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DNA Charge Transport: Conformationally Gated Hopping through Stacked Domains

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Abstract: The role of base motions in delocalization and propagation of charge through double helical DNA must be established experimentally and incorporated into mechanistic descriptions of DNA-mediated charge transport (CT). Here, we address these fundamental issues by examining the temperature dependence of the yield of CT between photoexcited 2-aminopurine (Ap*) and G through DNA bridges of varied length and sequence. DNA assemblies (35-mers) were constructed containing adenine bridges $Ap(A)_nG$ ($n = 0-9$, $3.4-34$ Å) and mixed bridges, ApAAIAG and ApATATG. CT was monitored through fluorescence quenching of Ap* by G and through HPLC analysis of photolyzed DNA assemblies containing Ap and the modified guanine, N^2-cyclopropylguanosine (^{CP}G); upon oxidation, the ^{CP}G radical cation undergoes rapid ring opening. First, we find that below the duplex melting temperature (~ 60 °C), the yield of CT through duplex DNA increases with increasing temperature governed by the length and sequence of the DNA bridge. Second, the distance dependence of CT is regulated by temperature; enhanced DNA base fluctuations within duplex DNA extend CT to significantly longer distances, here up to 34 Å in <10 ns. Third, at all temperatures the yield of CT does not exhibit a simple distance dependence; an oscillatory component, with a period of $\sim 4-5$ base pairs, is evident. These data cannot be rationalized by superexchange, hopping of a localized charge injected into the DNA bridge, a temperature-induced transition from superexchange to thermally induced hopping, or by phonon-assisted polaron hopping. Instead, we propose that CT occurs within DNA assemblies possessing specific, well-coupled conformations of the DNA bases, CT-active domains, accessed through base motion. CT through DNA is described as conformationally gated hopping among stacked domains. Enhanced DNA base motions lead to longer range CT with a complex distance dependence that reflects the roles of coherent dynamics and charge delocalization through transient domains. Consequently, DNA CT is not a simple function of distance but is intimately related to the dynamical structure of the DNA bridge.

Introduction

The DNA double helix mediates charge transport (CT) over a wide range of distance and time regimes.[1-11] Mechanistic

descriptions generally combine one-step superexchange-mediated tunneling and incoherent multistep hopping of localized charge (generally holes) to rationalize the varied features of DNA CT.[12-15] The fundamental difference between these two mechanisms is whether the charge actually occupies the DNA bridge. For CT through donor−bridge−acceptor systems, oc-

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cupancy of the bridge is related to the energetic barrier for charge injection from the donor to the bridge (ΔG_{inj}). Due to the different oxidation potentials of DNA bases in solution (G, $E_{ox} \approx 1.3$ V; A, $E_{ox} \approx 1.4$ V; C, $E_{ox} \approx 1.6$ V; T, $E_{ox} \approx 1.7$ V vs NHE),[16] and assuming the trend in potential to be maintained in DNA, there is a positive injection barrier for migration of a hole between guanines separated by A/T bridges. Consequently, holes were thought to hop between G "stepping stones" by tunneling through intervening A/T bridges. However, when guanines are separated by larger distances ($\sim \geq 4$ bases), tunneling through A/T base pairs was found to be unrealistic,[3b] and occupation of adenines was subsequently invoked to rationalize long-range CT between guanines. This thermally induced hopping (TIH) requires accessing the higher energy adenine bridge. However, once there, the hole is proposed to hop along the adenines in an essentially distance-independent fashion.

The notion of a transition from tunneling to thermal occupation of the DNA bridge governed by bridge length is an attractive one; it appears to rationalize the relatively steep distance dependence of CT rate constants reported in some time-resolved studies over distances $< \sim 20$ Å,[2b,4d,5c] with the shallow distance dependence of the yield of CT which has been observed as far as 200 Å.[3,9-11] However, neither tunneling nor TIH address mechanistically CT through DNA initiated by a donor that is energetically very near, or above, the A/T bridge. With no barrier to injection, bridge occupancy is likely, especially for donor−acceptor distances where tunneling may be slow. Experimental investigations have considered exergonic hole injection onto A/T bridges,[1b,2,3b,4,6,8] but these have led to some conflicting conclusions regarding the rate constants and distance dependence of CT. Moreover, localized hopping is not the only explanation for a shallow distance dependence; alternatively, charge occupying the DNA bridge could migrate in a more coherent, or bandlike, mechanism. The fundamental difference between these two mechanisms is whether the charge is localized at the first base or is more delocalized. This distinction, arguably at the root of DNA CT, remains ambiguous despite continued theoretical investigations.[17] To address this problem, we must include the unique features of DNA which render its CT chemistry distinct from other donor−bridge−acceptor assemblies, and biomolecules, such as proteins. In particular, we must ask how the inherent dynamics of the π-stacked DNA bases influence the extent of charge delocalization and the mechanisms of CT.

Recently, DNA base dynamics, which occur over a range of time scales relevant to CT,[18,19] have been incorporated into theoretical treatments of DNA CT.[20-22] For instance, rapid base motions have been shown to dramatically modulate electronic coupling between DNA bases.[20] Alternatively, in the model of Rudnick and co-workers, the rate of CT is limited by the requirement of an optimal alignment of bases, and thermally induced base fluctuations facilitate achieving this alignment.[21] We have previously demonstrated experimentally that DNA CT may be gated by picosecond motions of an intercalated ethidium photooxidant.[1d] More recently, we have extended this notion of conformational gating of CT to motion of the bases.[23] Using femtosecond spectroscopy, we examined the temperature-dependent rate constants for CT between photoexcited 2-aminopurine (Ap*) and G in DNA assemblies where the donor and acceptor were in contact or separated by a single intervening A. This identified a defining role for base fluctuations in CT dynamics. We proposed that CT occurs within DNA assemblies possessing specific, well-coupled conformations of the DNA bases (CT-active), accessed through base motion; the formation of CT-active conformations gates CT and governs the observed rate constants. This is distinct from models that invoke structural distortions, such as polarons, where thermally induced fluctuations (phonons) provide the energy necessary to overcome the barrier associated with self-trapping of the charge such that it can propagate.[10]

According to our model, the yield of CT reflects the probability of accessing CT-active conformations. We predicted that this would be strongly modulated by temperature, and by the distance between the donor and acceptor, because confor-

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mational gating should become more important as the number of bridging bases increase. Here, we examine the temperature-dependent yield of base–base CT through DNA bridges of varied length and sequence to test the predictions of our model, and to better understand the interplay between base dynamics, hole delocalization, and coherence of base and hole migration. We exploit Ap* as a dual probe to correlate structural dynamics of the DNA bases[18a,b,24] and base–base CT,[2,23,25−27] in well-defined DNA assemblies. We establish that temperature-induced base dynamics occur on the time scale of CT (<10 ns) and that these dynamics regulate the yield of CT as predicted by a model via CT-active conformations. Enhanced DNA base fluctuations, within a certain regime, can extend base–base CT to at least 34 Å, with a complex distance dependence that reflects the role of coherent dynamics and charge delocalization in DNA-mediated CT.

Experimental Section

Materials. DNA oligonucleotides were synthesized on an ABI DNA Synthesizer using standard solid-phase techniques. Phosphoramidites and 2.5 μmol CPG columns were from Glen Research. Oligonucleotides containing N^2-cyclopropylguanosine (^{CP}G) were prepared as previously described.[28] The crude tritylated DNA oligonucleotides were separated from failed sequences by reverse-phase HPLC (Hewlett-Packard) using a C-18 column with an acetonitrile/30 mM ammonium acetate gradient. Following detritylation, the DNA oligonucleotides were further purified by a second round of HPLC. Purity was assessed by HPLC and mass spectrometry (MALDI). After purification, the oligonucleotides were resuspended in 1 mL of Millipore water, dried via speedvac, and stored at −20 °C until use.

DNA Samples. DNA oligonucleotides were quantified using UV–vis spectroscopy with the following extinction coefficients for the nucleotides at 260 nm:[29] A = 15 400; G = 11 500; I = 11 000; C = 7400; T = 8700; Ap = 2500 L mol^{-1} cm^{-1}. Duplex solutions were prepared by combining equimolar amounts of the desired DNA complements and annealing with regulated cooling from 90 to 10 °C over a period of 3 h. Fluorescence measurements were made using 50 μM DNA in 100 mM sodium phosphate, pH 7, with the exception of excitation spectra, which were obtained using 2.5 μM DNA. This reduction in duplex concentration is necessary because the natural DNA bases, which are in greater abundance and have a much higher extinction coefficient than Ap at 260 nm, absorb the lower wavelength (<300 nm) light very strongly. The absorbance of each duplex at 325 nm was very similar (±<5%).

Thermal Denaturation. Thermal denaturation was monitored using a Beckman UV–vis spectrometer equipped with a melting temperature accessory. The melting profiles of the duplexes (50 μM) were obtained by slowly lowering the temperature (0.5 °C min^{-1}) from 90 to 10 °C and measuring the absorbance of Ap (325 nm) at each temperature. All duplexes displayed cooperative thermal denaturation profiles. Reported duplex melting temperatures were evaluated from the derivative of the melting profile obtained by monitoring the absorbance of Ap at 325 nm (unless otherwise stated). Due to the ratio of Ap to natural

DNA bases (1:69), it is not possible to monitor the absorbance of both Ap and the natural DNA bases in these DNA samples at a single concentration. The melting temperatures obtained by monitoring at the Ap site thus reflect the minimum temperatures for denaturation. However, duplexes containing Ap–T base pairs melt cooperatively, including the Ap region. It has previously been reported that monitoring absorbance of the DNA bases at 260 nm, or Ap at 325 nm, gives approximately the same melting temperature (generally ≤1 °C),[30] consistent with the notion that melting at the Ap–T site is essentially coincident with the global melting.

Fluorescence Experiments. Steady-state fluorescence measurements on Ap-containing DNA were conducted using an ISS K2 fluorimeter (5 mm path length) equipped with a Peltier-controlled thermostated sample holder (Quantum Northwest). Temperature fluctuations during spectral acquisition were less than ±0.02 °C. Emission spectra were obtained by exciting at 325 nm and monitoring the integrated emission between 340 and 500 nm. Excitation spectra were obtained by monitoring the emission at 370 nm while scanning excitation wavelengths between 240 and 350 nm. Fluorescence polarization measurements examined the polarized emission at 370 nm following excitation with polarized light at 325 nm.

Evaluation of the yield of CT from emission spectra is accomplished by comparing the observed fluorescence intensity in redox-active duplexes to otherwise identical duplexes in which the single G electron donor is replaced by inosine (I), an analogue of G which is inactive toward CT quenching of Ap*.[2] It is necessary to employ a redox-inactive reference to delineate CT from other modes of quenching. The fraction of fluorescence quenching due to CT is thus quantified as F_q, where $F_q = 1 - \Phi_G/\Phi_I$, and Φ_G and Φ_I are the relative quantum yields of Ap in the redox-active and redox-inactive oligonucleotides, respectively. Relative quantum yields are determined from the ratio of the integrated emission of the Ap-containing DNA to free Ap in identical buffer at each temperature. The reported F_q values are the average of 3−5 independent experiments; error bars represent the standard error of these F_q measurements.

Permanent Oxidation of ^{CP}G by Ap*. DNA duplexes (5 μM in 100 mM sodium phosphate pH 7) containing Ap and ^{CP}G were equilibrated in a temperature-regulated sample holder and irradiated with a mercury−xenon lamp (3 mW) at 325 nm (320 nm LP filter) for 5 min. The samples were then digested by 37 °C incubation with phosphodiesterase, P1 endonuclease, and alkaline phosphatase for 2 h to yield the free nucleosides. The nucleosides were separated and analyzed by reverse-phase HPLC as previously described.[28,31] Control experiments used free Ap base and ^{CP}G nucleoside each at a concentration of 500 μM in 100 mM sodium phosphate pH 7.

Results

Experimental Design. Our investigations employ Ap* as a dual reporter of DNA base stacking and DNA-mediated CT. In DNA, the decay of Ap* is multiexponential;[2,18a,b,26] CT between Ap* and G is delineated from other modes of quenching by comparing redox-active duplexes to otherwise identical duplexes in which the single G electron donor (G) is replaced by inosine (I), an analogue of G that is essentially inactive toward CT with Ap*.[2] The dominant consequence of this substitution is a change in redox potential ($E_{ox} \approx 1.3$ V[16] and 1.5 V[2a] vs NHE for G and I, respectively), and consequently in redox-active duplexes the lifetime and emission intensity of Ap* are reduced relative to the redox-inactive duplexes. Our premise that these differences in Ap* quenching are correlated to the change in CT driving force is validated by direct chemical evidence for CT

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Scheme 1. DNA Assemblies Used To Investigate the Influence of Temperature on the Yield and Distance Dependence of CT between Ap* and G
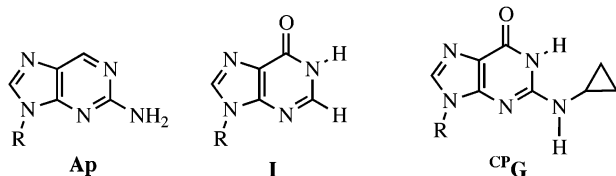
3'-C T A A T A T C T G T A T A A **C T (T)**$_n$**C C** A T A A T T C A T G T A A T G-5'
5'-G A T T A T A G A C A T A T T **I Ap (A)**$_n$ **Y I** T A T T A A G T A C A T T A C-3'

Y = I, G; n = 0-9

3'-C T A A T C T G T A T A A **C T T A T A C C** A T A A T T C A T G T A T G-5'
5'-G A T T A G A C A T A T T **I Ap A T A T Y I** T A T T A A G T A C A T A C-3'

3'-C T A A T C T G T A T A A **C T T T C T C C** A T A A T T C A T G T A T G-5'
5'-G A T T A G A C A T A T T **I Ap A A I A Y I** T A T T A A G T A C A T A C-3'

5'-A T A C G G C A A A **Ap A A A** ^{CP}**G** G C T C G T-3'
3'-T A T G C C G T T T   T T T T   C C G A G C A-5'

between Ap* and G in duplex DNA.[31] Here, we are interested in the yield of CT, evaluated from steady-state measurements as the fraction of fluorescence quenched, F_q, in redox-active relative to redox-inactive DNA assemblies ($F_q = 1 - \Phi_G/\Phi_I$). By calibrating with redox-inactive duplexes, we reveal the population undergoing ultrafast CT in our steady-state experiments. This is confirmed by the close match between the steady-state CT yields and the amplitude of k_{CT} derived from time-resolved experiments.[23]

To examine the influence of temperature-dependent base motions on CT in duplex DNA, 35-mer DNA assemblies (Scheme 1) with melting temperatures near 60 °C were employed. The temperature dependence of CT was examined through adenine bridges in Ap(A)$_n$G ($n = 0-9$, 3.4−34 Å) and mixed bridges in ApAAIAG and ApATATG, where the consecutive series of adenines, as well as the energetics, stacking, and flexibility of the DNA bridge, are altered. The base composition of all assemblies was fixed to ensure that the duplexes melt at approximately the same temperature. The influence of temperature on the yield of permanent oxidation by Ap* was evaluated using a duplex containing the modified G, N^2-cyclopropylguanosine (^{CP}G, Scheme 1) with a melting temperature of 65 °C (3 μM duplex monitored at 260 nm). Irradiation of Ap in this duplex initiates CT followed by rapid ring opening of the ^{CP}G radical cation and subsequent trapping by oxygen and/or water to yield oxidized products.[28,31]

Spectroscopic Characterization of Ap Duplexes as a Function of Temperature. Thermal denaturation profiles of the 35-mer DNA duplexes were obtained by monitoring the temperature-dependent absorbance of Ap at 325 nm. Each duplex exhibits cooperative thermal denaturation, yielding a single minimum in a plot of dA/dT versus temperature (peak width at half-height ≤ 5 °C), from which the duplex melting temperature (T_m) is obtained. As previously discussed, melting monitored at Ap is generally coincident with the global melting,[30] and the dynamics of Ap represent the dynamics of DNA bases throughout the duplex. For the Ap(A)$_n$Y and ApATATY assemblies, the T_m values of the fully matched duplexes are 63 and 61 °C for Y=G and Y=I, respectively.

The 2 °C difference in T_m between the Y=G and Y=I duplexes is anticipated on the basis of previous investigations[2] and is due, at least in part, to the loss of a hydrogen bond upon substitution of an I−C for a G−C base pair. The fact that this difference in T_m does not depend on the distance of the I/G site from Ap further supports the notion that melting at Ap is coincident with global duplex melting. The invariant melting temperatures are expected given the fixed base composition of these duplexes and indicate that the A-tract present in some assemblies (Ap(A)$_n$Y, $n \geq 5$) does not significantly impact the temperature of global duplex melting.[32] In these assemblies, as well as ApATATY, a small secondary transition centered at 55 °C was observed for both Y=I and Y=G, perhaps due to a minor structural transition within these duplexes. The ApAAIAY assemblies have slightly lower melting temperatures ($T_m = 58$, 59 °C for Y = I, G, respectively), which may be due to a disruption of the adenine stacking by the I−C base pair. We observe the same reduction in T_m with the insertion of an A−A mismatch in the ApA$_4$Y duplexes,[23] consistent with the influence of single base mismatches on base stacking, hydrogen bonding, and base dynamics.[18b,33,34]

The emission spectrum of neutral Ap* in solution (Figure 1a) exhibits a temperature dependence typical of many fluorophores and is comparable to previously reported studies of Ap*.[35] The decrease in emission intensity with increasing temperature is attributed to enhanced thermal deactivation and can be fit to a second-order polynomial. The emission spectra of Ap* in DNA display more complex behavior[35,36a] associated with the temperature dependence of Ap* emission and Ap* quenching (CT and non-CT) in duplex and single-stranded DNA (Figure 1b). To extract the influence of temperature on Ap* quenching, the emission in DNA is referenced to the emission of free Ap* at each temperature. The corresponding temperature-dependent quenching profile resembles an equilibrium melting curve (Figure 1c) and is very distinct from single-strand (ss) DNA. While the most dramatic change occurs through the helix-coil transition, temperature influences quenching prior to melting. In particular, differences between the temperature-dependent quenching in the Y=I and Y=G duplexes (Figure 1c) correspond to the temperature dependence of CT quenching (vide infra); consequently, the increase in emission intensity upon melting, which reflects the loss of duplex-specific Ap* quenching, is higher for the Y=G duplexes due to CT in double-helical DNA.

The apparent melting temperatures derived from emission are consistently a few degrees lower than those derived from the

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(33) (a) Hunter, W. N.; Brown, T.; Anand, N. N.; Kennard, O. *Nature* **1986**, *320*, 552−555. (b) Hunter, W. N.; Brown, T.; Kneale, G.; Anand, N. N.; Rabinovich, D.; Kennard, O. *J. Biol. Chem.* **1987**, *262*, 9962−9970. (c) Allawi, H. T.; SantaLucia, J. *Nucleic Acids Res.* **1998**, *26*, 4925−4934. (d) Peyret, N.; Seneviratne, P. A.; Allawi, H. T.; SantaLucia, J. *Biochemistry* **1999**, *38*, 3468−3477. (e) Marathias, V. M.; Jerkovic, B.; Arthanari, H.; Bolton, P. H. *Biochemistry* **2000**, *39*, 153−160.
(34) (a) Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2000**, *18*, 1096−1100. (b) Kelley, S. O.; Boon, E. M.; Barton, J. K.; Jackson, N. M.; Hill, M. G. *Nucleic Acids Res.* **1999**, *27*, 4830−4837.
(35) Law, S. M.; Eritja, R.; Goodman, M. F.; Breslauer, K. J. *Biochemistry* **1996**, *35*, 12329−12337.
(36) (a) Kawai, M.; Lee, M. J.; Evans, K. O.; Nordlund, T. M. *J. Fluoresc.* **2001**, *11*, 23−32. (b) Xu, D. G.; Nordlund, T. M. *Biophys. J.* **2000**, *78*, 1042−1058. (c) Nordlund, T. M.; Xu, D. G.; Evans, K. O. *Biochemistry* **1993**, *32*, 12090−12095.
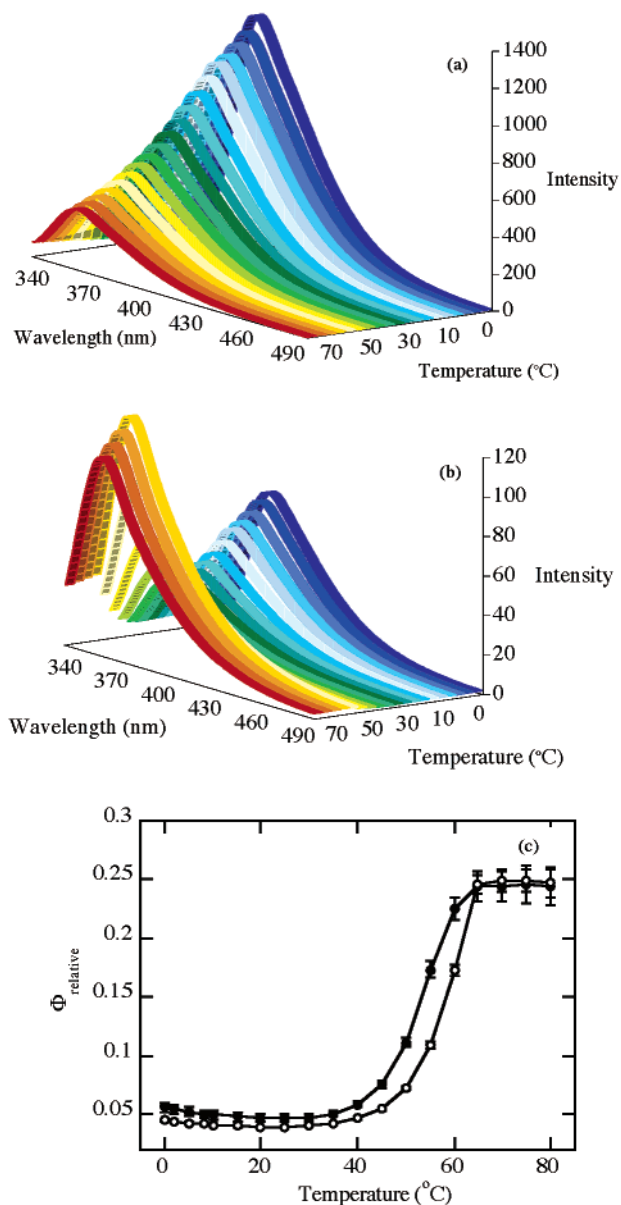
Figure 1. Temperature-dependent emission spectra of (a) Ap and (b) Ap in the DNA assembly ApAG (50 μM Ap and ApAG duplex in 100 mM sodium phosphate pH 7). The influence of temperature on the emission of Ap in DNA is determined relative to Ap at each temperature, as shown for the representative examples (c), ApA$_4$I ($\bullet$) and ApA$_4$G ($\bigcirc$).

Figure 2. Temperature-dependent changes in emission (a) and excitation (b) spectra of ApA$_4$G assemblies (50 and 5 μM duplexes, respectively, in 100 mM sodium phosphate pH 7). Arrows indicate the direction of increasing temperature. The temperature-induced shift in the excitation energy maximum ($\Delta\nu$, $\bigcirc$) correlates with the temperature-dependent fluorescence polarization ($\bullet$) of Ap in ApA$_4$G; the slightly lower transition temperatures observed for $\Delta\nu$ reflect the lower concentration of duplex used to measure excitation spectra (5 versus 50 μM for fluorescence polarization).

absorption,[37] and the emission profiles are generally broader (i.e., plots of dΦ/dT or dA/dT versus T). Because both measurements monitor Ap, these differences are not attributed to local melting at the Ap site, but to the distinct time scales probed by absorption and fluorescence techniques. Absorption occurs in 10^{-15} s and generates a snapshot of the average equilibrium configuration; molecular motions longer than 10^{-13} s are not detected. These motions do influence fluorescence occurring on the picosecond−nanosecond time scale, and transient states not reflected in the equilibrium may be captured in emission measurements. Consequently, the helix-coil transition and the equilibrium T_m are most accurately determined from

the temperature dependence of Ap absorbance. However, temperature-dependent base mobility in double helical DNA can readily be probed by fluorescence.[30,36]

Our steady-state fluorescence investigations reveal that temperature enhances DNA base motions well below the melting temperature and that these base motions modulate base stacking interactions during the lifetime of Ap*. For instance, the emission maximum of Ap* in duplex DNA undergoes a progressive blue shift (∼368 to 362 nm) with increasing temperature (Figure 2a).[38] Such a shift is not apparent in the temperature-dependent emission of free neutral Ap*. This blue

(37) It has previously been reported that the T_m of Ap DNA duplexes obtained by monitored Ap fluorescence is a few degrees lower than the melting temperature obtained by monitoring the absorbance of the natural DNA bases at 260 nm.[35]
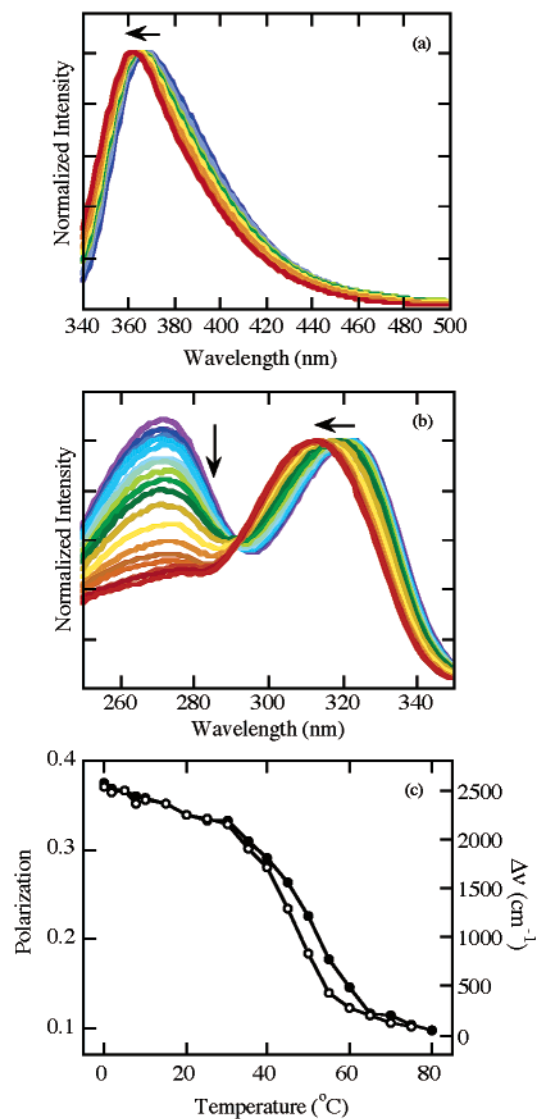
(38) This contrasts with earlier reports of only a 1 nm shift in the emission spectra of self-complementary Ap-containing DNA duplexes between 4 and 50 °C.[30] However, the fluorescence in these duplexes was attributed to unpaired Ap molecules (excitation maximum near 305 nm), which may already be significantly unstacked at low temperatures. The excitation maxima of Ap in our duplexes are coincident with the absorption maxima (315−320 nm), and fluorescence arises from H-bonded Ap.

shift is not associated with a progressive weakening of H-bonding, as comparable temperature-dependent shifts are observed for ssDNA. More likely, changes in base stacking and/or solvent accessibility with increasing base mobility are responsible.

Consistent with this interpretation are the temperature-dependent excitation spectra (Figure 2b) where both the long wavelength band ($\sim$310−320 nm), corresponding to direct excitation of Ap, and the short wavelength band ($\sim$270 nm), corresponding to energy transfer from the natural DNA bases, are valuable diagnostic tools.[2c,d,36] In DNA duplexes, the long wavelength maximum (λ_{max}) is red-shifted ($\sim$320 nm) relative to free neutral Ap* ($\sim$310 nm). This shift is generally ascribed to H-bonding and/or reduced solvent exposure associated with the duplex environment; more open configurations are less red-shifted. At low temperature, the red shift in λ_{max} (or ν_{max}) is most pronounced (Figure 2b), and with increasing temperature (0−80 °C), λ_{max} shifts progressively to the blue. This shift does not begin at a distinct temperature, but is instead continuous from 0 to $\sim$55 °C where it begins to level out (Figure 2c).[39] In ssDNA, the λ_{max} at $\sim$313 nm is similar to free Ap. An analogous temperature-induced blue shift in excitation maximum was previously reported for lower melting Ap-containing duplexes.[36b]

The relative intensity of the short wavelength band, associated with the efficiency of energy transfer and strength of base stacking interactions, is also strongly temperature dependent (Figure 2b), consistent with previous reports.[36] At 0 °C, we observe efficient energy transfer from photoexcited A to Ap, as anticipated given the presence of four consecutive adenines adjacent to Ap; adenines exhibit the highest energy transfer efficiency and are proposed to behave as funnels for energy transfer to Ap.[36c] The relative intensity of this band decreases progressively with temperature, both in the duplex regime and through the melting. Although there appears to be some energy transfer in ssDNA, it is markedly reduced relative to duplex DNA. The distinct isosbestic point in the variable temperature excitation spectra indicates that temperature-induced changes in λ_{max} are correlated with changes in energy transfer efficiency. We attribute these correlated changes to the fact that temperature-induced base dynamics alter base stacking interactions within double helical DNA.

Additional evidence that temperature-induced base motions are significant well below the melting is obtained from measurements of steady-state fluorescence polarization. Very low fluorescence polarization is observed for Ap in solution ($<$0.005), and for the poorly stacked DNA base analogue, 1-N^6-ethenoadenine, within DNA duplexes ($\sim$0.01).[2a] In contrast, significant steady-state fluorescence polarization ($\sim$0.2 to 0.4) is typically found for Ap which is base-paired and stacked within duplex DNA.[2,18a,b] Consistent with the temperature-dependent emission and excitation spectra, the fluorescence polarization in duplex DNA exhibits a progressive decrease with increasing temperature (Figure 2c). Although the temperature-induced
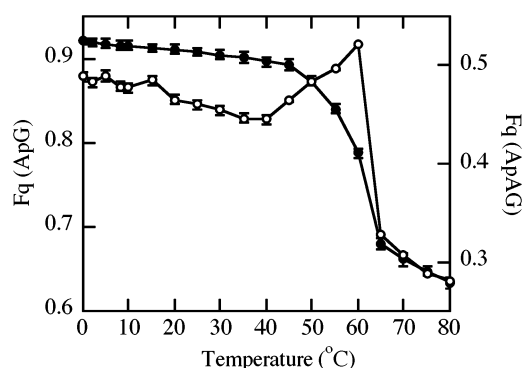

Figure 3. Fractional fluorescence quenching ($F_q = 1 - (\Phi_G/\Phi_I)$) as a function of temperature in ApY ($\bullet$) and ApAY ($\bigcirc$) assemblies. Error bars represent the standard errors of 3−5 independent measurements (50 μM duplexes in 100 mM sodium phosphate pH 7).

decrease in lifetime may increase the polarization, the temperature-dependent polarization closely parallels the shift in excitation maximum, indicating that motion is dominating the changes in polarization over this temperature regime. This confirms that base motions are largely responsible for the spectral shifts. The fluorescence polarization in the ssDNA ($>$$\sim$60 °C) is much less sensitive to temperature.

Influence of Temperature on Base−Base CT. Temperature Dependence of CT through A$_n$ Bridges: Influence of Bridge Length and Counterion. Having established that temperature significantly enhances base motions within the DNA duplexes, we next considered how these increased motions influence the yield of CT. Figures 3 and 4 present the temperature-dependent yield of CT (F_q) from Ap* to G through adenine bridges in the Ap(A)$_n$G assemblies, where $n = 0$−9. Several striking features are revealed by these data; clearly, temperature dramatically modulates the yield of CT in a manner that is governed by the length of the intervening bridge. In all assemblies, there is a sharp loss of CT quenching upon duplex melting that is entirely consistent with the temperature profile of k_{CT}.[23] This attests to the fact that DNA-mediated CT requires duplex DNA. Even for ApG where the donor and acceptor are in direct contact, the stacked arrangement of bases in double helical DNA is more favorable for CT than the average donor−acceptor geometry in the single strand. In fact, assemblies with more than one intervening adenine show no evidence of CT in ssDNA.

Given that CT reactions are unique to double helical DNA, the temperature dependence prior to duplex melting is significant. Here, for ApG, where the donor and acceptor are in direct contact, the yield of CT shows a gradual decrease with increasing temperature (Figure 3). For ApAG, where the donor and acceptor are separated by a single bridging base, the yield of CT gradually decreases until $\sim$35 °C, whereupon it increases until the sharp drop at the melting (Figure 3). For ApAAG, the yield of CT is initially almost temperature independent again until $\sim$35 °C where it abruptly increases. This rise in CT yield with increasing temperature is observed for bridges extending to nine intervening adenines (Figure 4), consistently beginning between 30 and 40 °C and continuing until near the melting, where the efficiency rapidly declines to essentially zero. Thus, increased temperature facilitates CT between Ap* and G over distances of at least 34 Å. Moreover, although the low temperature yield of CT diminishes as the bridge separating

(39) The method of difference spectroscopy was used to more precisely determine the relatively small ν_{max} shift in the excitation spectra as a function of temperature.[36a] The difference spectrum generated by subtracting any two spectra has an amplitude which is proportional to the spectral shift. The proportionality constant was determined by artificially shifting the high-temperature limit (80 °C) spectrum, and by subtracting the shifted spectra from the original spectrum. Plotting the wavelength shift as a function of difference amplitude yields a straight line with a slope equal to the proportionality constant.
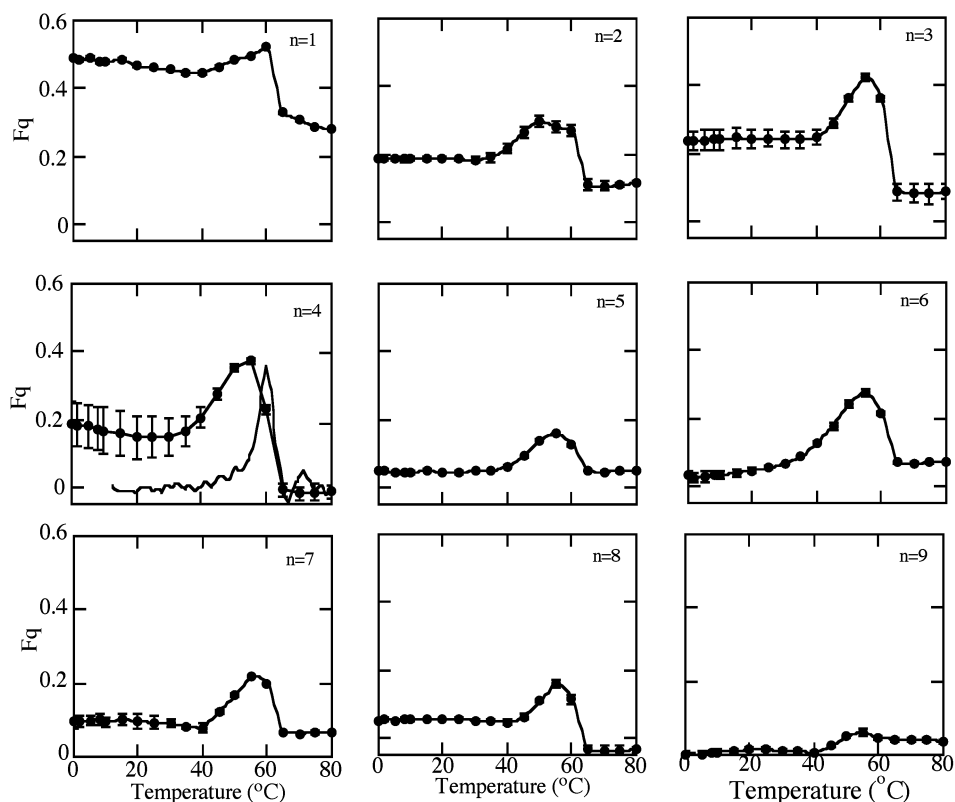
Figure 4. Fractional fluorescence quenching ($F_q = 1 - (\Phi_G/\Phi_I)$) as a function of temperature in ApA$_n$Y assemblies, $n = 1-9$. Error bars represent the standard errors of 3−5 independent measurements (50 μM duplexes in 100 mM sodium phosphate pH 7). For the $n = 4$ assembly, thermal denaturation, plotted as $-\mathrm{d}A_{325}/\mathrm{d}T$, is overlaid with the temperature dependence of F_q.

Ap and G is lengthened, the temperature-induced change in CT yield is particularly noteworthy at longer bridge lengths.

Water and counterions associated with the polyanionic backbone play important roles in the structure and dynamics of DNA.[40] Recent theoretical investigations have suggested that the dynamics of the DNA environment, particularly the water molecules, may modulate the relative redox potentials of DNA bases,[41] and even that dynamic fluctuations in the hydrated ionic configuration could gate hole migration in DNA.[42] Consequently, we considered the influence of the hydrated counterion on the yield of base−base CT and carried out an investigation with the Ap(A)$_4$Y assemblies. We observe that the yield of CT and its variation with temperature are equivalent when the Na$^+$ counterion is replaced by K$^+$ (data not shown, see Figure 4, $n = 4$).

Temperature Dependence for Different Bridge Sequences. Because the Ap(A)$_n$G assemblies possess a series of consecutive adenines, we considered whether the influence of temperature on the yield of CT might be unique to A$_n$ bridges. To eliminate this possibility, CT through mixed DNA bridges in ApAAIAG and ApATATG was examined (Figure 5). As expected from our previous investigations of CT between Ap* and G,[2d] and from studies of long-range DNA-mediated G oxidation,[3b] these sequences do not afford as efficacious CT as the well-coupled

(40) See, for example: (a) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984. (b) McConnell, K. J.; Beveridge, D. L. *J. Mol. Biol.* **2000**, *304*, 803−820. (c) Manning, G. S. *Biopolymers* **2003**, *69*, 137−143.
(41) Voityuk, A. A.; Siriwong, K.; Rösch, N. *Angew. Chem., Int. Ed.* **2004**, *43*, 624−627.
(42) Barnett, R. N.; Cleveland, C. L.; Joy, A.; Landman, U.; Schuster, G. B. *Science* **2001**, *294*, 567−571.
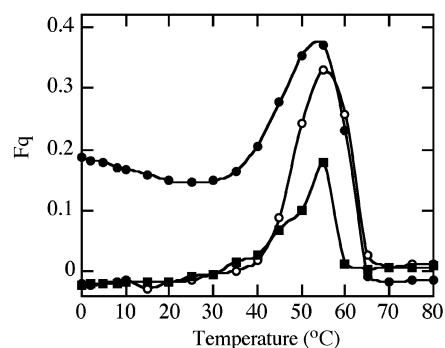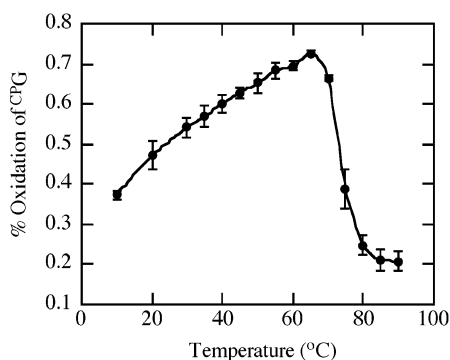

Figure 5. Fractional fluorescence quenching ($F_q = 1 - (\Phi_G/\Phi_I)$) as a function of temperature in ApA$_4$Y (●), ApATATY (○), and ApAAIAY (■) assemblies (50 μM duplexes in 100 mM sodium phosphate pH 7). The F_q values are the average of 3−5 independent trials.

adenine bridge. However, the influence of temperature on the yield of CT in these assemblies is clearly comparable to that observed in the Ap(A)$_n$G assemblies; in both ApAAIAG and ApATATG, the yield of CT rises sharply near 35 °C and continues to increase until duplex melting. The temperature-dependent yield of CT is not a manifestation of structural transitions within an A-tract sequence (vide supra), nor is this behavior restricted to A bridges. In fact, the influence of temperature on CT through ApATATG is so dramatic that at its maximum it closely approaches the yield in ApAAAAG. An analogous, dramatic increase in CT yield with temperature was also seen for CT through a bridge containing a single base pair mismatch, as described in our initial investigation.[23]

Temperature Dependence Monitored by the Cyclopropylamine Ring-Opening Reaction. We also evaluated the yield

Figure 6. Temperature dependence of oxidative damage induced by Ap* in the DNA duplex ApAAA^{CP}G (Scheme 1). The yield of ^{CP}G oxidation was evaluated following irradiation (325 nm, 5 min, ~3 mW), enzymatic digestion, and HPLC quantitation using adenosine nucleoside as an internal standard.[31] Shown is the average of three independent experiments with the associated standard error. Irradiations were conducted using 5 μM duplexes with T_m > 65 °C (T_m = 65 °C at 3 μM where the absorbance is < ~1.7). No loss of ^{CP}G is incurred by 325 nm irradiation of identical duplexes where Ap was replaced by A, nor following digestion of Ap/^{CP}G duplexes that were not irradiated. Over this temperature range, the yield of photooxidation of ^{CP}G nucleoside by free Ap is constant within experimental error (<±10%).

of Ap*-induced G oxidation as a function of temperature using a modified G, ^{CP}G, that undergoes ring scission upon oxidation,[28,43] which is competitive with back electron transfer.[31] Here, we are monitoring product formation, and not Ap* quenching, and require no reference duplex to evaluate CT yield. Certainly, the oxidized product is a consequence of hole injection, transport, and trapping, and the temperature dependence of product formation could contain unique contributions from each process. However, we observed no significant temperature dependence on the yield of CT between free Ap* and ^{CP}G nucleoside, indicating that ring scission and trapping is largely unaffected by temperature over this range (<10%, random variation). Consequently, the temperature profile of ^{CP}G oxidation in DNA represents the influence of temperature on the DNA-mediated CT chemistry, and not the trapping chemistry. However, unlike Ap* quenching, this will reflect the influence of temperature on both forward and back CT. As shown in Figure 6, a distinct increase in the yield of oxidative damage is observed for CT between Ap* and ^{CP}G in duplex DNA, which is lost upon duplex melting. Both of these features resemble the temperature dependence of CT between Ap* and G monitored by differential fluorescence quenching between I and G duplexes (F_q). Thus, steady-state fluorescence measurements do indeed provide a valid measurement of CT yield, even over a range of temperatures.

Distance Dependence of Base—Base CT. The experiments reported here monitor steady-state fluorescence and provide no direct measure of the rate constants for CT. According to simple Stern—Volmer analysis, the relative fluorescence intensities of the redox-inactive (Φ_I) to redox-active (Φ_G) duplexes can be written according to eq 1:

(43) N-Alkyl- and N-arylcyclopropylamine radical cations undergo rapid homolytic ring scission to the β-iminium carbon radicals; see, for example: Shaffer, C. L.; Morton, M. D.; Hanzlik, R. P. *J. Am. Chem. Soc.* **2001**, *123*, 349−350. While the rate constant for ring opening of the N^2-cyclopropylguanosine radical cation has not been measured, it is expected to be orders of magnitude faster than trapping of guanine radicals. The rate constant for ring opening of the neutral N-alkylcyclopropylamine radical is 7×10^{11} s^{-1}: Musa, O. M.; Horner, J. H.; Shahin, H.; Newcomb, M. *J. Am. Chem. Soc.* **1996**, *118*, 3862−3868.

$$(\Phi_I/\Phi_G) = (k_e + k_{nr} + k_q)/(k_e + k_{nr}) =$$
$$1 + k_q/(k_e + k_{nr}) = 1 + k_q/k_I \quad (1)$$

where k_e, k_{nr}, k_I, and k_q are the rate constants for Ap* emission, nonradiative decay, overall decay in redox-inactive (I) duplexes, and CT quenching, respectively. Consequently, $[(\Phi_I/\Phi_G) - 1]$ is proportional to k_q. Semilog plots of $[(\Phi_I/\Phi_G) - 1]$ versus separation distance (r) for CT between Ap* and G through $(A)_n$ bridges ($n = 0-9$) at temperatures between 0 and 55 °C are shown in Figure 7. Several significant features of these distance dependencies for CT through duplex DNA are immediately apparent. In particular, the distance dependence becomes more shallow as the temperature is increased, and the CT yield does not decay monotonically with distance; a distinct oscillatory feature is present in the distance dependence at all temperatures.

Because these plots exhibit an oscillatory component, the distance dependence cannot be fit exclusively by either a model of superexchange ($\ln[(\Phi_I/\Phi_G) - 1] \propto -\beta r$) or a hopping model ($\ln[(\Phi_I/\Phi_G) - 1] \propto -\eta \ln N$). If we ignore the oscillatory component, fitting to a superexchange model yields β values between 0.1 and 0.2, while fitting to a hopping model yields η values between 2 and 3. Consequently, we have fit these semilog plots to a model that reflects the influence of the number of bases on the probability of forming a CT-active conformation (vide infra).

Discussion

Three fundamental results have emerged from the current work. First, the yield of CT through duplex DNA increases with increasing temperature-induced base motions in a manner that is governed by the length and sequence of the DNA bridge. Second, the distance dependence of CT becomes more shallow at higher temperatures. At temperatures of highest CT efficiency, CT between Ap* and G proceeds over much longer distances than previously observed, here up to 34 Å. Third, at all temperatures, the yield of CT does not exhibit a simple exponential distance dependence, but instead displays a periodic oscillatory behavior.

Model for CT via CT-Active Conformations. All of these results confirm that base dynamics are critical to DNA CT and match the predictions of our previously proposed model for the role of base dynamics.[23] The tenets of this model are that a population of DNA molecules contain a heterogeneous distribution of local base conformations as a function of time, but that only specific conformations permit CT. Assemblies possessing a CT-active conformation at the moment of excitation undergo rapid CT. Some assemblies first lacking CT-active conformations may achieve these conformations via dynamic base motion during the lifetime of Ap*; CT in this population of assemblies is conformationally gated. This model is reminiscent of the behavior of ethidium-modified DNA duplexes where CT is gated by the motion of the intercalated photooxidant,[1d] except here it is the motion of the DNA bases that acts as the gate. The concept of gating by the formation of redox-active conformations is also significant in certain interprotein electron transfers.[44]

In this model, the yield of CT reflects the probability of being in a CT-active conformation at the moment of excitation or of accessing a CT-active conformation during the lifetime of Ap*. The population of assemblies in a CT-active conformation at the moment of excitation is expected to decrease with temper-
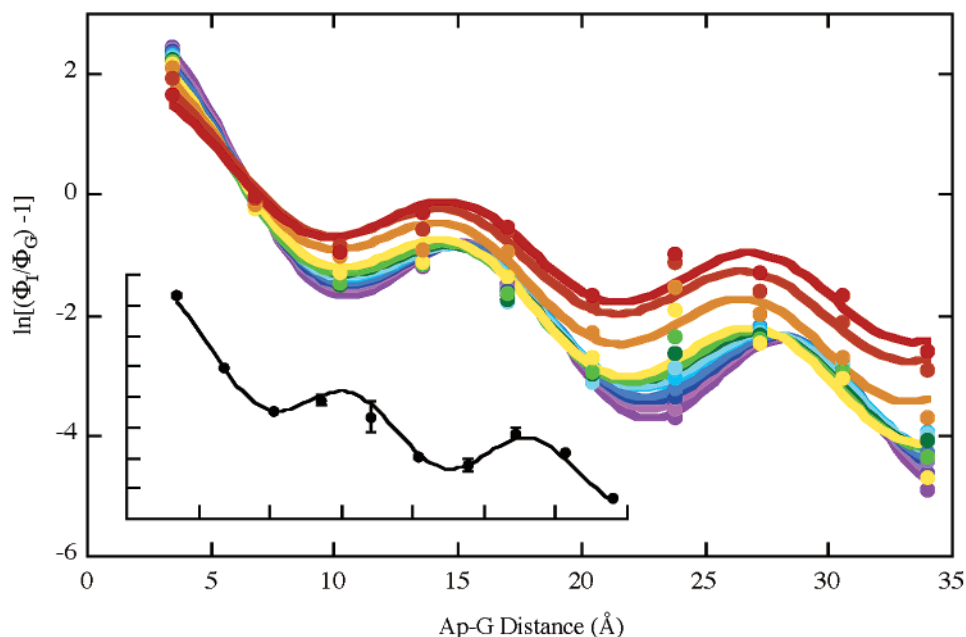
Figure 7. Influence of temperature on the distance dependence of the yield of CT (data points) through ApA$_n$Y duplexes. Shown is a plot of ln[(Φ_I/Φ_G) − 1] versus Ap−G distance for temperatures between 0 and 55 °C (increasing from violet to red). The distance is estimated from the average 3.4 Å interbase spacing of standard B-DNA. The lines represent a fit to the equation: ln[(Φ_I/Φ_G) − 1] = $C − \alpha$ ln(n) + A sin($2\pi/pn$), where n is the number of bases in a CT-active conformation ($n = r/3.4$ +1, where r is the Ap*−G distance in Å), α is the fall-off parameter of the monotonic component, and A and p are the amplitude and period, of the sinusoidal component, respectively (see text). The inset shows the distance dependence data and standard errors at 15 °C; the errors at other temperatures are comparable or smaller.

ature; increased base motion decreases the probability that a duplex is in a CT-active conformation at a given instant. This population is reflected by the ApG duplexes. The yield of CT in the duplex regime decreases with increasing temperature because the number of duplexes in CT-active conformations at the moment of excitation decreases. At 0 °C, >90% of the ApG duplexes undergo CT. This number is not 100% presumably because there are still thermal fluctuations and conformational sampling; thus, non-CT-active conformations also exist at the moment of excitation. Furthermore, even if we completely eliminate thermal fluctuations, the CT yield may never reach 100%. This is only expected if the static average DNA conformation is CT-active.

The population of duplexes accessing CT-active conformations after excitation should be enhanced by temperature; for duplexes initially in CT-inactive conformations, motion can increase the probability of achieving a CT-active conformation within the lifetime of Ap*. Consequently, one expects a certain regime where the yield of bridge-mediated CT increases with temperature. For all assemblies except ApG, where CT is not bridge mediated, this regime is observed at temperatures between ~35 and 55 °C. The upper temperature limit reflects duplex melting whereupon the possibility to form CT-active conformations is eliminated and the yield of CT falls to zero. At lower temperatures (~0−30 °C), the yield of bridge-mediated CT varies little with increasing temperature, suggesting that the decrease in the instantaneous population of CT-active duplexes

is similar to the increase in population of duplexes accessing CT-active conformations after excitation. The lower temperature limit thus reflects the temperature at which the latter becomes dominant, where increasing temperature sufficiently enhances base motions to allow conformational sampling of CT-active conformations. This lower limit should be defined by the inherent flexibility of the DNA duplex and should shift with duplex melting temperature. This is in fact observed in our previously reported study with a mismatched duplex.[23,45]

The fraction of gated, relative to direct, CT is expected to be more dramatic as the bridge is lengthened, and, indeed, this is what we observe. Because dynamics become more influential as CT proceeds through longer DNA bridges, the distance dependence of the CT yield is notably more shallow at higher temperatures (Figure 7). Increased base motions can extend DNA-mediated CT to longer distances. Significantly, we reached the same conclusion using nucleic acid structure, rather than temperature, to modulate DNA base dynamics.[2c]

At any given temperature, the bridge sequence should also influence the probability of accessing a CT-active state. The data indicate that this is the case; for instance, the probability varies according to AAAA > ATAT > AAIA. Interesting is the fact that enhanced motions play a larger role for CT through the ATAT and AAIA bridges; this is consistent with the notion that A$_n$ bridges are particularly rigid.[46] At low temperature, little CT between Ap* and G through ATAT and AAIA bridges is

(44) See, for example: (a) Lasey, R. C.; Liu, L.; Zang, L.; Ogawa, M. Y. *Biochemistry* **2003**, *42*, 3904−3910. (b) Liang, Z. X.; Jiang, M.; Ning, Q.; Hoffman, B. M. *J. Biol. Inorg. Chem.* **2002**, *7*, 580−585. (c) Liang, Z.; Nocek, J. M.; Huang, K.; Hayes, R. T.; Kurnikov, I. V.; Beratan, D. N.; Hoffman, B. M. *J. Am. Chem. Soc.* **2002**, *124*, 6849−6859. (d) Rabenstein, B.; Ullmann, G. M.; Knapp, E. W. *Biochemistry* **2000**, *39*, 10487−10496. (e) Ivkovic-Jensen, M. M.; Kostic, N. M. *Biochemistry* **1996**, *35*, 15095−15106.

(45) Similarly, a shift in the temperature dependence of the CT yield was observed when we lowered the duplex melting temperature (8 °C) by addition of glycerol (20%).

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evident, indicating that CT-active states are not likely within the lifetime of Ap*. With increasing temperature, heightened conformational motion of the bases in both AAIA and ATAT bridges facilitates access to conformations which afford fast CT. The temperature-induced enhancement in CT yield is so dramatic that the maximum yield of CT through the ATAT bridge approaches that through the AAAA bridge. Another striking example is the temperature dependence of CT through a mismatched DNA bridge.[23] Although the base sequence of the strand through which CT proceeds is unaltered, the disruption in stacking induced by the mismatch precludes CT through the mismatched duplex at low temperatures. Yet, enhanced motion of the DNA bases enables the mismatched duplex to find CT-active conformations and achieve an efficiency of CT comparable to the fully matched duplex. Here, in a DNA duplex that is inherently misaligned for CT, the motion of the DNA bases most radically regulates CT efficiency.

Our experimentally derived model fits well with a recent theory of DNA CT developed by Rudnick and co-workers.[21] Their treatment of DNA CT is founded on the premise that CT occurs only when neighboring base pairs are in a state of optimal alignment (θ^*). From this notion, a model emerges that predicts the two-stage kinetics observed experimentally for CT through DNA.[1d] The fast decay component corresponds to assemblies that possess sufficient energy and thus an optimal alignment for CT. The slower decay is then the product of the fast decay and the fraction of assemblies that subsequently reach an energy, $E(\theta^*)$. This conformationally gated rate constant is predicted to increase with increasing temperature because the fraction of assemblies possessing optimal alignments will be enhanced with temperature. The probability of achieving these alignments is the yield of gated CT in our qualitative model. Consequently, this theoretical treatment predicts enhanced yields of gated CT with increasing temperature, consistent with our experimental observations.

Conformationally Gated Charge Transport. The experimental distance dependence provides support for and insight into our model for conformationally gated CT (Figure 7). In this model, k_q, the rate constant for CT-induced quenching of Ap* in redox-active duplexes (proportional to $[(\Phi_I/\Phi_G) - 1]$), does not equal k_{CT}, but rather the rate constant for rate-limiting rearrangement. This interpretation is completely consistent with the temperature dependence of the measured rate constants for CT in ApG and ApAG duplexes.[23] These data cannot be explained by classic Marcus theory for nonadiabatic CT. However, if an adiabatic process, such as conformational rearrangement, is rate limiting, then the temperature dependence of k_{CT} can be considered in terms of transition state theory. The resulting Arrhenius−Erying plots (Supporting Information) yield similar activation enthalpies for ApG and ApAG (∼3 kJ/mol), but the activation entropy in ApAG (−42 J/(mol K)) is almost twice as large as that in ApG (−27 J/(mol K)). The negative entropy of activation is consistent with the rearrangement necessary to form the CT-active conformation, and the extent of this rearrangement is greater for ApAG as expected.

A plot of $\ln[(\Phi_I/\Phi_G) - 1]$ versus Ap−G separation (Figure 7) reflects the influence of the number of bridging bases on the rate constant, or probability (P), for achieving a CT-active conformation. As expected, this probability decreases as the number of bases in the CT-active conformation increases.

However, as is also evident in Figure 7, although increasing the number of base steps generally makes achieving CT conformations less probable, in some cases increasing the number of base steps increases this probability; for certain numbers of base steps, the probability of achieving a CT-active conformation is enhanced when this number is reached. To account for both of these observations, we have fit the data to an empirical function composed of a monotonic component that decays with the power of n (where n is the number of bases), and a nonmonotonic component, eq 2. We ascribe the nonmonotonic behavior to coherent motion of groups of DNA bases, a transiently formed well-stacked domain.

$$\ln P = \ln[(\Phi_I/\Phi_G) - 1] = C - \alpha \ln(n) + A \sin(2\pi/pn) \quad (2)$$

Here, A and p are the amplitude and period, respectively, of the sinusoidal function, n is the number of bases in a CT-active conformation ($n = r/3.4 + 1$, where r is the Ap*−G separation distance, taken from the average base step separation of 3.4 Å for B-DNA), and α is the fall-off parameter of the monotonic component. Our experimental data are well modeled by eq 2 (Figure 7, $R = 0.997$ (0 °C)−0.978 (55 °C) with α ranging from 2 to 3); these fits yield periods of 12−13 Å (r) at all temperatures, suggesting that the groups of ∼4−5 (n) bases act coherently as a domain.

Consideration of Other Mechanistic Models. Temperature-Induced Structural Transition. Importantly, our experimental data are inconsistent with alternative explanations. For instance, the variation in CT yield cannot be attributed to a temperature-induced structural transition within the A-tract present in some assemblies. Such a transition is not expected, or observed, in many of the duplexes investigated. Yet, the yield of CT through duplex DNA is consistently enhanced with increasing temperature, even through very short adenine bridges ($n \leq 4$), and mixed bridges. Furthermore, even in assemblies with longer adenine bridges ($n \geq 5$), any structural transition that may be occurring is observed near 55 °C (vide supra), which is well beyond the region where the CT yield begins to increase.

Ion-Gated Mechanisms. We propose that of the many conformations a DNA molecule can adopt, only those with particular arrangements of the DNA bases will be active toward CT, and that the base motion necessary to achieve these conformations gates CT. Alternatively, it has been suggested that it is not the conformation of the DNA bases that is important, but rather the configuration of the hydrated counterions; dynamic fluctuations in ionic configuration gate CT.[42] This model is based on a combined electronic structure, molecular dynamics investigation demonstrating that the spatial distribution of the hole in DNA varies with ionic configuration. While it is undoubtedly true that hydrated counterions are key to the structure and dynamics of DNA, it is the electronic structure within the DNA π-stack that governs DNA-mediated CT. Small changes in base conformation are expected to have a much greater impact on this electronic structure[20] than comparable changes in ionic configuration.

Moreover, our model for gating through base motions emerges from several pieces of experimental evidence and is supported by theory.[21] First, by using Ap* as a dual probe of structural dynamics and base−base CT, we have shown that temperature-induced changes in CT yield are coincident with changes in base mobility on the time scale of CT. Second, the

influence of temperature on the yield of CT is strongly dependent on the number of bases separating the donor from the acceptor; the temperature profile for direct CT (ApG) is quite distinct from bridge-mediated CT, and the influence of temperature-induced motions tends to increase with n. This is expected for conformational gating mediated through base motion. In contrast, gating through ionic fluctuations is not expected to be dependent on the number of bases between the donor and acceptor. Third, the CT yield and temperature dependence are independent of the identity of the monovalent counterion (Na^+ or K^+). While this observation does not completely eliminate some mechanistic role for counterions, it is consistent with rate-limiting base motions, but is difficult to rationalize for an ion-gated mechanism. Changing the size of the counterion alters its rate of diffusion as well as the strength and nature of its interactions with both its hydrating sphere and with DNA. If ionic motions are gating CT, one would expect these differences to be manifested in the CT yields and temperature dependence.

One experiment has been conducted to test the ion-gated mechanism. In this experiment, a segment of the negatively charged phosphate backbone between two GG sites, distal and proximal to an endcapped anthraquinone photooxidant, was replaced with neutral methyl phosphonates; this substitution was found to reduce the yield of permanent G oxidation at the distal site.[42] While the methyl phosphonates will alter the configuration of ions associated with the DNA backbone, it is equally true that they will influence the structure and dynamics of the DNA. For instance, backbone substitution with methyl phosphonate has been shown to bend the DNA up to $\sim20°$ toward the neutral face.[47] Consequently, these data do not discriminate between gating by ionic versus base dynamics and can easily be rationalized by our model of conformationally gated CT.

Temperature-Induced Transition from Superexchange to TIH. The temperature-induced increase in the yield of CT cannot be understood as a consequence of a transition from superexchange to TIH. First, the temperature dependence correlates with the dynamics of the DNA assemblies, and not with bridge energetics, as expected for a transition from superexchange to TIH. For instance, incorporation of a mismatch alters the duplex dynamics, as evident by the lower melting temperature, but leaves the energy of the DNA bridge essentially unchanged. Consequently, the temperature of transition from superexchange to TIH should not be altered by a mismatch, yet the opposite is observed.[23,45] Conversely, the modified bridge energy of the ApATATG assembly does not induce a shift in the temperature dependence of CT as would be expected for a transition to TIH. This is consistent with the fact that the ApA$_4$G and ApATATG duplexes have the same T_m; the temperature dependence of CT yield parallels the duplex dynamics.

More fundamentally, CT is initiated by Ap* (1.5 V vs NHE),[2a] which is capable of oxidizing both A (1.4 V vs NHE) and G (1.3 V vs NHE);[16] thermal energy is not required to occupy the bridge, and increasing the temperature should not significantly influence bridge occupation. In the Ap−bridge−G assemblies, CT occurs from Ap* to a single G. There is no

hopping between G sites, and endergonic oxidation of A by the guanine radical cation/radical is not required. Moreover, if one supposes that Ap* is energetically below the DNA bridge, and that thermal energy is required for occupancy, the design of our experiments should preclude observing the transition from superexchange to TIH. The yield of CT is determined by comparing the fluorescence quenching in redox-active to redox-inactive duplexes, which differ only by the exchange of a single base, the hole acceptor. The bridge, and the pathway for promotion and hopping of a localized charge, is the same in both of the duplexes. Consequently, localized hopping is not included in our yield determinations.

Delocalization of Charge. How then can this process, which results in CT between bases over 34 Å in less than 10 ns, be described mechanistically? In our previous time-resolved investigations, we examined the rate constant for CT between Ap* and G through short adenine bridges (≤3) at ambient temperature.[2b] In redox-active duplexes, there are two channels for CT, charge injection and superexchange, while in the redox-inactive duplexes only the charge injection channel exists. Thus, the rate constants for superexchange in the redox-active duplexes were obtained by subtracting the rate constants for the corresponding redox-inactive duplexes, suggesting a $\beta \approx 0.6$ Å^{-1}. However, the distance dependence of k_{CT} is more shallow when both superexchange and charge injection channels are considered. At higher temperatures, it is now necessary to account for CT over significantly longer distances where a mechanism that does not involve occupation of the DNA bridge is difficult to rationalize. For superexchange to account for CT over distances up to 34 Å in the lifetime of Ap* ($\sim$5 ps to 10 ns in DNA), β-values $\leq 0.1-0.2$ Å^{-1} would be necessary; such a weak distance dependence suggests that another mechanism may be operative. Moreover, because Ap* may have sufficient energy to oxidize A, a superexchange mechanism need not be invoked.

While our CT reactions likely involve occupation of the DNA bridge, injection cannot be onto a single, electronically isolated base (here, A is present in both redox-active and redox-inactive duplexes); injection must be sensitive to distant bases (here, I versus G). This sensitivity could arise if the charge is injected into a *domain: a transiently extended π-orbital defined dynamically by DNA sequence over which charge can delocalize.* A domain may include the hole acceptor (G), or may dynamically couple with a domain that includes the acceptor in the lifetime of Ap*. A domain, or collection of domains modulating electronic communication, constitutes a CT-active conformation.

The notion that the injected charge delocalizes over domains is also suggested by our experimental distance dependence where the yield of CT oscillates with a period of $12-13$ Å. We attribute this to the coherent motion of a group of $\sim4-5$ base pairs (vide supra). This cooperativity among bases along the helical axis is communicated through interaction of their π-orbitals. Thus, it is this coherent motion that leads to the formation of transiently extended π-orbitals. Consequently, we propose that a domain for our Ap(A)$_n$Y duplexes consists of $\sim4-5$ base pairs.

That electronic charge can delocalize in duplex DNA is hardly surprising. The delocalized charge and corresponding conductive properties of synthetic π-stacked materials as solids or in solution are well known.[48] Interesting are recent reports of

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delocalized excitation and charge (holes) over groups of five fluorenes in polyfluorenes that are π-stacked in the solid state and solution.[49] As different coupling mechanisms may be responsible for delocalization of excitation or charge, there is no reason to expect that the extent of delocalization will be the same. The authors attribute the exciton and hole delocalization length to an intrinsic feature of the π-stacked fluorene polymer, either a dynamic persistence length imposed by thermal vibrations, or an inherent length of electronic interaction.[49a] Exciton delocalization in DNA[50] was proposed over 40 years ago and has been the subject of renewed investigations, including a report[50c] that the number of coherently coupled chromophores is as high as 4−8 for dynamically fluctuating (MD simulated) duplex DNA. Yet, a similar MD study of the influence of dynamics on hole delocalization found that the hole is largely localized to a single base, at least for the lowest-lying HOMO.[51] However, a number of other theoretical analyses predict delocalization of charge in DNA.[17] Recently, Renger and Marcus applied a variable-range hopping model to experimental yields of DNA hole transport and found that delocalized bridge states are required to account for the distance dependence.[17a]

Charge delocalization in DNA has not yet been established experimentally. We recently proposed that the extent of hole delocalization modulates the relative yields of hole propagation and back reaction with the reduced hole donor.[1b] A role for polarons in DNA CT has also been invoked by experimentalists[10] and studied theoretically.[52] A polaron is a structural distortion created by a charge over which the charge can delocalize; this creates a shallow energy minimum in which the hole becomes self-trapped. This is distinct from a domain, a transiently extended π-orbital over which charge can delocalize. A domain is a sequence-dependent feature of DNA that exists in the absence of a charge. The charge can delocalize over the extended π-orbital without distorting the domain structure and without becoming trapped. Thermally induced coherent base motions lead to the formation of domains, and CT-active conformations allow the charge to "hop" among domains; DNA CT can therefore be mechanistically described as conformationally gate hopping among well-stacked domains. In the phonon-assisted polaron-like hopping model proposed by Schuster and co-workers,[10] thermal energy is instead used to overcome the energy barrier of self-trapping for the charge to propagate.

We believe that the data of Schuster and co-workers, concerning the distance and sequence dependence of the yield of DNA-mediated guanine oxidation, could be equally well understood in terms of conformationally gated domain hopping. The results presented here provide experimental evidence for the existence of domains, and we need not invoke polarons in a mechanistic description of our data. At least three distinct experimental observations point to conformationally gated domain hopping, rather than phonon-assisted polaron hopping: (i) Charge *injection* from Ap* is sensitive to distant bases. This is expected if injection is to a transient extended π-orbital, but does not make sense in terms of a polaron that forms only in response to the charge *after* it is injected. (ii) The temperature dependence of the yield of CT in duplex DNA is nonmonotonic and exhibits two distinct regimes. This behavior fits our model of gated CT where thermal energy provides access to active conformations of the DNA bases; in the phonon-assisted model, where thermal energy is used to surmount a barrier, one expects a monotonic increase in the yield of CT with increasing temperature. (iii) The temperature dependence of the yield of CT is not influenced by the nature of the monovalent counterion. This is predicted by the conformationally gated domain hopping model where the significant contribution of the ionic environment is its influence on DNA base dynamics. We do not know how the phonon-assisted polaron hopping model should depend on ionic environment. However, the structural distortions associated with the polaron and its propagation through the DNA are expected to require significantly more rearrangement of the ions and solvent; a corresponding dependence on the ionic environment would not be surprising.

Implications. Incoherent hopping of localized charge is not the only explanation that accounts for a shallow distance dependence of DNA CT. Our results reveal a mechanism where charge is delocalized over domains, transient π-orbitals extending over ∼4−5 base pairs, and charge propagation occurs via hopping among these domains. Formation of, and communication between, domains is gated by base motion and leads to CT-active conformations. DNA CT can thus be described as conformational gated hopping among domains. Thermal fluctuations govern the rate constants,[23] yields, and distance dependence of CT between bases in DNA by modulating base dynamics. This is distinct from thermally induced hopping or phonon-assisted hopping where thermal energy is instead used to surmount an energy barrier.

Our mechanistic picture has significant implications for ongoing investigations of DNA CT. As in other conjugated donor−bridge−acceptor molecules,[53] conformational flexibility and gating in DNA can generate complex distance and temperature dependencies. Also, to understand differences in the rate constants and yields of CT in different DNA constructs, one must consider how the construct influences DNA dynamics. For instance, the exceptional rigidity of the stilbene modified hairpins employed by Lewis and co-workers may contribute to the steep dependence of k_{CT} on distance.[4]

Our model of conformationally gated domain hopping also raises many interesting questions. In particular, what do CT-active conformations look like? Is a CT-active conformation unlikely? Or is it true, as the current results suggest, that these conformations are frequently accessed, particularly at physiologically relevant temperatures? How is this influenced by DNA-binding proteins and the cellular environment? As the data

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presented here emphasize, to address these questions we must incorporate the unique features of DNA, particularly the dynamics of π-stacking interactions, into models of DNA CT.

Supporting Information Available: Arrhenius−Erying plots for ApG and ApAG DNA duplexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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