Long-Range Triplet Energy Transfer between Metallointercalators Tethered to DNA: Importance of Intercalation, Stacking, and Distance

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The DNA base stack offers a unique medium for long-range charge-transfer (CT) reactions.[1] Photoinduced electron transfer (ET) between ethidium and rhodium intercalators tethered to DNA is remarkably fast ($>10^{10}$ s^{-1}) and exhibits a shallow distance dependence over 17−36 Å.[2] DNA-mediated hole transfer (HT) between intercalators[3−6] and modified DNA bases can also be observed over long molecular distances. These[1−6] and other[7] DNA-mediated CT reactions are remarkably sensitive to the stacking of the reactants and the intervening bases. Other groups have described similar long-range CT through DNA;[8,9] however, the role of the DNA base stack in mediating CT continues to be the subject of considerable experimental[1,9−14] and theoretical[1,15] scrutiny. Herein, we demonstrate DNA-mediated triplet energy transfer (TET) over 31−44 Å between metallointercalators tethered to opposite ends of DNA duplexes.

In classic studies[16] of TET, Closs and Miller showed that TET consists of two concerted CT reactions, HT and ET.[17] The net result of TET is quenching of the donor emission and sensitization of the excited state of the acceptor. We reasoned that if the DNA base stack facilitates both long-range HT and ET, it should similarly facilitate TET. TET between polypyridyl complexes of Ru(II) and Os(II) is well-known.[18] Tor showed quenching between Ru(II) and Os(II) complexes when the reactants were covalently bound but not intercalated in synthetic DNA duplexes.[19] Therefore, we tethered $[M(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ (M = Os, Ru; Figure 1A) complexes, which bind avidly to DNA by intercalation,[6,7,20] to the 5′ terminus of oligonucleotides to provide well-defined assemblies for studies of long-range TET.[21]

For the 16 base pair (bp) duplex **1**, shown schematically in Figure 1, 32% of the Ru(II) donors are quenched by the Os(II) acceptor over an intraduplex separation of at least 37 Å.[22] The luminescence of a sample with the metallointercalators isolated on separate duplexes (8 μM duplex; 4 μM Ru, 4 μM Os) exhibited no quenching; Ru/Os quenching is intraduplex. Sensitization of Os(II) emission was observed with the Ru/Os pair bound to DNA, consistent with TET; however, some reductive quenching by ET cannot be ruled out.[23] In addition, the excited state lifetime of Ru(II) in the singly modified assembly and that of Ru(II) in the doubly modified duplex are not significantly different,[25] which suggests a rapid reaction. Similar dynamics were seen with ET and were proposed to result from gating by base stacking.[2,3]

To demonstrate the importance of Ru/Os stacking in these reactions, we substituted $[Os(bpy)_2(bpy')]^{2+}$ as the acceptor in tethered duplexes bearing Ru(II) and Os(II), **2**. The excited-state characteristics of $[Os(bpy)_2(bpy')]^{2+}$ closely resemble those of the dppz complex,[7,18] but $[Os(bpy)_2(bpy')]^{2+}$ does not intercalate.[7,26] Only 9% of the ruthenium emission was quenched by $[Os(bpy)_2(bpy')]^{2+}$, which reflects a diminution of 72% in the yield of TET compared to that for the intercalated acceptor **1** (Figure 1). This low yield of TET with $[Os(bpy)_2(bpy')]^{2+}$ underscores the importance of intimate association with the DNA base stack to achieve efficient CT between reactants bound to DNA.[1,7]

TET between metallointercalators is also sensitive to the stacking of the bases that intervene between the Ru(II) and Os-

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(21) Conjugates were prepared by coupling $[M(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$, as the *N*-hydroxy succinimidyl ester, to aminoalkylated DNA (Wachter, L.; Jablonski, J.-A.; Ramachandran, K. L. *Nucleic Acids Res.* **1986**, *14*, 7985−7995) on a solid support;[6] Holmlin, R. E.; Tong, R. T.; Yao, J. A.; Barton, J. K., unpublished results.
(22) Studies of Ru(II)-induced DNA damage indicate intercalation at the third base step from the duplex terminus.[6] With intercalation at the third base step of a 16 bp duplex and 3.4 Å stacking separation, the donor and acceptor are separated by 37 Å (Figure 1).
(23) Since the emission intensity of the Ru(II) complex substantially exceeds that of Os(II), $[Ru(phen)_2(dppz)]^{2+}$ was titrated into solutions of an Os-modified 16-mer. In this experiment (8 μM Os, 3 μM Ru, Fq = 0.25), we observed a spectrum with both shape and maximum consistent with emission from Os(II) (Supporting Information). Both TET ($-\Delta G \approx 0.4 \pm 0.1$ eV) and reductive quenching by ET ($-\Delta G \approx 0.3 \pm 0.1$ eV) are favored thermodynamically;[7,24] however, we did not detect either Ru(I) or Os(III) as products of ET by transient absorption on the μs time scale under conditions where these species have been detected for different reactions.[24]
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(25) The excited state of $[Ru(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ (Δ-4 isomer; see Table 1) tethered to the 16 bp in Figure 1 decays as a triexponential with $\tau1$ = 736 ns (58%), $\tau2$ = 196 ns (35%), $\tau3$ = 14.7 ns (7%) without Os(II), and $\tau1$ = 710 ns (43%), $\tau2$ = 207 ns (45%), $\tau3$ = 17.7 ns (12%) with the osmium intercalator, as measured by phase modulation (ISS Inc., Urbana-Champaign, IL). By time-resolved luminescence spectroscopy on the ns time scale, titrations (noncovalent) of Os(II) into solutions of Ru(II) (10 μM) bound noncovalently to DNA (0.5 mM bp) reveal a substantial loss in initial emission intensity as well as some dynamic changes in the excited-state lifetime (Fq (1 equiv Os(II)) = 0.4; for [Os] = 0 μM: $\tau1$ = 635 ns (21%), $\tau2$ = 139 ns (79%); [Os] = 10 μM (1 equiv): $\tau1$ = 555 ns (19%), $\tau2$ = 96 ns (81%)).
(26) The excited-state lifetime of $[Os(bpy)_2(bpy')]^{2+}$ both tethered to DNA (23 ns) and bound noncovalently to DNA (25 ns) is enhanced relative to that of the complex free in solution (15 ns), which indicates that the complex is most likely groove-bound in the presence of DNA. The diminution in TET arises from the lack of intercalation and not because $[Os(bpy)_2(bpy')]^{2+}$ is displaced from the duplex bearing Ru(II).
(27) This sensitivity to bulges rules out a through-space mechanism for quenching, but the modest diminution likely indicates that significant stacking is maintained.

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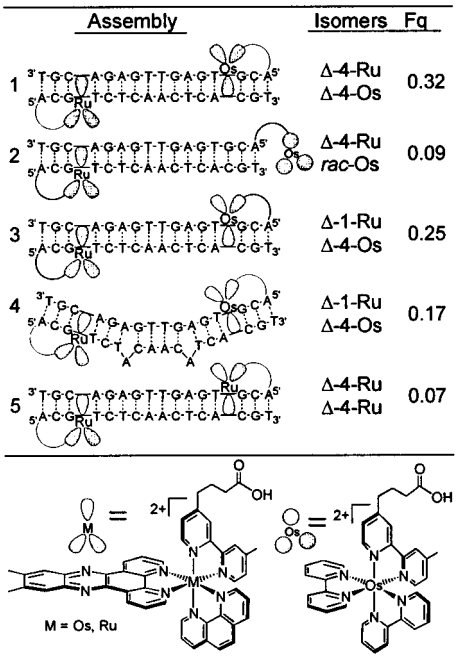

Figure 1. Fraction of $[Ru(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ quenched (Fq = $1\text{-}I_{Ru-Os}/I_{Ru}$) by Os acceptors tethered to opposite ends of DNA assemblies. The complexes $[M(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ (M = Os, Ru; Me_2-dppz = 7,8-dimethyl dipyridophenazine; bpy′ = 4-butyric acid-4′-methyl-2,2′-bipyridine) and $[Os(bpy)_2(bpy')]^{2+}$, are also illustrated schematically. Descriptions of the assemblies follow: (**1**) Duplex (16 bp) bearing two metallointercalators as reactants separated by 37 Å. (**2**) The same 16-mer with $[Os(bpy)_2(bpy')]^{2+}$ instead of $[Os(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ as the acceptor.[26] (**3**) Modified 16-mer with different isomers of $[M(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ compared to **1**. (**4**) Os-modified 16-mer hybridized to a Ru-modified 18-mer to produce two single-base A bulges in the intervening base stack. (**5**) Duplex (16 bp) bearing two $[Ru(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ complexes tethered to opposite 5′ termini. The error in Fq is estimated to be ±10%.These results illustrate (i) the two intercalators behave independently on the helix; (ii) the requirement of intercalated reactants to achieve long-range TET; and (iii) the importance of having a fully stacked pathway for TET. Conditions were [duplex] = 8 μM in 5 mM sodium phosphate, 50 mM NaCl, pH 7.0 at 20 °C. Emission intensities were determined by integrating the area under the emission (Ru) spectrum from 540 to 800 nm with λ_{exc} = 440 nm. Fq was independent of excitation wavelength.

(II) complexes. The introduction of two single-base, adenine bulges, **4**, resulted in a 24% reduction in the yield of TET compared to that for the duplex without bulges, **3** (Figure 1).[4,5] Moreover, melting the duplex leads to a complete elimination of the TET reaction (Supporting Information). Thus, the DNA base stack provides the pathway for this long-range TET reaction.[27]

Owing to the asymmetry of $[M(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$, four different stereoisomers are isolated in the conjugation reactions with DNA.[28] The yield of TET depends very strongly on which of the 16 possible combinations of isomers is reacting (Table 1). Across this series of assemblies, Δ isomers always produce the greatest yield of TET. Moreover, ruthenium isomers that exhibit the greatest emission intensity, which reflects deeper intercalation,

Table 1. Yield of Triplet Energy Transfer for $[M(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ Isomers Tethered to DNA as a Function of Absolute Configuration and Linker Orientation[a,b,c]

	fraction quenched by Os(II)				
Ru/Os	Δ-1-Os	Λ-2-Os	Λ-3-Os	Δ-4-Os	I_{Ru}[c,d]
Δ-**1**-Ru	0.19	0.19	0.21	0.25	0.76
Λ-**2**-Ru	0.11	0.11	0.16	0.17	0.54
Λ-**3**-Ru	0.19	0.11	0.15	0.25	0.67
Δ-**4**-Ru	0.26	0.24	0.28	0.31	1.21

[a] Entries correspond to the fraction of Ru emission quenched (Fq = $1 - I_{Ru-Os}/I_{Ru}$) by osmium in a doubly modified duplex constructed by hybridizing a Ru-modified conjugate from the column (left) to an Os-modified conjugate from the row (top).[28] Conditions were as described in the Figure 1 caption. [b] Since the extinction coefficient for each of the Os-modified strands is the same while the yield of TET is isomer-dependent, the inner-filter effect (light stealing by Os(II)) is ruled out as a cause for emission quenching. We also ruled out an inner-filter effect by comparing ruthenium luminescence with and without a solution of Os(II) in a cuvette along the excitation pathway. [c] Steady-state emission intensity of duplexes singly modified with $[Ru(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$. [d] Excited state lifetimes are not strictly correlated with the yield of TET. For example, with Δ-4-Ru paired with Δ-4-Os the yield of TET is the same as with Δ-1-Ru paired with Δ-4-Os, despite a shorter excited-state lifetime for Δ-1-Ru compared to Δ-4-Ru.

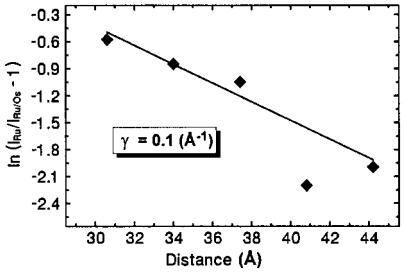

Figure 2. Plot of the distance dependence of the yield TET between metallointercalators tethered to DNA duplexes ranging from 14 to 18 bp in length. The data are given for the mixture of later moving Ru and Os isomers (Λ-3 + Δ-4). The fraction quenched (Fq) at each donor/acceptor separation[22] are: (i) 31 Å: Fq = 0.36; (ii) 34 Å: Fq = 0.30; (iii) 37 Å: Fq = 0.26; (iv) 41 Å: Fq = 0.10; (v) 31 Å: Fq = 0.12. The plot extrapolates to full quenching at short distance. The slope ($-\gamma = -0.1$ $Å^{-1}$) of the line reflects a remarkably shallow distance dependence.

also give rise to the highest yield of TET. These observations reflect the sensitivity of TET to the DNA binding properties of the reactants and show that, even among intercalators, deeper intercalation results in more efficient TET.[1,7]

Finally, we determined the distance-dependence of DNA-mediated TET in a series of Ru and Os-modified duplexes ranging from 14 to 18 bp in length. In Figure 2 we plot a logarithmic function of the steady-state yield of TET as a function of distance. From this plot, a slope ($-\gamma$) of -0.1 $Å^{-1}$ is obtained.[29,30] Values of γ for DNA-mediated ET with intercalators[2,3] have indicated a similarly shallow distance-dependence in yield.

This work represents the first systematic characterization of TET through the DNA base stack. Like other DNA-mediated CT reactions, TET between intercalators is only weakly sensitive to distance but exquisitely sensitive to stacking of the reactants and the intervening medium.

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Supporting Information Available: A plot of the temperature dependence of TET and emission spectra for an Os-modified duplex ± $[Ru(phen)_2(dppz)]^{2+}$ (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(28) $[M(phen)(bpy')(Me_2\text{-}dppz)]^{2+}$ gives two diastereomers (orientation of carboxylate with respect to dppz plane) and their enantiomers (4 isomers). We use a notation that differentiates each sample by the absolute stereochemistry about the octahedral metal center (Δ or Λ, determined by circular dichroism) and the order in which each product was isolated by HPLC. We have not yet assigned the orientational isomers.

(29) For TET mediated by σ-bonded systems, the decay in electronic coupling with distance is reflected by the product of β (Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265−322) for ET and β for HT.[16]

(30) As with ET,[1−3,7] we find that TET appears to be too fast to measure the rate of reaction since the quenching is manifested primarily as changes in initial intensity; β is not obtained. Instead we rely on a parallel measure in changes in the *yield* of CT with distance (γ).