Electron Transfer

Ultrafast Dynamics in DNA-Mediated Electron Transfer: Base Gating and the Role of Temperature**

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Electron transfer (ET) in double-helical DNA has been the subject of major interest, both experimentally and theoretically.[1,2] The role of dynamic base motions in DNA ET is not fully understood, as it involves a range of timescales and different motions. We previously showed that motions of an intercalated photooxidant on the picosecond timescale can modulate ET in what we termed the gate effect.[3] More recently, incorporation of DNA base dynamics into theoretical treatments of DNA ET[4,5,6] suggests not only that rapid base motions dramatically modulate electronic coupling between DNA bases,[5] but that these motions may facilitate DNA-mediated ET.[6] Herein, to explore the effects of base dynamics, we examine ET between DNA bases directly as a function of temperature through femtosecond spectroscopy.

Our investigations employ photoexcited 2-aminopurine (Ap*) as a dual reporter of DNA base dynamics and DNA-mediated ET. Ap undergoes normal Watson–Crick pairing with T and is well-stacked.[7] ET reactions between Ap* and nucleotides,[8] as well as Ap* and bases in DNA[9–11] have been extensively characterized. In DNA, ET between Ap* and G can be distinguished from other modes of quenching by comparing redox-active G-containing duplexes to otherwise identical duplexes in which the G is replaced by inosine (I), an analogue of G that is essentially inactive towards ET with Ap*.[9a] Femtosecond transient absorption spectroscopy was used to determine the rate constant for ET, k_{ET}, which was evaluated as k_G–k_I (where k_G and k_I are the rate constants for G- and I-containing duplexes, respectively); other nonradiative processes were examined and they contribute in a relatively minor way, primarily because of the thermodynamically favorable oxidation/reduction in these systems.[8–10,12] On the other hand, steady-state measurements determine the yield of ET according to the fraction of fluorescence quenched, F_q, in redox-active relative to redox-inactive DNA assemblies ($F_q = 1 - \Phi_G/\Phi_I$). In this case, to probe the influence of temperature-dependent base dynamics on ET in duplex DNA, 35-mer DNA assemblies (Scheme 1) with melting temperatures of approximately 60 °C were used.[13]

ApG/ApI

5'-GATTATAGACATATTI**ApY**ITATTAAGTACATTAC-3'
3'-CTAATATCTGTATAAC**T** **C**CATAATTCATGTAATG-5'

ApAG/ApAI

5'-GATTATAGACATATTI**ApAY**ITATTAAGTACATTAC-3'
3'-CTAATATCTGTATAAC**T** **TC**CATAATTCATGTAATG-5'

Scheme 1. Sequences of the 35-mer Ap-oligonucleotides and complementary strands used to investigate the influence of temperature on the rate constants and yields of ET in DNA (Y = I, G). The single strands referred to in the text correspond to the Ap-containing strands.

Figure 1 shows the decay of Ap* following excitation (325 nm, ~100 fs) of Ap in ApG and ApAG 35-mers between 0 and 78 °C. Consistent with all other time-resolved investigations of Ap* in DNA, these decays are characterized by the ultrafast decay due to ET followed by ns-type decays. In both assemblies, but particularly in ApG, a distinct fast decay component can be resolved below the T_m. This fast quenching is not found in the reference duplexes, ApI and ApAI, for which ET is not thermodynamically favorable.

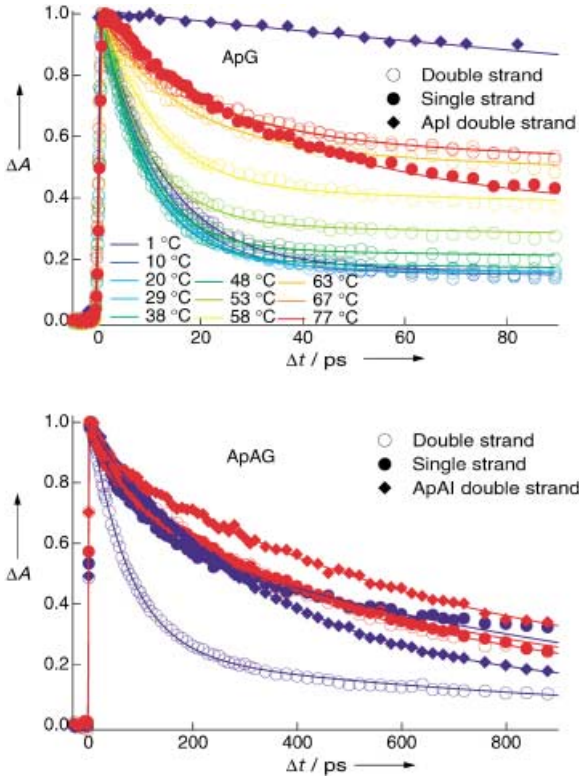

Figure 1. Time-resolved decay of excited Ap in ApG (top) and ApAG (bottom) 35-mers as a function of temperature. Samples were excited with a femotsecond pulse at 325 nm and the decay of Ap* was monitored by transient absorption at 600 nm.

The rate constant for ET, along with the relative amplitudes of the ET component (and slower decay compo-

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nents), exhibits a pronounced temperature dependence (Figure 2). These may be characterized within three temperature regimes. At lower temperatures, in which the assemblies are in the duplex form, k_{ET} exhibits an increase with temperature, more pronounced for ApG than ApAG, whereas the amplitudes of the fast ET and slower decay components show a shallow decrease and shallow rise, respectively. Through the duplex-melting regime, both the rate constant and the amplitude for fast ET decrease dramatically with a correspondingly sharp increase in the amplitude of the slower components. At higher temperatures, significantly less variation is evident. Steady-state measurements of the ET yields (i.e. F_q, data not shown) in ApG and ApAG parallel the amplitude (C_1) of k_{ET} observed in time-resolved experiments. For both ApG and ApAG, the yield of ET decreases markedly through the melting and displays little variation at higher temperatures.

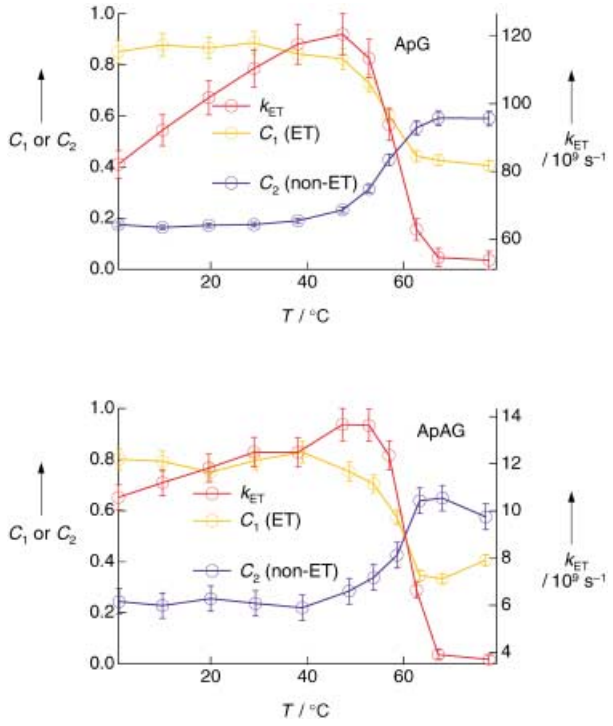

Figure 2. Rate constants for ET, k_{ET}, and the amplitudes of the fast (C_1) and slower (C_2) decay components for ApG (top) and ApAG (bottom) 35-mers as a function of temperature. The decays for both assemblies were analyzed by using a biexponential model. The rate constants and amplitudes are the average of two independent trials.

Given the significant changes in rates and amplitudes associated with melting, a comparison with the single-stranded 35-mer is warranted. In the single-stranded ApG, the rate constant and amplitude of the fast component at ambient temperature decrease by ~50% relative to duplex ApG. Notably, in the single-stranded ApAG there is no significant fast decay component. In fact, the decay times in these single strands (~20 and 420 ps for ApG and ApAG, respectively) are similar to those observed after melting of the DNA duplexes. Thus we conclude that stacking is a prerequisite for efficient ET and that for single-stranded DNA the temperature effect is much-less pronounced than for duplex DNA.

In the premelting regime, we first considered the change in rates with temperature by using the Marcus equation.[14] For a driving force of -200 mV,[9a] we obtained electronic couplings and reorganization energies of 92 cm^{-1} and 0.67 eV, respectively for ApG, and 14 cm^{-1} and 0.51 eV, respectively for ApAG. These values, particularly the electronic couplings, are significantly low, especially given other experimental estimates of electronic couplings in DNA,[15] and that the lowest calculated electronic couplings between adjacent bases are about 200 cm^{-1}.[16] In Figure 3, we calculate the predicted dependence of rate on temperature using the known driving force of -200 mV, and a calculated reorganization energy, assuming that the driving force and the reorganization energy are, to a first approximation, functions only of the donor and acceptor, and thus are relatively independent of temperature in the premelting regime.

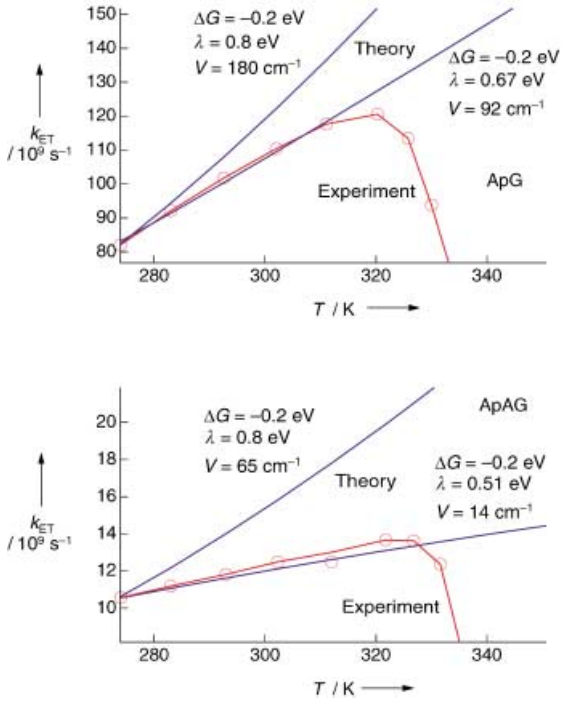

Figure 3. Measured (red lines) and calculated (blue lines) rate constants for ET in ApG and ApAG duplexes are shown for two sets of values (see text).

From the calculated (B3LYP6-311G* level) energy of one-electron-reduced Ap at the geometry of neutral Ap, and the energy of the relaxed (optimized) Ap radical anion, we estimate the reorganization energy[16d] for Ap radical anion formation to be 0.31 eV. This, combined with an experimentally determined reorganization energy for the formation of the G radical cation (0.47 eV),[17] gives a total reorganization energy of 0.8 eV (neglecting solvent effects), which is close to the experimentally determined base-intercalator reorganization energies.[15b] The results are shown in Figure 3, where the experimental temperature dependence of k_{ET} is compared

with the calculated change in the ET rate constant. However, this approach neglects the temperature-dependent motion of the DNA bases, which should influence base–base coupling and the change of rates with temperature. The importance of donor, acceptor, and bridge dynamics in bridge-mediated ET reactions of biological assemblies was recently discussed, and modifications of the Marcus theory have been incorporated into the theoretical description of ET in proteins.[18]

From the results presented herein we propose the following model: On the timescale of ET, a DNA solution consists of assemblies with a heterogeneous distribution of base conformations. In some of these assemblies, the donor, the acceptor, and the DNA bridge, if present, are in conformations that upon excitation, facilitate immediate ET. This we assign to direct ET, observed as the fastest decay component in our femtosecond measurements; these conformations can be termed ET-active. Other DNA assemblies do not adopt ET-active conformations at the time of excitation, but can undergo significant base motions, and therefore conformational reorganization, during the lifetime of Ap*. Some of these assemblies may not assume ET-active conformations and, consequently, Ap* decays through non-ET pathways. In DNA assemblies that do adopt ET-active conformations within the lifetime of Ap*, Ap* decays through ET that is gated by initial reorganizational motion. It is this population of assemblies in which DNA dynamics have the most profound impact on ET. This gating concept has been proposed and confirmed by anisotropy measurements on the femtosecond timescale.[3] The model presented herein is reminiscent of the behavior of ethidium-modified DNA duplexes, except that it is the motion of the DNA bases themselves that act as the gate. A recent theoretical study of DNA conformational order has actually reproduced the biphasic temporal behavior and is entirely consistent with the gating model.[6]

In ApG duplexes, in which the donor and acceptor are in direct contact, the probability of being in an ET-active conformation is high and ET occurs in 10 ps, leaving little time for conformational reorganization. In ApAG, however, the ET is mediated by the DNA bridge and requires ~100 ps, during which time some reorganizational motion may occur. Therefore, it is not surprising that for ApAG duplexes, k_{ET} is even less dependent on the temperature and that the deviation from the conventional model is more pronounced (Figure 3). In this case, we are observing ET in those assemblies that assume ET-active conformations at the moment of excitation, as well as assemblies that undergo fast conformational reorganization prior to ET. The temperature dependence of k_{ET} thus reflects both the direct ET and the rate-limiting reorganizational motions.

The variation in yield of ET with temperature is also consistent with this model. In the steady-state experiments, one probes the fraction of assemblies that are not ET active, and the active fraction (F_q) is deduced by subtraction. Thus there is a parallel with C_1 (Figure 2), the fraction undergoing ET, provided that there are no other significant channels on this timescale. In ApG, a relatively large population of assemblies assumes ET-active conformations at the time of excitation, and thus dynamical motions are not needed for ET. For ApAG, the yield of ET is nearly parallel with C_1, but with some subtle differences: It initially decreases (~10%) with increasing temperature, but then rises (~20%). This increase in yield with increasing temperature is consistent with a gating mechanism. For assemblies initially in ET-inactive conformations, enhanced motion results in a higher probability of an ET-active conformation within the lifetime of Ap*. Consequently, increased base dynamics, within a certain regime, can enhance the efficiency of bridge-mediated ET.

According to the model, the fraction of gated ET, relative to direct ET, should increase with the number of bases that separate the donor and acceptor. In fact, our investigations of longer DNA bridges strongly support this interpretation. In DNA duplexes with four adenine residues between Ap and G, the yield of ET, determined through steady-state measurements, exhibits a marked rise with increasing temperature. This increase begins at ~30 °C below the melting and continues almost to the melting, at which point the yield of ET plummets as the duplex is lost (Figure 4). The increase in ET yield indicates that enhanced base dynamics can facilitate DNA-mediated ET. Additional evidence is derived from the temperature dependence of the ET yield through the same length DNA bridge but with an A–A mismatch (Figure 4). Although the mismatch does not change the base sequence of the strand through which ET proceeds, the disruption in stacking induced by the mismatch results in significantly less ET quenching at low temperatures than in the fully matched DNA. In fact, there is no observable ET at low temperatures in the mismatched duplex. Remarkably, however, with increasing temperature the enhanced motion of the DNA bases enables the mismatched duplex to access ET-active conformations and reach an efficiency that is comparable to that of the fully matched duplex. Again, ET is lost as the

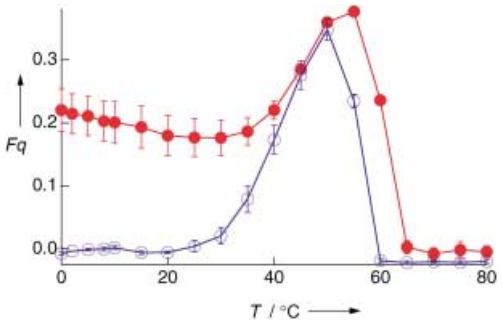

Figure 4. Temperature-dependent yield of ET between Ap* and G through four intervening adenine residues (closed, red circles) and through four intervening adenine residues in which the duplex possesses an A–A mismatch at the third adenine (open, blue circles). The yield of ET is defined by F_q, where $F_q = 1 - \Phi_G/\Phi_I$, and Φ_G and Φ_I are the relative fluorescence quantum yields in the G- and I-containing duplexes, respectively (relative to free Ap in sodium phosphate (pH 7; 100 mM) at each temperature). The ApAAAAY assemblies (Y = I, G) are 35-mers with the same A–T content and melting temperatures as the ApY and ApAY assemblies. The F_q values are the average of three or four independent trials.

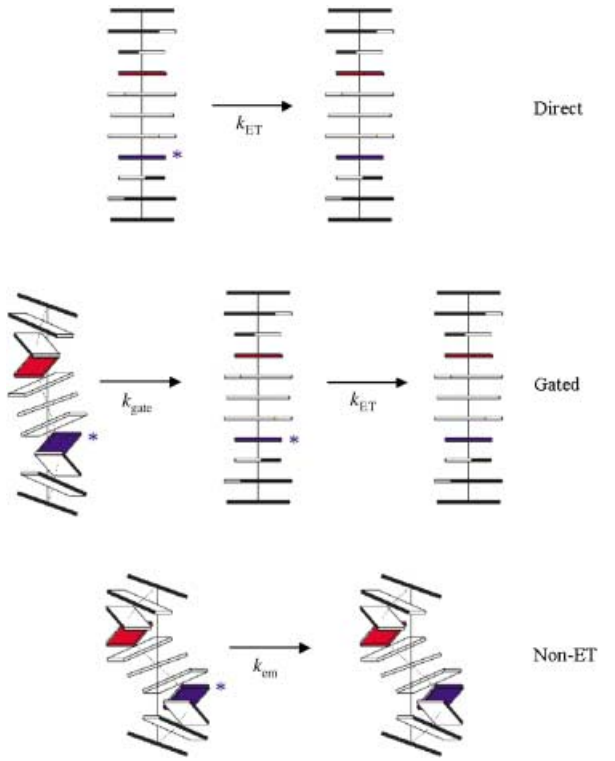

Figure 5. Schematized model depicting the role of DNA base dynamics in DNA-mediated ET. In an ensemble mixture, certain DNA assemblies may adopt conformations that, upon excitation, facilitate direct ET (k_{ET}). Other DNA assemblies do not assume such conformations at the time of excitation, but can reorganize during the lifetime of Ap* (k_{gate}). Assemblies may not attain ET-active conformations and Ap* decays through non-ET pathways such as emission (k_{em}).

duplex melts (~3 °C lower owing to the mismatch) to yield the single strands. Our model is illustrated in Figure 5.

In summary, we used femtosecond spectroscopy to show that ultrafast DNA dynamics play a defining role in DNA-mediated ET. This role originates from the fact that base motions occur on the ET timescale. As ET occurs only through DNA assemblies that have a specific, well-coupled alignment of the DNA bases, motions of the DNA bases that lead to these ET-active conformations can serve as a gate for ET reactions, and thus modulate the rate constants and yields of ET. These data provide compelling experimental evidence that DNA ET cannot be approximated by models designed for more static donor–acceptor assemblies. Fluctuations of DNA bases must be a part of the descriptions of ET dynamics, especially because conformational gating necessarily becomes more important as the DNA bridge is lengthened.

Experimental Section

The femtosecond transient absorption setup has been described previously.[19] Briefly, a pump pulse at 325 nm (< 0.2 μJ per pulse) was used to selectively excite Ap, and then a probe pulse (< 0.01 μJ per pulse) at 600 nm with variable time delay relative to the pump pulse was used to measure the transient absorption initiated by the pump pulse. The polarization of the probe pulse was at the magic angle (54.7°) relative to that of the pump pulse to avoid contributions from the reorientation motion of the molecules. The sample was contained in a stirred 5-mm quartz cell. The temperature was controlled by using a 4-position cell holder under thermostat control, and the temperature was measured with a thermocouple in an identical cuvette. Estimated uncertainties in the determined lifetimes are less than 5% for ApG, and less than 10% for ApAG, and experiment-to-experiment variations in lifetimes are less than 5%. Steady-state fluorescence measurements were conducted on an ISS K2 fluorimeter (pathlength: 5 mm) equipped with a peltier-controlled sample holder (Quantum Northwest) under thermostat control. DNA oligonucleotides were synthesized (trityl on) on an ABI DNA Synthesizer by using standard solid-phase techniques. They were then purified twice on a Hewlett Packard HPLC on a reverse-phase C-18 column with an acetonitrile/ammonium acetate gradient, and subsequently analyzed by mass spectrometry (MALDI). DNA oligonucleotides were quantified through UV/Vis spectroscopy and annealed with regulated cooling from 90–10 °C over a period of 3 h. Duplex melting temperatures were evaluated from the temperature derivative of the absorbance of Ap at 325 nm. Time-resolved and steady-state measurements were carried out on DNA duplexes (50 μM) in sodium phosphate (pH 7; 100 mM).

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