Flipping the Molecular Light Switch Off: Formation of DNA-Bound Heterobimetallic Complexes Using $Ru(bpy)_2tpphz^{2+}$ and Transition Metal Ions

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Over the past 15 years, several groups have reported on the DNA-binding properties of polypyridyl complexes of Ru(II).[1] Of these, complexes containing the ligand dppz (dppz = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine) have emerged as the most promising metal-based molecular probes of DNA.[2] The finding of strong binding constants (10^5-10^8 M^{-1}) for $Ru(L)_2dppz^{2+}$ (L = bpy (bpy = 2,2′-bipyridine) or phen (phen = 1,10-phenanthroline)) in the presence of DNA, in conjunction with extensive spectral analysis, supports an intercalation binding model for these complexes.[2,3] These complexes function as "molecular light switches" in aqueous solution, exhibiting negligible luminescence in the absence of DNA and strong luminescence upon addition of DNA. Protection of the phenazine nitrogens from solvent appears to be necessary for complexes of this type to luminesce in DNA solutions.[4]

The search for other "molecular light switch complexes" has led us to the investigation of $Ru(bpy)_2tpphz^{2+}$ (tpphz = tetrapyrido[3,2-*a*:2′,3′-*c*:3″,2″-*h*:2″,3″-*j*]phenazine.[5] In most respects, $Ru(bpy)_2tpphz^{2+}$ possesses properties similar to those of $Ru(L)_2dppz^{2+}$, including the "molecular light switch" property,[4] placement of the phenazine nitrogens in a similar orientation, and planar architecture[6] of the π-extended ligand. In contrast to $Ru(L)_2dppz^{2+}$, $Ru(bpy)_2tpphz^{2+}$ contains a phenanthroline-like coordination site at the periphery of the tpphz ligand where metal complexation can occur.[7] Spectroscopic evidence and correlation of data obtained for other Ru(II) complexes interacting with DNA[8] suggest that $Ru(bpy)_2tpphz^{2+}$ binds to DNA (at high [DNA-P]/[Ru] ratios) primarily by intercalation of the tpphz ligand between adjacent base pairs of the duplex, similar to several other Ru(II) complexes that have been investigated previously.[9] In addition, the peripheral coordination site on the tpphz ligand appears to remain accessible while $Ru(bpy)_2tpphz^{2+}$ is bound to DNA, evidenced by spectral changes consistent with metal coordination to tpphz as transition metal ions are added to DNA-bound $Ru(bpy)_2tpphz^{2+}$.

This report focuses on the formation of DNA-bound heterobimetallic complexes using $Ru(bpy)_2tpphz^{2+}$ [10] and Cu^{2+}. We propose that coordination of Cu^{2+} occurs while the tpphz portion of $Ru(bpy)_2tpphz^{2+}$ is intercalated between the base pairs of DNA, and addition of Cu^{2+} causes loss of luminescence and absorption spectral shifts consistent with metal complexation, coupled with *retention* of binding by intercalation. We also propose that Cu^{2+} coordinates to tpphz from the opposite side of the helical axis with respect to the intercalated $Ru(bpy)_2tpphz^{2+}$, analogous to a molecular nut (the Cu^{2+} ion) and bolt (the $Ru(bpy)_2tpphz^{2+}$ complex) through DNA.

Absorption spectra[12] for aqueous and calf thymus DNA (Sigma) solutions of $Ru(bpy)_2tpphz^{2+}$ in 5 mM Tris, pH 7.4 buffer are shown in Figure 1a. At [DNA-P]/[Ru] = 50, 10 μM Ru, essentially all of the complex is bound, and hypochromism of the bands assigned as tpphz intraligand (IT) transition bands ($n-\pi^*$ and $\pi-\pi^*$)[5] in the range 350−390 nm is evident. The hypochromism observed is not altered as a function of ionic strength (up to 1 M NaCl), contrary to weaker binding complexes such as $Ru(phen)_3^{2+}$ [13] which lose their hypochromism under high-salt conditions. Estimates of the binding constant of $Ru(bpy)_2tpphz^{2+}$ to DNA are 10^5-10^6 M^{-1}.

Coupled with hypochromism, strong luminescence[12] centered at 617 nm is found for DNA-bound $Ru(bpy)_2tpphz^{2+}$ at [DNA-P]/[Ru] = 50, with a monoexponential luminescence lifetime[12] (630 ± 13 ns) exceeding the lifetime of the complex in CH_3CN (213 ± 10 ns). It is thus apparent that monomeric $Ru(bpy)_2tpphz^{2+}$ binds strongly to DNA, suggesting intercalation as a probable binding mode.

The absorption spectra in Figure 1b show DNA-bound $Ru(bpy)_2tpphz^{2+}$ at [DNA-P]/[Ru] = 50, 10 μM Ru with and without 10 μM Cu^{2+} at 25 °C. Clearly, the IT band at 384 nm shifts to higher energy, but no evidence for cation exchange as a result of added Cu^{2+} is apparent, as the hypochromism of the IT band is retained after the addition of Cu^{2+}. Similarly, the IT band of the dimeric complex $[(bpy)_2Ru(tpphz)Ru(bpy)_2]^{4+}$ in CH_3CN is found to be higher in energy relative to monomeric $Ru(bpy)_2tpphz^{2+}$ in CH_3CN, with an IT band at 370 nm for the dimer and 380 nm for the monomer.[5]

In association with the IT band energy shift, Figure 2 indicates that total loss of luminescence occurs with increasing amounts of Cu^{2+}. A Cu^{2+} titration using a 10 μM Ru, [DNA-P]/[Ru] = 50 solution indicated that total loss of luminescence occurred at

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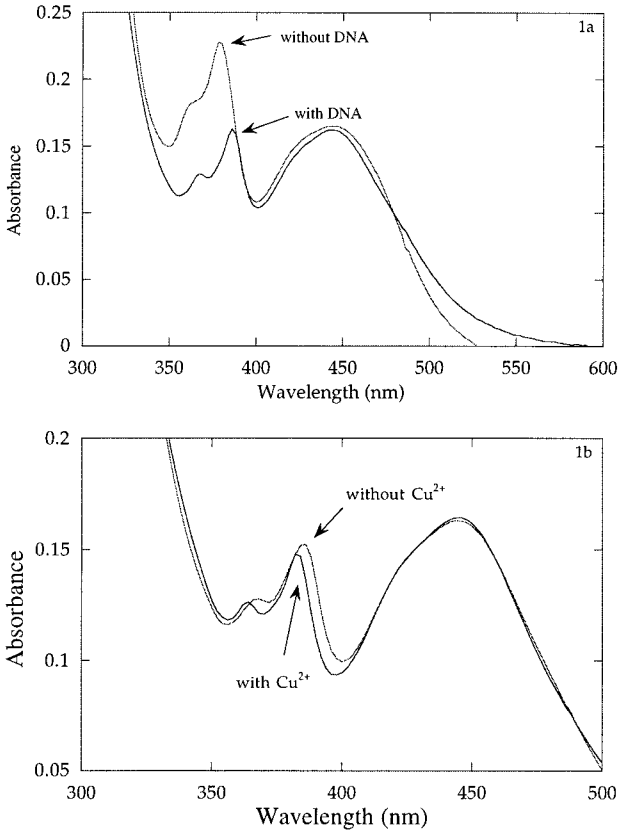

Figure 1. (a) $Ru(bpy)_2tpphz^{2+}$ (10 μM) with and without 500 μM calf thymus DNA, 5 mM Tris buffer, pH 7.4, [DNA-P]/[Ru] = 50 at 298 K. Reagent blanks containing 5 mM Tris and 500 μM DNA were used for the DNA-containing solutions. (b) Same with 10 μM Cu^{2+} added. Spectra were collected after 5 min at 298 K. A reagent blank containing 5 mM Tris and 500 μM DNA was used.

a ca. 1:1 $[Ru(bpy)_2tpphz^{2+}]/[Cu^{2+}]$ ratio. During the titration, while intensity of the luminescence decreased as a function of Cu^{2+}, it was noted that the luminescence lifetime of the complex in DNA remained constant as Cu^{2+} was added. Similar quenching of luminescence due to transition metal complexation has also been observed with other Ru(II) systems, for example, with aqueous solutions of $Ru(bpy)_2ppz^{2+}/Cu^{2+}$.[15]

The quenching observed for this system is not consistent with quenching by diffusion-contolled intermolecular electron or energy transfer found in other systems which involve Ru(II) polypyridyls such as aqueous $Ru(bpy)_3^{2+}$ and $Ru(phen)_3^{2+}$.[16] Typical experiments with $Ru(bpy)_3^{2+}$ and quenchers such as ferricyanide demonstrate a decrease in luminescence intensity coupled with decreasing luminescence lifetime as a function of increasing quencher concentration, characteristic of diffusional quenching. For systems like aqueous $Ru(bpy)_3^{2+}$, higher concentrations of quencher are generally required to see decreases in luminescence intensity and lifetime. With the DNA-bound $Ru(bpy)_2tpphz^{2+}$ system, a decrease in luminescence intensity of DNA-bound $Ru(bpy)_2tpphz^{2+}$ upon addition of Cu^{2+} is coupled with a nondecreasing luminscence lifetime, indicative of strong associational

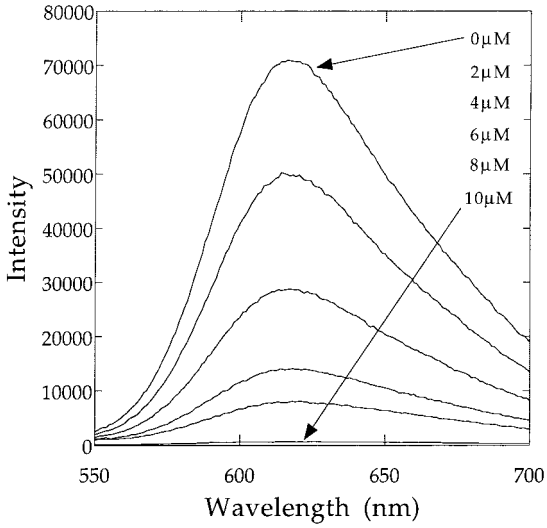

Figure 2. $Ru(bpy)_2tpphz^{2+}$ (10 μM) in 500 μM calf thymus DNA, 5 mM Tris buffer, pH 7.4, [DNA-P]/[Ru] = 50 at 298 K. Increasing amounts of Cu^{2+} up to 10 μM were added. All samples were excited at 450 nm and collected under the same instrumental conditions.

quenching.[17] This observation is consistent with formation of a nonluminescent species, most likely the heterobimetallic dimer $[Ru(bpy)_2(tpphz)Cu]^{4+}$. It is apparent that as Cu^{2+} becomes available, it coordinates to the $Ru(bpy)_2tpphz^{2+}$, initiating associational quenching. Those $Ru(bpy)_2tpphz^{2+}$ ions which are not coordinated to Cu^{2+} (due to insufficient availability of Cu^{2+}) remain luminescent in the DNA (with the same lifetime) until sufficient Cu^{2+} is made available for coordination to $Ru(bpy)_2tpphz^{2+}$.

In summary, $Ru(bpy)_2tpphz^{2+}$, a highly luminescent DNA probe, can be rendered nonluminescent yet remain intercalated in DNA by addition of equimolar concentrations of Cu^{2+}. Formation of a DNA-bound heterobimetallic complex is evidenced by the retention of hypochromism of the IT bands, binding which is independent of salt concentration up to 1 M, and spectral shifts reminiscent of metal ion complexation, coupled with luminescence quenching at [Ru]/[Cu] = 1. Studies using DNA-bound $Ru(bpy)_2dppz^{2+}$ under identical conditions showed no evidence of luminescence quenching upon addition of Cu^{2+}, presumably because $Ru(bpy)_2dppz^{2+}$ lacks a vacant coordination site. A plausible binding model for the $[Ru(bpy)_2(tpphz)Cu]^{4+}$ heterobimetallic complex situates the $Ru(bpy)_2tpphz^{2+}$ region of the complex intercalated between the base pairs of DNA, and places the tpphz coordination site (where Cu^{2+} coordinates) on the opposite side of the helix with respect to $Ru(bpy)_2tpphz^{2+}$. It is likely that $Ru(bpy)_2tpphz^{2+}$ is oriented such that the periphery of the tpphz ligand protrudes out of the opposite side of the helix sufficiently to allow for coordination of Cu^{2+} to occur. It is evident from this binding model that if $Ru(bpy)_2tpphz^{2+}$ intercalates from the major groove, then Cu^{2+} must be coordinated in the minor groove, and conversely, if $Ru(bpy)_2tpphz^{2+}$ intercalates from the minor groove, then Cu^{2+} must be coordinated in the major groove.

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Supporting Information Available: Synthetic and spectroscopic details and comments. This material is available free of charge via the Internet at http://pubs.acs.org.

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