Ultrafast Electron−Transfer Reactions between Thionine and Guanosine Bases

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Ultrafast Electron-Transfer Reactions between Thionine and Guanosine Bases

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Femtosecond electron-transport processes in DNA have received renewed attention both experimentally[1−3] and theoretically.[4−8] From an experimental perspective, optical excitation of an intercalated dye is one method by which electrons or holes can be rapidly injected into DNA strands.[3] Moreover, it has been argued recently that DNA flexibility is the key to its functionality and intercalated dyes can be used as probes of these dynamics.[9,10] Fiebig et al.[11] have recently reported on the photophysics of ethidium complexes with mononucleotides and polynucleotides in water. In this study it was necessary to consider the interplay between orientational motion and electron transfer, since both occurred on a picosecond time scale. In contrast, we present data here with thionine, which illustrate that both the forward and backward processes can be extremely rapid on the time scale of orientational motion, provided that both the energetics and the geometry are favorable.

The excited state of DNA itself is extremely short-lived[12] and it has been suggested that this may protect the molecule from photodamage. However, photodynamic degradation of DNA may also be induced by ultrafast redox reactions.[13] Here, the phenothiazine family of dyes has attracted considerable attention[13−18] because the excited states of the dyes are strongly quenched when they bind near guanine bases. This quenching is believed to be due to electron transfer from the guanine to the dye excited state.[19] Quenching by adenine is less favorable.[17,20] We report here, for the first time, that for thionine this process and the subsequent back reaction to reform the ground state (eq 1) both occur on a femtosecond time scale in the polynucleotide, $[poly(dG\text{-}dC)]_2$, which adopts a B-DNA structure,[21] and slightly more slowly in a thionine-5′-guanosine monophosphate (GMP) complex and in thionine bound to DNA.

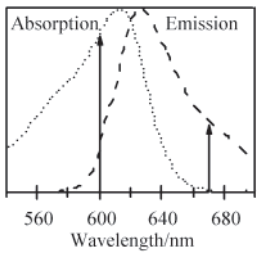

Figure 1. The absorption spectrum of the thionine-$[poly(dG\text{-}dC)]_2$ complex and the emission spectrum of free thionine in water. Pump and probe wavelengths are shown. (The absorption spectrum of free thionine is blue shifted by ca. 15 nm, but the shape remains almost unchanged.)

$$Th^+*,G \xrightarrow{k_f} [Th^\bullet,G^{\bullet+}] \xrightarrow{k_r} Th^+,G \qquad (1)$$

Solutions of thionine (50 μM, Aldrich) in 5 mM phosphate buffer (pH = 6.9) containing either $[poly(dG\text{-}dC)]_2$ (0.5 mM), GMP (100 mM), or calf-thymus DNA (ca. 1.2 mM) (Sigma) were studied. Approximately 98% of the thionine is bound, and 1:2 complexes are expected to predominate for GMP.[22] Binding to the polynucleotide and to DNA is intercalative under these conditions, supported by experiment[18] and force field-based energy calculations on methylene blue with a decamer of alternating G−C bases.[15]

Samples were excited with 25 fs pulses of 600 nm light from a Ti:sapphire pumped, noncollinear, optical parametric amplifier. The transient species, so-formed, were monitored using a second parametric amplifier, also with 25 fs resolution, either by following the reformation of ground-state thionine at 600 nm, or by loss of its singlet state by observing the stimulated emission at 670 nm. At this latter wavelength neither the ground state (Figure 1) nor the excited state absorb significantly. The relative polarization of the pump and probe was set to 54.7° ("magic angle") to remove any contributions from orientational effects.

As shown in Figure 2a the stimulated emission signal indicates that the thionine excited state, when bound to the polynucleotide, reacts with guanine and is strongly quenched with a single-exponential lifetime of 260 fs. The lifetime of free thionine in the absence of the polynucleotide is 320 ps,[17,23] more than a factor of 1200 longer. Monitoring the transient bleaching at 600 nm allows one to follow reformation of the ground state as the reaction products recombine. The signal recovers with a single-exponential lifetime of 760 fs, and this decay will be a lower limit for the return electron-transfer rate, k_r. We estimate the error on the measured lifetimes to be <10%. Similarly, Figure 3 shows that with the mononucleotide, GMP, the excited-state lifetime is

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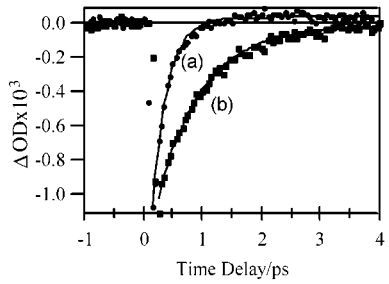

Figure 2. Thionine intercalated with $[poly(dG\text{-}dC)]_2$ showing (a) the loss of the stimulated emission signal at 670 nm (260 fs) and (b) the ground-state recovery at 600 nm (760 fs).

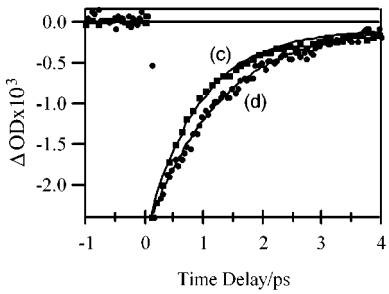

Figure 3. Thionine complexes with GMP showing (c) the loss of stimulated emission at 670 nm (880 fs) and (d) the ground-state recovery at 600 nm (1.2 ps).

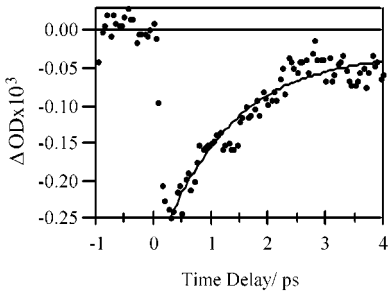

Figure 4. Data showing the ground-state recovery (1.2 ps) at 600 nm of thionine complexed with calf-thymus DNA.

880 fs and the bleach recovery is 1.2 ps. Preliminary data (Figure 4) show the ground-state recovery of thionine bound to C−T DNA also to be ca. 1.2 ps, indicative of rapid quenching mainly by G−C base pairs but with a longer component of ca. 20% (essentially a constant baseline on the time scale of the experiment) due to dyes that are not bound sufficiently close to a guanosine base. We note that all of these rates are fast, due to the favorable Gibbs energy and the short distance between dye and base. In comparison, charge transfer along the DNA chain would occur on the tens of picosecond time scale.[1] Our results also show that both the forward and reverse reactions are extremely rapid on the time scale of molecular reorientation. The rotation time of thionine in water at 298 K is 63 ps,[24] and it is impossible that the dye molecules rotate through any significant angle on the time scale of electron transfer.

Taking the reduction potential for the thionine ground state, E_A, as −0.03 V,[25] its excited-state energy, E_S as 2.03 V,[17] and the oxidation potential for guanine, E_D, in $[poly(dG\text{-}dC)]_2$ as 1.15 V (vs NHE)[26] the Gibbs energy for the forward electron transfer, ΔG_f is calculated to be −0.85 eV, and for the reverse, ΔG_r is −1.17 eV. If the observed quenching is due to electron transfer, within the limits of a classical Marcus treatment we might assume that the forward and return rate constants, k would be given by

$$k = \frac{4\pi^2}{h\sqrt{4\pi\lambda k_B T}}|V|^2 \exp\left(-\frac{(\Delta G + \lambda)^2}{4\lambda k_B T}\right)$$

Solving for λ, the reorganization energy, and $|V|$, the electronic coupling matrix element at 298 K, using the measured forward, k_f, and return, k_r, rates from the $[poly(dG\text{-}dC)]_2$ measurements, gives crude estimates of $\lambda \approx 0.87$ eV ($\sim$7000 cm^{-1}) and $|V| \approx 0.015$ eV ($\sim$120 cm^{-1}). The maximum classical rate, that is, when the driving force is equal to the reorganization energy, ($\Delta G = -\lambda$) corresponds to a rate on the order of 1/260 fs, placing our measured forward rate near the maximum on the Marcus curve.[27] These parameters are typical of weakly coupled donor−acceptor pairs, suggesting that a nonadiabatic treatment is not unreasonable, notwithstanding that the observed rates are on the femtosecond time scale. It is clear that the quenching for the mononucleotide, while still very fast (880 fs), is slower than that for the polynucleotide (260 fs). The oxidation potential for free GMP is thought to be somewhat higher (1.29 V)[24] than for guanine in $[poly(dG\text{-}dC)]_2$ (1.16 V), giving values for the forward and reverse ΔG of −0.71 and −1.32 V, respectively. Using these potentials we would predict slower rates for the forward and return steps in the GMP complex, assuming that the reorganization energy and the electronic coupling are similar to the polynucleotide, in agreement with our experimental observation.

Solution of the Marcus equation for the thionine−GMP complex gives a similar reorganization energy of 8000 cm^{-1} and a 100 cm^{-1} coupling energy. The slower measured rates could also reflect a slightly larger separation between donor and acceptor in the GMP complex than when intercalated in the polynucleotide. Using the same values of λ and $|V|$ from the polynucleotide measurements and the quoted driving force (−0.2 eV), we can also estimate the electron-transfer rate for the ethidium−ZTP complex, which would be ca. 60 ps in agreement within error of the experimental value.[11]

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