Electron Transfer between Metallointercalators Bound to DNA: Spectral Identification of the Transient Intermediate

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Double-helical DNA represents a useful model system to explore how extended π-stacked arrays mediate electron transfer reactions.[1] Our recent experiments have utilized bis(phenanthroline)(dipyridophenazine)ruthenium(II) [$\mathrm{Ru(phen)_2dppz^{2+}}$] and bis(9,10-phenanthrenequinone diimine)(phenanthroline)rhodium(III) [$\mathrm{Rh(phi)_2phen^{3+}}$], both of which intercalate avidly into DNA.[2,3] Previously, we reported quenching of this photoexcited ruthenium(II) donor by the rhodium(III) acceptor with τ < 300 ps over a distance of 40 Å in a covalent assembly.[3] In this work, the mechanism for quenching was assigned as electron transfer because of the strong thermodynamic driving force and the lack of spectral overlap necessary for energy transfer; analogous photophysical studies[4] of covalently-linked ruthenium(II) and rhodium(III) polypyridyl complexes without DNA have also supported an electron transfer mechanism. Using donor and acceptor analogues, [$\mathrm{Ru(DMP)_2dppz^{2+}}$] (DMP = 4,7-dimethylphenanthroline) and [$\mathrm{Rh(phi)_2bpy^{3+}}$] (bpy = 2,2′-bipyridine),[5] we now demonstrate that this quenching mechanism is indeed electron transfer and present the first direct evidence for electron transfer between metallointercalators bound to DNA.

When bound to DNA, photoexcited $\mathrm{Ru(DMP)_2dppz^{2+}}$ (*Ru(II)) is efficiently quenched by Δ-$\mathrm{Rh(phi)_2bpy^{3+}}$ (Rh(III)) as follows:[6]

$$\mathrm{^*Ru(II) + Rh(III) \rightarrow Ru(III) + Rh(II)} \qquad (1)$$

$$\mathrm{Ru(III) + Rh(II) \rightarrow Ru(II) + Rh(III)} \qquad (2)$$

Upon intercalation[7] (nucleotide/Ru = 100), the decay of the $\mathrm{Ru(DMP)_2dppz^{2+}}$ metal-to-ligand charge transfer (MLCT) excited state is biexponential with lifetimes of 32 ns (27%) and 107 ns (73%), as measured by single photon counting. Upon addition of Δ-$\mathrm{Rh(phi)_2bpy^{3+}}$, the luminescence intensity decreases markedly, with only a minimal change in the luminescent lifetimes, consistent with quenching on a time scale that is fast relative to the measurement (<10 ns). The fraction quenched is 0.4 at 2 equiv of Δ-$\mathrm{Rh(phi)_2bpy^{3+}}$ (Figure 1), where only 25% of the donor/acceptor separations are within 20 Å, assuming random binding.[8] Similarly fast luminescent quenching with τ < 300 ps is found[2,3] for the $\mathrm{Ru(phen)_2dppz^{2+}}$/$\mathrm{Rh(phi)_2phen^{3+}}$ donor–acceptor pair bound to DNA, and this observation was recently confirmed by picosecond transient absorption spectroscopy.[9]

An electron transfer intermediate is observed on the microsecond time scale by transient absorption spectroscopy as shown in Figure 1. In the absence of quencher, the 440 nm bleach signal for *Ru(II) returns to the base line in <1 μs, as expected for the kinetics of the MLCT excited state. However, an additional, slower component to the signal ($\tau \gg \tau$(MLCT)) grows in upon titration with Rh(III). If this long-lived transient is in fact an intermediate formed by redox quenching, then its signal size should increase in proportion to the amount of quenching during the titration. This correlation is presented quantitatively in Figure 1(inset).[10] The parallel increases in the fraction quenched and in the transient signal height for times greater than 1 μs clearly demonstrate that the 440 nm transient is directly linked to the quenching, and this observation indicates the formation of an electron transfer intermediate.[11]

The spectral characteristics of the intermediate are also consistent with its assignment as a product of electron transfer. The wavelength dependence for the transient formed by Rh(III) quenching of *Ru(II) is typical of oxidation of ruthenium polypyridyl complexes.[12,13] Figure 2 illustrates transients at two wavelengths. The inset compares the signal height of this long-lived transient at several wavelengths to the Ru(III) − Ru(II) difference spectrum for the DNA-bound donor, generated by electron transfer to the nonintercalating and spectrally silent quencher, $\mathrm{Ru(NH_3)_6^{3+}}$. These data clearly indicate formation of an intermediate containing Ru(III) upon quenching of *Ru(II) by Rh(III). It is noteworthy that the luminescence quenching is faster for the intercalating acceptor compared with the nonintercalating acceptor,[2] but that the decay kinetics of the electron transfer intermediate are of the same order with both acceptors.

While the quenching by Δ-$\mathrm{Rh(phi)_2bpy^{3+}}$ is fast (<10 ns), the decay of the intermediate has observable lifetimes ranging from 1 μs to >1 ms.[14] We estimate that the long-lived signal accounts for approximately 30% of the quenched material,[15] neglecting contribution from Rh(II);[16] some of the intermediate may return to the ground state with $\tau < \tau$(MLCT). The complex

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(6) From cyclic voltammetry, the 3+/2+ reduction potentials of $\mathrm{Ru(DMP)_2dppz^{3+}}$ and $\mathrm{Rh(phi)_2bpy^{3+}}$, respectively, were found to be +1.3 and −0.3 V versus Ag/AgCl in DMF, leading, with E_{00} of 2.2 V (560 nm), to driving forces of 0.6 and 1.6 V for the reactions in eqs 1 and 2, respectively.

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(10) The kinetics of the long-lived signal are independent of quencher concentration at this level of signal/noise.

(11) These data are consistent with *Ru(II)/Rh(III) → Ru(III)/Rh(II) electron transfer, rather than reaction of the ground state donor with the excited state acceptor, as seen recently in a Ru(II)−Rh(III) dyad.[4c]

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(13) The strong similarity between the *Ru(II) − Ru(II) and Ru(III) − Ru(II) difference spectra does not permit one to follow Ru(III) formation at an excited−ground isosbestic point, the standard method for monitoring a fast-appearing intermediate. See: Wallin, S. A.; Stemp, E. D. A.; Everest, A. M.; Nocek, J. M.; Netzel, T. L.; Hoffman, B. M. *J. Am. Chem. Soc.* **1991**, *113*, 1842. Detection of a spectral intermediate is therefore restricted to time scales longer than the excited state decay.

(14) For signals in the presence of Rh(III), the data at $t > \tau$(MLCT) in Figure 1 may be fitted by a biexponential with $\tau_1 = 4$ μs (60%) and $\tau_2 =$ 136 μs (40%).

(15) This estimate assumes that the signal is due solely to the Ru(III) − Ru(II) absorbance change, as obtained by $\mathrm{Ru(NH_3)_6^{3+}}$ quenching.

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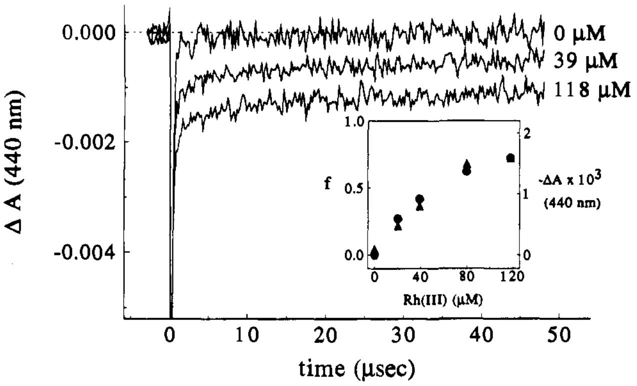

Figure 1. Transient absorption kinetic profiles at 440 nm during a titration of Ru(DMP)$_2$dppz^{2+} (20 μM) with Δ-Rh(phi)$_2$bpy^{3+} in DNA (1 mM base pairs). The partial recovery of ground state Ru(DMP)$_2$dppz^{2+} is monitored at 440 nm with 0, 39, and 118 μM Δ-Rh(phi)$_2$bpy^{3+}. For clarity of presentation, the early portions of the signals corresponding to the excited state decay are truncated. In the inset, the fraction of luminescence intensity quenching (●) and the absolute signal height of the 440 nm transient at 6 μs (▲) are plotted as a function of quencher concentration. Excitation was achieved with an excimer-pumped dye laser, which gave 4.5 mJ/10 ns pulse at 480 nm (Coumarin 480). Luminescence profiles were monitored at 628 nm, the emission maximum for intercalated Ru(DMP)$_2$dppz^{2+}. Intensity quenching was obtained by integrating the time-resolved decays (400 shots). Transient absorption measurements utilized a pulsed xenon arc lamp as the probe, and individual data traces typically required ~2500 shots. Because of overlap in absorption spectra of donor and acceptor, it was necessary to correct the data for interference from the Δ-Rh(phi)$_2$bpy^{3+} excited state. Measurements were carried out with sonicated calf thymus DNA in aerated 5 mM Tris, 50 mM NaCl (pH 8.5) at ambient temperature.

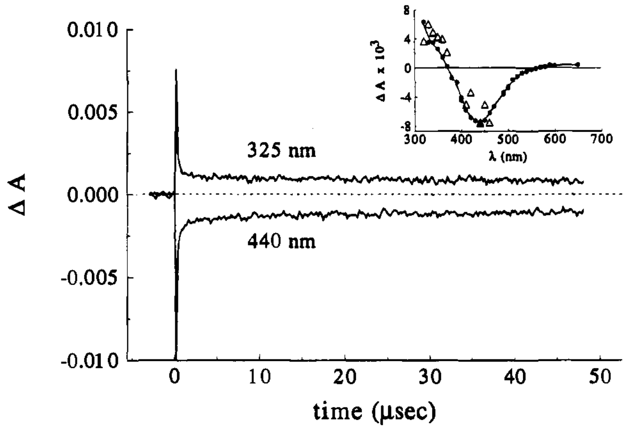

Figure 2. Kinetic profiles at 325 and 440 nm for transients formed upon 480 nm excitation of Ru(DMP)$_2$dppz^{2+} (20 μM) in the presence of Δ-Rh(phi)$_2$bpy^{3+} (118 μM) and DNA (1 mM base pairs). Conditions are as in Figure 1. The inset shows the Ru(III) − Ru(II) difference spectrum (●), obtained by measuring ΔA at $t \gg \tau$(MLCT) for intercalated *Ru(DMP)$_2$dppz^{2+} (10 μM) quenched by Ru(NH$_3$)$_6^{3+}$ (300 μM). Also shown are the absorbance changes extrapolated to $t = 0$ for *Ru(DMP)$_2$dppz^{2+} quenched by Δ-Rh(phi)$_2$bpy^{3+} (Δ). Signal heights were normalized to those of Ru(NH$_3$)$_6^{3+}$ data for comparison. Conditions for the inset were 1.5 mJ/pulse, 470 nm excitation, 0.5 mM base pairs sonicated calf thymus DNA.

kinetics of the back electron transfer are understandable, given that (i) the *Ru decay in the absence of quencher is biexponential, (ii) there are multiple binding orientations for the two intercalated metal complexes,[7,8b,17] and (iii) there are a large number of donor−acceptor separation distances. We can account for the difference between the photoinduced and recombination rates, given that the donor for the forward reaction is in an excited state and is, therefore, better coupled to proposed high-energy bridging states in DNA.[18] The recombination reaction, on the other hand, occurs from a ground state donor [Rh(II)] which is low in energy compared to a molecular bridge.[19] Furthermore, the return to the ground state by back electron transfer through DNA is not the only possible fate for the charge-shifted intermediate. For the small fraction of intermediate which persists after 1 ms, dissociation of the complexes from the helix will occur,[17,20] likely resulting in a second-order reaction or perhaps even decomposition of Ru(III) via oxidation of H$_2$O.[21]

In conclusion, these results confirm that electron transfer is the mechanism for the fast quenching between ruthenium(II) and rhodium(III) metallointercalators bound to DNA. We may now use this system to delineate those factors which govern the ability of the DNA π-system to serve as a charge transport medium, both for the photoinduced forward and thermal back electron transfer reactions.

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(16) The Δε for the intermediate also contains a Rh(II) − Rh(III) contribution, which is likely positive between 300 and 450 nm. Hence, the yield of the electron transfer intermediate may be higher than estimated. Monomeric Rh(II) complexes are not well-characterized because of their instability.

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