Short-Circuiting the Molecular Wire: Cooperative Binding of Δ-[Ru(phen)$_2$dppz]$^{2+}$ and Δ-[Rh(phi)$_2$bipy]$^{3+}$ to DNA

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In the the rapidly growing literature on electron transfer (ET) mediated by double-helical DNA, considerable disagreement persists in the estimates of the distances over which fast ET may occur.[1−3] Recently, Barton et al. reported[3a] total luminescence quenching of [Ru(phen)$_2$dppz]$^{2+}$ (RU)[4] by [Rh(phi)$_2$bipy]$^{3+}$ (RH), when the complexes were covalently tethered to opposite 5′-ends of a 15 base pair DNA duplex, and interpreted their results as indicative of fast long-range (>40 Å) ET from the intercalated excited state electron donor *RU[5] to the intercalated acceptor RH.[6] The quenching reaction between RU and RH *noncovalently* bound to a large excess of DNA was subsequently studied in detail by these authors and indeed showed very rapid ET from *RU to RH, which under the assumption of a random distribution of intercalated donors and acceptors supports the notion of highly efficient ET through the stack of DNA bases.[3b−f] However, the closely related intercalator [Rh(phen)$_2$phi]$^{3+}$ was surprisingly enough reported to not quench DNA-bound RU luminescence, although it was as active as RH in the quenching of *RU in dodecylsulfate micelles.[3d]

Further, the efficiency of luminescence quenching of *RU by RH was found to depend sensitively on RH and RU chirality and base pair composition.[3d,f] These peculiarities of the quenching reaction prompted us to critically examine the most efficient luminescence quenching system, Δ-RU + Δ-RH in the prescence of [poly(dA-dT)]$_2$, specifically regarding the hypothesis of a random distribution of bound metal complexes.[7] We show here that, even in the presence of a vast excess of binding sites, most acceptors will still be bound adjacent to the donors due to a strong mutual cooperativity in the binding of Δ-RU and Δ-RH to this DNA, implying that the trivial explanation of a short distance between the donor and the acceptor is likely to be responsible for the high ET rates reported.

Circular dichroism spectroscopy (CD) is particulary sensitive to the nondegenerate exciton coupling expected to arise when two different chromophores are close in space. Figure 1 shows the CD spectrum[8] of equimolar amounts of Δ-RU and Δ-RH mixed with [poly(dA-dT)]$_2$ at P/M = 25 (P/M is the ratio of nucleotide to *total* metal complex) compared to the sum of the spectra of Δ-RU and Δ-RH bound separately to [poly(dA-dT)]$_2$ at P/M = 50. A significant perturbation of the spectra of concurrently bound RU and RH is noted. Also shown are the difference spectra between P/M = 25 and P/M = 50 for separately bound Δ-RU and Δ-RH, respectively, which demonstrate that the observed perturbation is due solely to a RU−RH interaction. The perturbation is very similar to that observed in the spectrum of RU and RH mixed with [poly(dA-dT)]$_2$ at P/M = 6, where the complexes are forced to bind in close proximity, indicating that the RU−RH interaction is indeed an exciton coupling due to adjacent binding of complexes also at P/M = 25. The magnitude of the perturbation at P/M = 25 gives a rough estimate for the RU−RH cooperativity parameter ω_{RU-RH} of 20−100.

The cooperativity parameter ω_{ab} is the dimensionless equilibrium constant for binding of ligands a and b as the closest possible ab pair versus isolated binding of a and b. There is disparity in the literature about the binding site size *n* for these complexes with reported values ranging from 2 to 4 base pairs.[2b,3a,b,5,6c] However, according to our binding analyses (Supporting Information), data for both complexes are best fitted with $n = 2.3$, close to the nearest-site exclusion mode of binding ($n = 2$) commonly exhibited by intercalators. Hence, the closest possible proximity in this case is likely a 2 base pair separation of the intercalated ligands, and complexes thus bound side-by-side are referred to in this paper as "adjacent". Notably, if the Δ-RU:Δ-RH pair is intercalated with the ancillary ligands in the same groove, the short distance between the metal centers (*ca.* 10 Å) may permit direct physical contact between the two complexes. A least-squares projection analysis of the spectra from a titration of Δ-RU-saturated [poly(dA-dT)]$_2$ with Δ-RH indicates that the latter complex completely displaces Δ-RU in a 1:1 ratio for P/Rh >12.5 (Supporting Information).

In Figure 2 we demonstrate that this binding cooperativity consistently explains the efficient photoinduced ET observed between the *RU and the RH complex in presence of DNA, while random binding models fail. Figure 2 shows how the luminescence intensity[9] of Δ-RU bound to [poly(dA-dT)]$_2$ at different P/Ru ratios decreases as a function of the amount of Δ-RH added, confirming that the rhodium complex is indeed a remarkably efficient quencher of the ruthenium complex luminescence in a DNA medium. In previous studies, per-

(1) For work on systems not involving RU and RH see: (a) Baguley, B. C.; Le Bret, M. *Biochemistry* **1984**, *23*, 937. (b) Brun, A. M.; Harriman, A. *J. Am. Chem. Soc.* **1992**, *114*, 3656. (c) Meade, T. J.; Kayyem, J. F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 352. (d) Warman, J. M.; de Haas, M. P.; Rupprecht, A. *Chem. Phys. Lett.* **1996**, *249*, 319. (e) In our laboratory, the quenching of Δ-RU by the intercalating viologen *N*,*N*′-dimethyl-2,7-diazapyrenium gave no indication for long-range (>20 Å) electron transfer: Tuite, E.; Lincoln, P.; Becker, H.-C.; Nordén, B. unpublished work.

(2) (a) Risser, S. M.; Beratan, D. N.; Meade, T. J. *J. Am. Chem. Soc.* **1993**, *115*, 2508. (b) Schulman, L. S.; Bossmann, S. H.; Turro, N. J. *J. Phys. Chem.* **1995**, *99*, 9283. (c) Felts, A. K.; Pollard, W. T.; Friesner, R. A. *J. Phys. Chem.* **1995**, *99*, 2929. (d) Priyadarshy, S.; Risser, S. M.; Beratan, D. N *J. Phys. Chem.* **1996**, *100*, 17678.

(3) (a) Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Gathlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025. (b) Murphy, C. J.; Arkin, M. R.; Gathlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5315. (c) Stemp, E. D. A.; Arkin, M. R.; Barton, J. K. *J. Am. Chem. Soc.* **1995**, *117*, 2375. (d) Arkin, M. R.; Stemp, E. D. A.; Turro, C.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 2267. (e) Holmlin, R. E.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 5236. (f) Arkin, M. R.; Stemp, E. D. A.; Holmlin, R. E.; Barton, J. K.; Hörmann, A.; Olson, E. J. C.; Barbara, P. F. *Science* **1996**, *273*, 475.

(4) Abbreviations: phen, 1,10-phenanthroline; dppz, dipyrido[3,2-*a*:2′,3′-*c*]phenazine; bipy, 2,2′-bipyridyl; phi, phenanthrene-9,10-diimine.

(5) (a) Friedman, A. E.; Chambron, J.-C.; Sauvage, J.-P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960. (b) Hiort, C.; Lincoln, P.; Nordén, B. *J. Am. Chem. Soc.* **1993**, *115*, 3448. (c) Haq, I.; Lincoln, P.; Suh, D.; Nordén, B.; Chowry, B. Z.; Chaires, J. B. *J. Am. Chem. Soc.* **1995**, *117*, 4788. (d) Tuite, E.; Lincoln, P.; Nordén, B. Submitted. (e) Lincoln, P.; Broo, A.; Nordén, B. *J. Am. Chem. Soc.* **1996**, *118*, 2644. (f) Chambron, J.-C.; Sauvage, J.-P. *Chem. Phys. Lett.* **1991**, *182*, 603.

(6) (a) Pyle, A. M.; Long, E. C.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 4520. (b) Pyle, A. M.; Morii, T.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 9432. (c) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2302. (d) Uchida, K.; Pyle, A. M.; Morii, T.; Barton, J. K. *Nucleic Acids Res.* **1989**, *17*, 10259.

(7) All experiments, unless otherwise noted, were performed at 20 °C in a pH 6.9 buffer, 5 mM in sodium phosphate and 45 mM in sodium chloride (52 mM total sodium ion concentration), prepared from triple-deionized water (Milli-Q). Double-stranded [poly(dA-dT)]$_2$ (Pharmacia) was dissolved in buffer without further purification. The preparation of homochiral Δ-[Ru(phen)$_2$dppz]Br$_2$ (Δ-RU) has been described elsewhere (ref 5b). [Rh(phi)$_2$bipy]Cl$_3$ was prepared by a slight modification of the published procedure (Pyle, A. M.; Chiang, M. Y.; Barton, J. K. *Inorg. Chem.* **1990**, *29*, 4487) and recrystallized from methanol/ether. Fractional precipitation of a solution of the racemate with sodium arsenyl-D-(−)-tartrate, until the precipitate and the remaining solution showed the same optical activity, afforded pure Δ-RH enantiomer, which was isolated as solid arsenyltartrate by further addition of sodium arsenyl-D-(−)-tartrate to the mother liquid. This arsenyl-D-(−)-tartrate salt of Δ-RH was then used in the experiments.

(8) CD spectra were recorded on a Jasco J-700 spectropolarimeter.

(9) Steady state luminescence intensities were measured on air-equilibrated solutions on a Spex Fluorolog τ2 spectrofluorimeter with an excitation wavelength of 440 nm and corrected for excitation light absorption and sample dilution. Although slow attainment of equilibrium in a similar system has been reported (ref 2b), we found the quenching to be complete within the time for mixing (*ca.* 15 s) and the intensity to be stable for at least several hours. However, experiments performed in a buffer of lower ionic strength (5 mM sodium phosphate, pH 6.9) showed a minor slow component of the quenching with a half-life of about 15 min.

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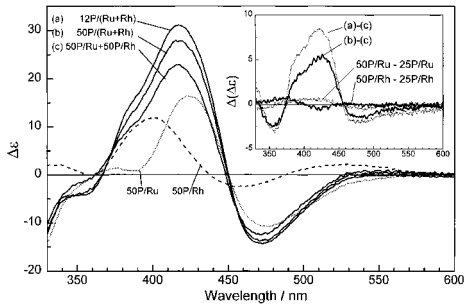

Figure 1. Circular dichroism spectra showing short-distance interactions between Δ-[Ru(phen)$_2$dppz]$^{2+}$ (RU) and Δ-[Rh(phi)$_2$bipy]$^{3+}$ (RH) at low DNA-binding densities. (a) 12P/(Ru + Rh) (solid line): Spectrum of Δ-RU (10 μM) and Δ-RH (10 μM) in the presence of [poly(dA-dT)]$_2$ (AT, 120 μM nucleotides). (b) 50P/(Ru + Rh) (solid line): Spectrum of Δ-RU (10 μM) and Δ-RH (10 μM) in the presence of AT (500 μM nucleotides). 50P/Ru (dotted line): Spectrum of Δ-RU (10 μM) in the presence of AT (500 μM nucleotides). 50P/Rh (dashed line): Spectrum of Δ-RH (10 μM) in the presence of AT (500 μM nucleotides). (c) 50P/Ru + 50P/Rh (solid line): Sum of the spectra 50P/Ru and 50P/Rh. Inset shows the difference spectra a−c (dotted line) and b−c (solid line) and the difference spectrum of Δ-RU (10 μM) in the presence of different concentrations of AT (500 and 250 μM, dotted line) and the difference spectrum of Δ-RH (10 μM) under the same conditions (solid line). The circular dichroism has been normalized to molar differential absorptivity ($\Delta\epsilon$).

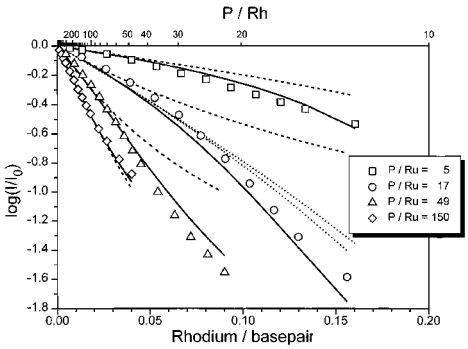

Figure 2. Semilogarithmic plots of normalized emission intensities of Δ-[Ru(phen)$_2$dppz]$^{2+}$ (RU) bound to [poly(dA-dT)]$_2$ duplex (AT) observed at 630 nm as function of added Δ-[Rh(phi)$_2$bipy]$^{3+}$ (RH): (□) 27.5 μM RU and 137 μM AT (P/Ru = 5); (○) 2.5 μM RU and 42.5 μM AT (P/Ru = 17); (△) 2.5 μM RU and 122 μM AT (P/Ru = 49); (◇) 8.5 μM RU and 1.27 mM AT (P/Ru = 150). Emission intensities are normalized with respect to emission in the absence of quencher. Typically, 3 mL of sample was titrated with 10 μL aliquots of Δ-RH stock solution. The curves show the best global fits of three different models to the experimental data (see text): (dotted line) random-binding model; (dashed line) random-binding model where intercalating RU blocks ET path; (solid line) RU−RH cooperative binding model. Due to the high binding constants,[5c,6c] quantitative binding of both complexes was assumed in calculations of normalized emission intensities, except in the titration at P/Ru = 5, where RH was allowed to completely displace RU after the free binding sites were saturated, as suggested by absorption titration data (Supporting Information).

formed at the very high P/Ru ratio of 100 to supress random RU−RH contacts, single luminescence titrations of RU with RH have been excellently fitted by a sphere-of-action quenching model with no indication of RU−RH cooperativity.[2b] Accordingly, assuming a random distribution of the complexes on the polynucleotide, the data for P/Ru = 150 imply a remarkable "sphere-of-action" for the rhodium quencher with a radius of more than 100 Å! However, the effects of cooperative binding depend sensitively on the binding densities of both compounds and significant cooperativity should be manifest as deviations from the behavior expected for random binding, provided both P/Rh and P/Ru ratios are varied. Indeed, when the P/Ru ratio is varied, we find that the large sphere-of-action radius calculated at P/Ru = 150 is inconsistent with the behavior at lower P/Ru ratios: at P/Ru = 5 the quenching can be explained simply by the displacement of one RU by one RH (*vide supra*) and concommitant quenching of adjacent complexes only. In order to quantitatively analyze the quenching data, we have developed a generalized McGhee−von Hippel approach,[10] which allows exact calculations of the probability of quenching directly from binding density data (Supporting Information).

The random-binding single-sphere-of-action model fails completely to describe the observed quenching (the best global fit to the data, giving a sphere-of-action radius of 8 base pairs or *ca.* 27 Å, is shown by dotted lines in Figure 2), since it predicts the quenching at a given P/Rh ratio to be practically independent of the P/Ru ratio.[2b] An explicit P/Ru dependence is obtained if one assumes that the ET path is blocked by any intercalated Δ-RU that may sit between donor and acceptor. The dashed curves in Figure 2 show the best global fit to the data for this model with a sphere-of-action radius for ligand-free DNA stretches of 38 base pairs or *ca* 130 Å. Further, the fit of this model is poor, especially at lower P/Rh ratios.

By contrast, a good global fit to the experimental data (solid lines) was obtained with a cooperative binding model in which quenching of Δ-RU was allowed only by adjacently bound Δ-RH. The only adjustable parameter ω_{RU-RH} had the rather high value of 55 for the best fit. Including more parameters to allow RU−RU cooperativity and different RU−RH cooperativity parameters for the bipy and the phi face of the unsymmetrically bound RH complex did not improve the fit. Moreover, if longer range quenching than between adjacently bound complexes was included the fit actually deteriorated.[11]

This result may seem at variance with a RH photofootprinting study that did not detect perturbations in the cleavage pattern in the presence of RU.[3f] However, both RH and RU are known to be largely sequence neutral in their DNA binding; therefore, cooperativity would not be expected to give any large perturbations. Further, it remains undetermined to what extent the variations in the photocleavage pattern observed with RH reflect the binding density or the DNA reactivity.

In conclusion, we have strong spectroscopic evidence that Δ-[Ru(phen)$_2$dppz]$^{2+}$ and Δ-[Rh(phi)$_2$bipy]$^{3+}$ bind cooperatively to a [poly(dA-dT)]$_2$ duplex and that the remarkable quenching properties of Δ-RH can be quantitatively described by a cooperative binding model where ET only occurs from donors bound adjacent to acceptors. This "short circuiting" offers an explanation for the Δ-RH-loading-invariant electron transfer rates found by others[3f] and the highly stereospecific quenching efficiency. The results prompt for further studies on well-characterized covalently linked systems or on systems in which the electron donor and acceptor show true uncorrelated binding. The strong RU−RH cooperativity is an interesting phenomenon that warrants further study to give us better insight into the mechanisms of DNA binding of metal complexes.

Note Added in Proof: In a later submission, Barbara and co-workers (*J. Phys. Chem.*, in press) reach the same conclusion regarding donor−acceptor cooperativity in this system by analyzing the data from ref 3f.

Supporting Information Available: A theoretical quenching model and analysis of competitive RU−RH binding from spectral data (9 pages). See any current masthead page for ordering and Internet access instructions.

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(10) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469 (erratum: *Ibid*, **1976**, *103*, 679).

(11) For samples quenched to 50%, 17% reduction of both the short and the long lifetimes was observed for P/Ru = 5, while no effect on lifetimes were noted at P/Ru = 150. The lifetime quenching could result from some longer-than-nearest neighbor ET or could be a consequence of a slight change in the intercalation geometry of Δ-RU in the Δ-RU- and Δ-RH-saturated polynucleotide. Due to this uncertainty, we considered attempts to estimate an ET distance decay parameter (β) value an over-interpretation of data and have modeled with all-or-none quenching only.