of ^{CP}C or ^{CP}G[b]
		$[Rh(phi)_2(bpy)]^{3+}$		57.6[c]
C-1	3′-TGCTCGGCATCAGT^{CP}CGGCATA-5′ 5′-ACGAGCCGTAGTCA GCCGTAT-3′	$[Ru(phen)(dppz)(bpy')]^{2+}$[d]	-Q[e]	2.5[f]
			+Q	4.8
		$[Rh(phi)_2(bpy)]^{3+}$		100
G-2	3′-TGCTCGGCATCAGTCG^{CP}GCATA-5′ 5′-ACGAGCCGTAGTCAGC CGTAT-3′	$[Ru(phen)(dppz)(bpy')]^{2+}$[d]	-Q	9.1
			+Q	87.5

[a] DNA strands were synthesized as described in the Experimental Section. [b] Ru photooxidations are averaged over at least three data sets. Deviations are less than 10%. [c] Amount of cyclopropylamine-modified nucleoside remaining after 10 min of irradiation. Duplexes (5 μM) were irradiated with 5 μM $[Rh(phi)_2(bpy)]^{3+}$ in 50 mM NaCl and 20 mM sodium phosphate buffer. Details are in the Experimental Section. [d] All ruthenium experiments were carried out anaerobically under Ar. See Experimental Section for details. [e] Q refers to quencher, $Ru(NH_3)_6^{3+}$. Details are in the Experimental Section. [f] Amount of cyclopropylamine-modified nucleoside remaining after 30 min of irradiation. Duplexes (5 μM) were irradiated in 50 mM NaCl and 20 mM sodium phosphate buffer. Concentration of $[Ru(phen)(dppz)(bpy')]^{2+}$ is 5 μM, and that of $Ru(NH_3)_6^{3+}$ is 50 μM if added. Details are in the Experimental Section.

course of charge transport. Significantly, these measurements can be made in solution under physiological conditions on well-characterized DNA duplexes.

Indeed, cyclopropylamine-substituted adenosines in DNA were first used to establish that charge transport through DNA cannot involve hopping only among low-energy guanine sites.[41] Our own first studies using ^{CP}C showed furthermore that hole occupation is not restricted to purines in DNA.[24] Instead, a significant population of radical density on the cytosines must also occur, despite the relatively high oxidation potential of isolated pyrimidine nucleosides. Because of these experiments, we have proposed that radicals must delocalize within transient domains of the DNA duplex, domains that include pyrimidines as well as purines. The relative efficiencies of decomposition of the cyclopropylamine-substituted bases can therefore be used to probe these domains and the relative extent of hole delocalization into domains as a function of sequence.

A consideration in utilizing such trapping chemistry is whether the trapping reaction itself serves as a driver, thus perturbing rather than reporting upon hole occupation. The range of efficiencies reported here as a function of even subtle variations in sequence indicates that the ring-opening reaction cannot be driving hole transport; if that were the case, decomposition efficiencies would all be the same. Instead we can, therefore, utilize the reaction as a reporter of hole occupancy and thus, for ^{CP}C, of hole delocalization.

Energetic Considerations. It has been proposed that flanking bases can serve to modulate oxidation potentials.[42] Based upon theoretical calculations by Voityuk et al.,[42] a hole on the middle cytosine in the trinucleotide, 5′-CCC-3′, has an energy ~0.5 eV higher than that in 5′-GCG-3′. Here we have compared the decomposition efficiency of ^{CP}C in the 5′-C^{CP}CC-3′ and 5′-G^{CP}-CG-3′ segments, and we find that ^{CP}C decomposes slightly more efficiently with flanking guanines. The result is, then, not inconsistent with the calculation. The magnitude of energy lowering of cytosines by flanking bases, nonetheless, is not sufficient to account for the similarity in efficiency of decomposition of ^{CP}C and ^{CP}G within the duplex.

In an effort to obtain some limit on the extent of stabilization of cytosines within DNA, we examined ^{CP}C ring opening by two noncovalently bound oxidants, a ruthenium(III) oxidant, generated in situ by flash-quench, and our rhodium photooxidant, $[Rh(phi)_2(bpy)]^{3+}$. With a reduction potential of 1.6 eV, the Ru(III) complex is able to promote oxidation of guanine in DNA;[33] with rhodium as a photooxidant (>1.9 eV), all bases can be oxidized.[31]

With the noncovalently bound intercalators, consistent with these potentials, both are able to promote ring opening of ^{CP}G in **G-2**. However, only the rhodium complex can promote efficient decomposition of ^{CP}C in **C-1**. These results suggest that, while the energy of ^{CP}C may be lowered significantly owing to delocalization, the energy of ^{CP}C is still higher than that of ^{CP}G.[43]

Domain Formation Is Sensitive to DNA Sequence. Although some experimental results showing the sequence and distance dependence in DNA-mediated CT can be rationalized using models based upon energetics alone,[4,15] others do not directly fit these models.[9,45,46,47] Our experiments utilizing ^{CP}C as a probe of hole occupancy demonstrate that the hole occupies both purines and pyrimidines during the course of CT through DNA.[24] All the bases in DNA duplexes are involved in CT, not only guanines and adenines. But what determines the extent

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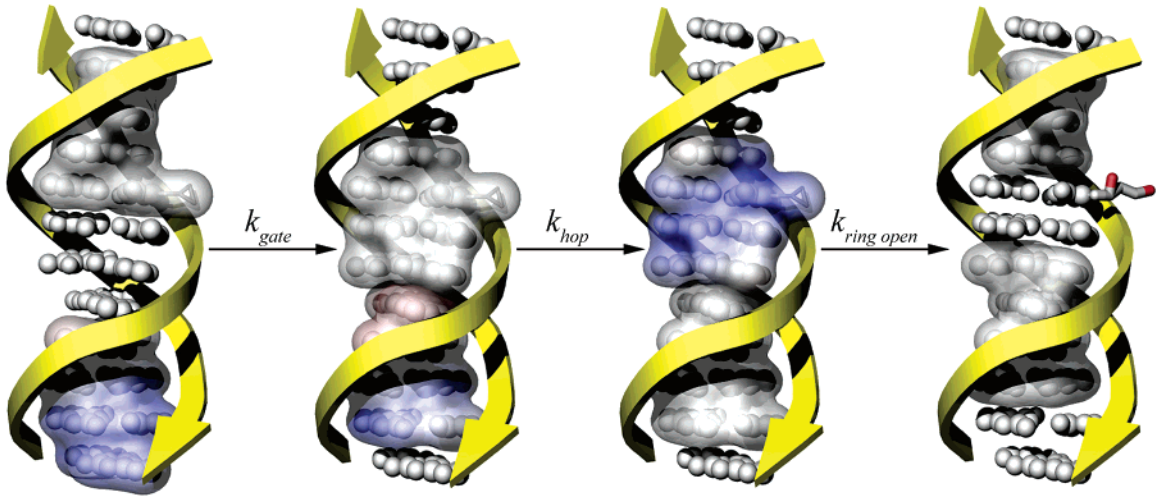

Figure 4. Schematized model for conformationally gated DNA CT among delocalized domains. The charge delocalizes over transiently extended π-orbitals within a domain (blue); as the domain enlarges and dissolves, charge is transported, gated by coherent base motions. DNA domains form transiently, governed by DNA sequence and dynamics. When the radical reaches the cyclopropylamine-substituted base, hole density is reported through ring opening.

of delocalization? Do some sequences promote greater delocalization, and hence greater stabilization, of pyrimidine radicals than do others?

Domain formation is sensitive to the sequence of DNA. Our data show, for example, that the arrangement of the A-tract modulates the hole density on ^{CP}C. As evidenced in Tables 1 and 2, holes delocalize not only over the purine-containing strands, but also over those containing pyrimidines, although the holes are not equally distributed between the strands. ^{CP}C surrounded by two A_4 segments decomposes 5 times faster than that with two flanking T_4 segments. In **AQ13**, an alternatively stacked (purine-pyrimidine)$_3$ segment dramatically attenuates the hole density on ^{CP}C. A CT-active conformation facilitating CT across a domain is easily accessed in the A-tract of **AQ7**, which contains only purine stacking. In contrast, when purine−purine stacking is interrupted by pyrimidines, such as in **AQ13**, larger domains facilitating CT are less likely to be reached.

Among the most noteworthy results is that seen in comparing ^{CP}C decomposition between **AQ4** and **AQ6**. These two assemblies have identical sequences intervening between the photooxidant and ^{CP}C; they differ in that **AQ4** contains a T4 tract distal to ^{CP}C, whereas **AQ6** contains an A4 tract. Yet ^{CP}C decomposition and therefore hole density on ^{CP}C are markedly attenuated for **AQ4** versus **AQ6**. Thus, it must be the larger DNA sequence of the assembly that governs CT, not just the intervening bases. Earlier we had seen that ^{CP}C decomposition is affected also by base substitutions on both strands.[24] These effects can be understood in the context of dynamic delocalized domains in the duplex that form and dissolve over time. The results here underscore the fact that the full sequence of the assembly must govern such dynamical interchanges.

The adenine tract can tolerate small perturbations without disrupting domain formation. As seen in Table 2, a one base perturbation of a six-base A-tract slightly attenuates the hole density on the following ^{CP}C. The most pronounced effect we observe here occurs with guanine substitution, which is likely the result of a combination of energetic factors, guanine competing for the hole, and the disruption of adenine stacking.

Domain sizes in adenine tracts have been found to be four to five bases in length based upon the aminopurine fluorescence quenching experiments in DNA.[22] In the **AQC*n*** assemblies (Table 3), ^{CP}C is placed after the second adenine in a seven-base adenine tract. Based on ^{CP}G decomposition, it is apparent that ^{CP}C and ^{CP}G affect one another in the same domain when there are fewer than two bridging adenines. However, when more than two adenines separate ^{CP}G from ^{CP}C, the decomposition efficiency of ^{CP}G is attenuated to half of that in **AQC0**. We understand this result in terms of the lack of interchange between cyclopropylamine−nucleosides in these assemblies with longer distances separating cyclopropylamine-substituted bases; the cyclopropylamine-substituted bases must now fall into separate domains. This observation then supports the domain size in the A-tract being at least three bases.

The Delocalized Domain Model for CT. Two mechanisms of charge transfer in DNA have been proposed that incorporate dynamic structural distortions. The first one is ion-gated polaron hopping.[26,27] This mechanism describes a structural distortion over several DNA base pairs to self-trap the charge and generate a polaron-like species in response to charge injection.[26] In this model, charge is transported from one polaron to the next by thermal activation. The second mechanism, our model,[22,24] involves CT among sequence-dependent delocalized domains. Domains are transiently formed extended π-orbitals that depend on the dynamics and sequence of DNA. Contrary to the polaron model as first described,[26] here domains are related to the structure and the internal dynamical motion of bases across the DNA duplex. Holes and electrons are transported as delocalized domains form and dissolve depending upon the sequence-dependent stacking within the duplex.

Figure 4 shows our model for CT through delocalized domains. In this model a charge is injected into a domain and delocalizes over the transiently extended π-orbitals without distorting the domain structure. The charge can then either be trapped by a cyclopropylamine base if the domain contains one or be transported through delocalization into the next domain. The transport is gated by coherent base motion. When two domains come together, forming a well-stacked CT-active conformation, transport can occur. It is the sequence-dependent and dynamic structure of DNA that determines the efficiency of CT in this model. The dynamical motion of DNA base pairs

helps to achieve the CT-active conformations. In this model, then, time-dependent structural distortions in DNA should vary with DNA sequence, irrespective of whether charge is being transported. Our time-resolved kinetic studies of base−base CT as a function of temperature have been consistent with this model, where CT is gated by base motions,[21] and our CT studies at 77 K have earlier shown the requirement for conformational motion for CT.[23] Here it is apparent that CT is furthermore remarkably sensitive to DNA sequence and not owing to energetic considerations alone.

Can we distinguish between the polaron and domain models on the basis of these data? As described above, previous studies[1−5] have used double or triple guanine sites as traps for charge transfer events, but this method has a shortcoming of an extremely slow trapping rate, and thus leads to data representing the average of CT events occurring over many time scales. In the study here we make use of a much faster trap, cyclopropylamine-substituted bases, which can be oxidized and ring-opened within $\sim 10^{-11}$ s;[39] this fast trap allows us to monitor hole delocalization over even transiently formed domains. What we observe with this fast trap is that the entire sequence of the DNA duplex contributes to the formation and disintegration of domains. Furthermore, in the polaron hopping model, charge is transferred by thermal activation among low-energy purine sites, and what is usually measured is the result of trapping at these low-energy sites. Here we measure instead hole density on the bridge, kinetically trapped by the ring-opening reaction.

It should be noted that recently a solvated polaron model[27] has been distinguished from that described earlier based upon charge-dependent distortions in the DNA.[26] Here, the solvated polaron state is formed by injection of a charge carrier into an appropriate configuration reached by fluctuation of the DNA bases and solution environment surrounding the DNA. Our results are not sufficient to differentiate between these models.

We can, however, consider some differences expected on the basis of these various proposals. For example, in considering the series of assemblies **AQ9**, **AQ10**, **AQ8**, and **AQ11** (Table 2), while the G inserted in **AQ9** would be expected to stabilize polaron formation, the higher energy C, T, and T2 inserts should interfere with propogation of the polaron. Yet when we measure hole density at the distal site along the bridge through ^{CP}C decomposition, the opposite trend is revealed. An *increase* in ^{CP}C decomposition is seen along the series **AQ9**, **AQ10**, **AQ8**, **AQ11.** In addition, for the polaron hopping model, polarons in an adenine tract are considered to drift step by step: as one adenine adds to the polaron, another is released. Therefore, as long as the A-tract remains intact, the oxidation yield should be constant. However, here, we clearly see differences of decomposition efficiency in **AQC*n*** assemblies, even with the fast hole trap. In fact, not only does a distal change in sequence affect the hole delocalization of nearby ^{CP}C, but also the distance between ^{CP}C and ^{CP}G along the tract affects the decomposition in a discrete way. We consider that these observations can best be rationalized through a delocalized domain model.

Here we have illustrated the remarkable dependence of DNA CT upon sequence. Importantly, our model of conformationally gated CT among delocalized domains predicts a rich sequence and structure dependence. Certainly, then, we can understand both the data presented here and earlier data showing the sensitivity of DNA CT to stacking in the context of our model; other models based largely upon energetic considerations associated with base oxidation do not provide a similar reconciliation of the data. Clearly, the sequence dependence of DNA CT must be taken into account in any mechanistic descriptions going forward and in viewing the possible applications and biological implications of DNA CT.

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