Proton Transfer Quenching of the MLCT Excited State of $Ru(phen)_2dppz^{2+}$ in Homogeneous Solution and Bound to DNA

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Received February 2, 1995[⊗]

Abstract: The bimolecular quenching of the metal-to-ligand charge transfer (MLCT) excited state of $Ru(phen)_2(dppz)^{2+}$ (phen = 1,10-phenanthroline, dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) by proton transfer has been investigated in homogeneous acetonitrile solutions and in the presence of calf thymus DNA. In acetonitrile the monoexponential decay of the MLCT excited state emission of $Ru(phen)_2(dppz)^{2+}$ is dynamically quenched by proton donors with $pK_a = 4.7-15.7$. The emission lifetimes and quenching when the complex is bound to DNA have been measured for racemic mixtures, as well as Δ- and Λ-$Ru(phen)_2(dppz)^{2+}$, and the values compared. In the presence of DNA the biexponential decay of the emission is quenched dynamically and three times slower than in acetonitrile when the quencher, in this case hydroquinone, is hydrophilic. Static quenching is observed in the presence of DNA when a hydrophobic proton donor, *o*-chlorophenol, is utilized. The static quenching with *o*-chlorophenol is shown to arise solely from the quenching of the long-lived component. These observations are explained in terms of two different modes of binding between the complex and DNA, as well as the different affinities for the aqueous medium of the quenchers.

Introduction

The binding of Ru(II) complexes to DNA has been the subject of intense investigation, owing to their stereo- and sequence-specific interaction with the double helix.[1-5] Two of the most interesting complexes investigated are $Ru(phen)_2(dppz)^{2+}$, whose structure is shown in Figure 1, and $Ru(bpy)_2(dppz)^{2+}$ (phen = 1,10-phenanthroline, bpy = 2,2′-bipyridine, and dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine), which have the highest binding affinity for DNA ($K_b \geq 10^6\ M^{-1}$) and intercalate in the double helix.[6-9] The importance of understanding the geometry and structure of the intercalation modes of these complexes, as well as the extent of protection of the dppz ligand by DNA, is necessary in the design of new complexes with better binding characteristics and to provide a basis for the observed fast electron transfer through DNA when these complexes are utilized.[10] There is evidence from photoluminescence and NMR studies[6-9] which indicates the existence of two different binding geometries between $Ru(L)_2(dppz)^{2+}$ complexes (L = bpy, phen) and DNA, although studies utilizing the linear dichroism technique suggest that only one binding mode is present.[11] However, all studies agree on the existence of at least one intercalative binding mode; the present study provides clear evidence that at least one other binding geometry of $Ru(phen)_2(dppz)^{2+}$ to DNA exists where the dppz ligand is protected from the solvent.

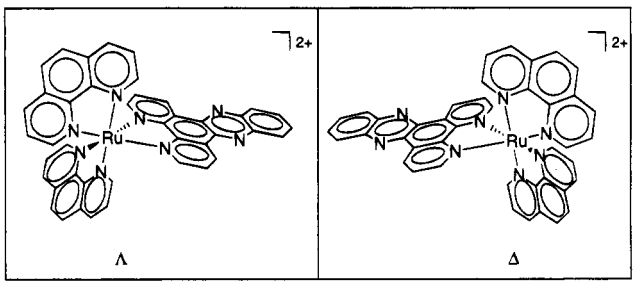

Figure 1. Structural representation of Λ- and Δ-$Ru(phen)_2(dppz)^{2+}$.

The strong binding of $Ru(L)_2(dppz)^{2+}$ complexes to DNA gives rise to the "molecular light switch effect", where the nearly undetectable emission from the triplet metal-to-ligand charge transfer (MLCT) excited state of $Ru(L)_2(dppz)^{2+}$ in water becomes strongly enhanced upon binding, assigned to intercalation of the planar dppz ligand between the base pairs of DNA.[6-9]

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⊗ Abstract published in *Advance ACS Abstracts,* August 15, 1995.

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However, the exact nature of this intercalation, which takes place with a binding constant of 6×10^7 M^{-1}, remains to be determined.[6-9,11] The interaction of the dppz ligand with DNA may be probed by monitoring the changes in the photophysical properties and excited state reactivity of $Ru(L)_2(dppz)^{2+}$ upon addition of DNA, and these results may be applied to elucidation of the mode of binding of this complex to DNA.

The MLCT excited state of Ru(II) diimine complexes is characterized by a strong long-lived luminescence, from a state from which the promoted electron is localized on one of the ligands. In heteroleptic complexes, where the ligands are substituted bipyridines and phenanthrolines, the promoted electron is expected to be mainly localized on the most easily reduced ligand.[12-15] For example, in $Ru(L)_2(dppz)^{2+}$ the lowest energy MLCT transition is localized on the dppz ligand,[16] as determined from emission and transient absorption studies, and therefore the excited state may be written as $*Ru^{III}(L)_2(dppz^{\bullet-})^{2+}$.[14-17] This excited state structure suggests that the reactivity of the reduced ligand may be monitored specifically, and can therefore be utilized to probe the intercalation of the dppz ligand in DNA.

The emission of the MLCT excited state of dppz-containing Ru(II) complexes in acetonitrile is quenched by H_2O, and less effectively by D_2O, which is indicative of proton complexation and/or transfer to the nitrogens of the reduced dppz ligand.[5] Excited-state proton transfer is commonly observed in organic molecules,[18-24] however only examples of the analogous ground-state reaction are prominent in transition metal inorganic complexes.[25-27] The basicity of Ru(II) complexes with ligands which contain non-coordinating nitrogens has been shown to increase in their MLCT excited state,[28-31] and the proton transfer quenching of the MLCT excited state of $Re(py)_4O_2^+$ has been reported for proton donors of pK_a values ranging from -1.0 to 15.5.[32] In the latter complex the charge transfer transition places the negative charge on the oxygens, which transforms them into avid proton abstractors. A similar quenching mechanism may be postulated for the MLCT excited state of dppz-containing Ru(II) complexes, where the nitrogens in the reduced dppz ligand may abstract protons from acids.

Two distinct binding modes have been proposed for $Ru(L)_2(dppz)^{2+}$ complexes bound to DNA based on the negligible quenching of the MLCT excited state by ferrocyanide, as well as from the photophysical properties of complexes containing substituted dppz ligands, which include a biexponential emission decay.[7,8] Both binding modes are proposed to be intercalative in nature, the difference between the two being the extent of the exposure of the dppz nitrogens to the solvent. The more exposed dppz structure possesses a shorter lifetime, owing to the quenching by water in the bulk solution. Another interpretation has been proposed by Nordén based on linear dichroism results, which are consistent with only one binding geometry of $Ru(phen)_2(dppz)^{2+}$ with DNA.[11] Nordén also observed a biexponential decay of the DNA-bound complex, however, even for the separated Δ and Λ enantiomers. The authors attribute the two observed lifetimes to differences in the base pair sequence about the binding site, with both emitting species having a similar binding constant and geometry (dppz axis perpendicular to the long axis of DNA).[11] Emission from racemic dppz complexes bound to synthetic polynucleotides of different sequences, however, also exhibits a biexponential decay.

The two different proposals of the binding of the complex to DNA may be probed by monitoring the quenching of each lifetime component of $*Ru(phen)_2(dppz)^{2+}$ bound to DNA with reactants in the aqueous phase and those bound more closely to the helix. If there are indeed two binding geometries to DNA with significantly distinct exposures of the dppz nitrogens, then each lifetime component would be expected to exhibit different excited state reactivity toward proton transfer quenching. To this end we have utilized a neutral hydrophobic and a hydrophilic proton donor, which act as proton donating quenchers of the MLCT excited state of $Ru(phen)_2(dppz)^{2+}$ molecules bound to DNA. Since the proton donors most plausibly quench through interaction with the dppz nitrogens, these experiments may be utilized to measure the depth of the intercalation of the ligand or exposure of the nitrogens to the solvent. On the other hand, if the two lifetime components are simply due to the difference in the surrounding DNA base pairs in binding modes with similar geometry and binding constant as proposed by Nordén, then negligible difference would be expected between the quenching rate constants of each lifetime component, since access to the nitrogen atoms responsible for quenching is expected to be comparable for similar binding geometries. As part of a systematic program we have explored the proton transfer quenching of the MLCT excited state of $Ru(phen)_2(dppz)^{2+}$ utilizing proton donors with pK_a values in the 4.7–15.7 range in acetonitrile.

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Table 1. Short and Long Emissive Lifetimes (τ_q^{sh} and τ_q^{lo}, respectively) of Λ- and Δ-Ru(phen)$_2$(dppz)$^{2+}$, as well as the Racemic Mixture, Bound to DNA, Along with Their Respective Pre-exponential Coefficients, A^{sh} and A^{lo}

enantiomer	τ_q^{sh}/ns	A^{sh}/%	τ_q^{lo}/ns	A^{lo}/%
racemic	93	63	512	37
Δ	97	57	473	43
Λ	61	43	346	57

Experimental Section

Materials. The quenchers were purchased from Aldrich and were either recrystallized or used without further purification. The ligand dppz was synthesized by the refluxing 1,10-phenanthroline-5,6-dione with *o*-diaminobenzene in ethanol.[33,34] The complex Ru(phen)$_2$(dppz)$^{2+}$ was synthesized from its Ru(phen)$_2$Cl$_2$ precursor by a standard procedure[13,35] and was subsequently purified utilizing reversed-phase HPLC (0.1 M triethylammonium acetate/CH$_3$CN eluent adjusted to pH = 6.0) with a C18 column (Vydac). Calf thymus DNA was purchased from Sigma and purified by standard methods.[36] Δ- and Λ-Ru(phen)$_2$(dppz)$^{2+}$ were separated by passing 0.12 M potassium antimonyl tartrate (Aldrich) in water through a Sephadex SP C-25 cation exchange column. Once separation occurred the tartrate was washed with water, and both bands were eluted with sodium chloride gradient. The isomers separated in this manner contain ~75% of the desired enantiomer.

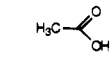
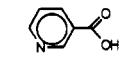
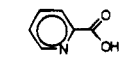
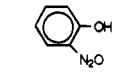
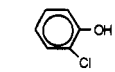
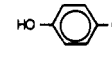
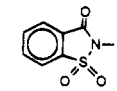
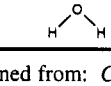
Methods. Steady-state emission spectra were collected with a Perkin Elmer LS-5 spectrometer with λ_{exc} = 440 nm. Time-resolved luminescence decays were measured with a single photon counting instrument which has been previously described in detail.[37] Absorption spectra were measured with a Perkin Elmer 3840 diode array spectrometer. All experiments in which DNA was utilized contained 80 μM base pair concentration and were performed in 5 mM Tris, 50 mM NaCl buffer at pH = 6.9. All measurements were performed at room temperature in a 1 × 1 cm quartz cuvette, and in all quenching experiments the Ru(phen)$_2$(dppz)$^{2+}$ concentration was 8 μM.

Results and Discussion

Emission Lifetime in Acetonitrile and DNA Solutions. The luminescence of *Ru(phen)$_2$(dppz)$^{2+}$ decays monoexponentially in homogeneous solvents, such as acetonitrile, ethanol, and propanol, with typical lifetimes in the 170−210 ns range.[6,8,16] However, for aqueous solutions which contain DNA, a biexponential decay is observed, with lifetimes (sh = short lived, lo = long lived) τ_{sh} = 93 ns (63%) and τ_{lo} = 512 ns (37%) in 5 mM tris, 50 mM NaCl (pH = 6.9).[6−9,11,38]

We have conducted experiments with either Δ- or Λ-Ru(phen)$_2$(dppz)$^{2+}$ and with the racemic mixture. The short and long lifetime components of the emission decay of each solution containing calf-thymus DNA are listed in Table 1, along with the pre-exponential factors corresponding to each component. These results are similar to those recently reported by Nordén, where the long component of Δ-*Ru(phen)$_2$(dppz)$^{2+}$ is significantly longer than that of the Λ enantiomer.[11] It is evident from Table 1 that the lifetimes and pre-exponential factors observed for the racemic mixture correspond to those of Δ-*Ru(phen)$_2$(dppz)$^{2+}$. This result can be explained by the difference in emissive quantum yields between the two enantiomers, since that of Δ-*Ru(phen)$_2$(dppz)$^{2+}$ is ten times greater than that for Λ-*Ru(phen)$_2$(dppz)$^{2+}$.[11] Owing to this difference in quantum yields, the emission due to the Λ-enantiomer is expected to be negligible compared to that of Δ-*Ru(phen)$_2$(dppz)$^{2+}$ in racemic mixtures. Indeed this appears to be the case, since the decay can be fit satisfactorily to a biexponential function and both lifetime components and their pre-exponential factors correspond to Δ-Ru(phen)$_2$(dppz)$^{2+}$. This preferential emission by the Δ-isomer in the presence of right-handed double helical DNA is completely consistent with earlier studies of Ru(phen)$_3^{2+}$.[1]

Table 2. Bimolecular Rate Constants for the Quenching of Ru(phen)$_2$(dppz)$^{2+}$ by Several Proton Donors Obtained from the Slopes of Stern-Volmer Plots of the Emission Intensity, $k_q(I)$, and Lifetime, $k_q(t)$, as a Function of Quencher Concentration

Quencher	Molecular Structure	pKa[a]	$k_q(t)$ / M^{-1}s^{-1}	$k_q(I)$ / M^{-1}s^{-1}
acetic acid		4.77	5.4×10^9	5.4×10^9
nicotinic acid		4.85	7.2×10^8	6.0×10^8
picolinic acid		5.52	3.6×10^9	3.5×10^9
2-nitrophenol		7.17	3.8×10^8	5.7×10^8
2-chlorophenol		8.49	4.5×10^8	5.2×10^8
hydroquinone		10.4	1.1×10^9	1.1×10^9
saccharin		11.7	1.1×10^9	8.2×10^8
water[b]		15.7	2.2×10^{6b}	2.2×10^{6b}

[a] Values obtained from: *CRC Handbook of Chemistry and Physics* 71st ed.; Lide, D. R., Ed.; CRC Press: Boston, 1990−1991. [b] From ref 6.

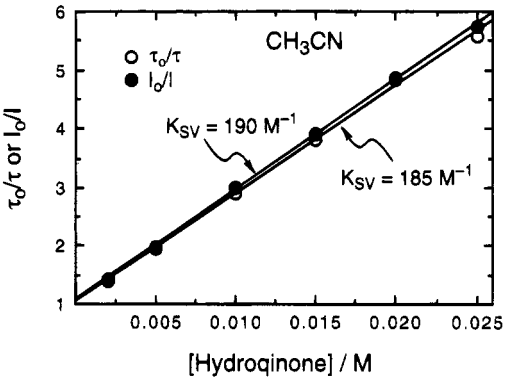


Figure 2. Stern−Volmer plots of the quenching of the emission lifetime and intensity of *Ru(phen)$_2$(dppz)$^{2+}$ by hydroquinone in acetonitrile.

Quenching in Acetonitrile. The triplet MLCT excited state of Ru(phen)$_2$(dppz)$^{2+}$ is effectively quenched in acetonitrile by proton donors with pK_a values ranging from 3.0 to 15.5. Listed in Table 2 are the quenchers as well as their pK_a values and molecular structure, and bimolecular quenching rate constants determined from the slopes of the linear Stern−Volmer plots of the measured lifetimes and emission intensities. A typical Stern−Volmer plot is shown in Figure 2, where it is apparent that the experimental slopes of both the time-resolved and steady-state measurements are nearly superimposable. The difference in the slopes of the steady-state and time-resolved

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(38) The percentages of each component were deduced solely from the pre-exponential factors of a sum of two exponential decays.

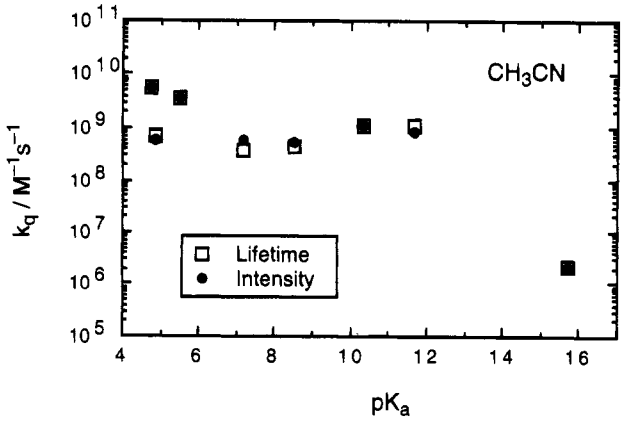

Figure 3. The bimolecular quenching constants, k_q, obtained from lifetime and emission intensity data for the reaction of the MLCT excited state of Ru(phen)$_2$(dppz)$^{2+}$ in acetonitrile plotted as a function of the proton donors' pK_a.

Stern–Volmer plots for each quencher is within 10% (except in the case of 2-nitrophenol), as is evident from the quenching rate constants listed in Table 2. These results are indicative of dynamic bimolecular quenching exclusively, so that no significant ground state hydrogen-bonded assembly (subject to static quenching) is present. A plot of the quenching rate constants (listed in Table 2) as a function of quencher pK_a is shown in Figure 3. It is evident from this plot that all the quenchers in the pK_a range from 3 to 12 are close to the diffusion controlled quenching regime with rate constants between 4×10^8 and 5×10^9 M^{-1} s^{-1}, indicating that the rate constants are not highly dependent on the structure of the quencher as long as a proton source is available (Table 2). This plot resembles that reported for Re(py)$_4$O$_2^+$, where the decrease in the quenching rate was not observed for pK_a < 12.[33]

Other intermolecular quenching mechanisms are possible for MLCT excited state of Ru(II) complexes in addition to proton transfer, which include energy and electron transfer.[13,14] Several control experiments have been conducted with molecules of similar electronic energy levels and redox properties as the proton donors listed above in order to examine the possibility of electron or energy transfer as alternative quenching mechanisms of the MLCT excited state of Ru(phen)$_2$(dppz)$^{2+}$. These molecules included *p*-dimethoxybenzene, chlorobenzene, 2-chloroanisole, methyl nicotinate, and nitrobenzene. No quenching of the lifetime or emission intensity of *Ru(phen)$_2$(dppz)$^{2+}$ was observed for these potential quenchers in acetonitrile solutions at concentrations similar to those shown in Figure 2. Since the structure of each of these molecules has the same likelihood of quenching via energy or electron transfer as their phenolic or acid analogs, it may therefore be concluded that the quenching shown in Figures 2 and 3 does not occur via electron or energy transfer, but rather through a proton transfer mechanism.

Since the deactivation of the MLCT excited state for Ru(phen)$_2$(dppz)$^{2+}$ does not take place through energy or electron transfer and all the effective quenchers possess a readily transferable proton, it may be concluded that the quenching is indeed due to proton transfer to the reduced dppz ligand. This conclusion is supported by the slower quenching by D_2O relative to H_2O, with $k_q(H)/k_q(D) = 2.2$, where the abstraction of the heavier isotope is expected to be slower.[24,26,27] Furthermore, for water or acetic acid as quenchers, it is unlikely that quenching mechanisms other than proton transfer are operative.

Quenching in the Presence of DNA. (a) Quenching of Emission Lifetimes. As discussed above, the emission decay of racemic mixtures of *Ru(phen)$_2$(dppz)$^{2+}$ is biexponential in DNA solutions, with typical lifetimes of $\tau_{sh} = 93$ ns (63%) and

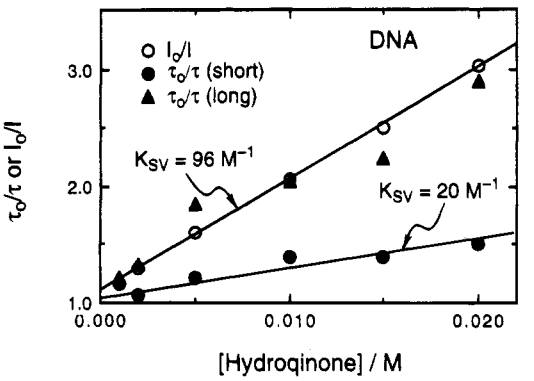

Figure 4. Stern–Volmer plot for the quenching of the short and long lifetime component and the emission intensity of 8 μM *Ru(phen)$_2$(dppz)$^{2+}$ by HQ in the presence of 80 μM calf-thymus DNA (in 5 mM trizma buffer, 50 mM NaCl).

Table 3. Bimolecular Rate Constants for the Quenching of Ru(phen)$_2$(dppz)$^{2+}$ Bound to DNA by Hydroquinone (HQ) and *o*-Chlorophenol (Cl-phenol) Obtained from the Slopes of Stern–Volmer Plots of the Short and Long Lifetime Components, k_q^{sh} and k_q^{lo}, Respectively, as a Function of Quencher Concentration

quencher	enantiomer	k_q^{sh}/M^{-1} s^{-1}	k_q^{lo}/M^{-1} s^{-1}
HQ	racemic	3.3×10^8	3.0×10^8
	Δ	3.0×10^8	1.8×10^8
	Λ	4.9×10^8	1.4×10^8
Cl-phenol	racemic	4.5×10^8	8.2×10^7
	Δ	4.2×10^8	1.5×10^8
	Λ	2.4×10^7	2.4×10^7

$\tau_{lo} = 512$ ns (37%) in 5 mM tris, 50 mM NaCl (pH = 6.9). The long and short components are assigned to two different binding modes because they are quenched independently.[7,8] The relative quenching of the two components, as well as the comparison to quenching in the absence of DNA, should yield information regarding the structures of the binding modes of the emissive species giving rise to each decay component. Therefore, one may utilize proton donors with different proton donating abilities to probe the dynamic quenching rate constant of each lifetime component. The quenching rate constant may then be related to the extent of the exposure of the dppz nitrogens to the solvent in each binding mode. To this end, quenching with two proton donors, hydroquinone (HQ) and *o*-chlorophenol (Cl-phenol), has been conducted. HQ and Cl-phenol differ in their affinity toward both water and the hydrophobic binding regions of DNA, and therefore their quenching behavior is expected to be different toward the two binding modes of Ru(phen)$_2$(dppz)$^{2+}$ particularly if one is more exposed to water than the other.

The bimolecular quenching rate constants for the MLCT excited state of 8 μM Ru(phen)$_2$(dppz)$^{2+}$ in the presence of 80 μM DNA by HQ are listed in Table 3 for each enantiomer and the racemic mixture. The quencher is water soluble and not expected to bind to DNA, and therefore the observed quenching is expected and found to be dynamic in nature. Both the short and long lifetime components are quenched dynamically, and the corresponding Stern–Volmer plots for the racemic mixture are shown in Figure 4. The quenching rate constants obtained from the slopes of the plots in Figure 4 are $k_q^{sh} = 3.3 \times 10^8$ M^{-1} s^{-1} and $k_q^{lo} = 3.0 \times 10^8$ M^{-1} s^{-1}, for the short and long lifetime components, respectively. Thus, at a fixed [HQ] both components are quenched with rate constants which are 3–4 times slower than those measured in acetonitrile ($k_q^{ac} = 1.1 \times 10^9$ M^{-1} s^{-1}).

When the quencher Cl-phenol, which has a more hydrophobic

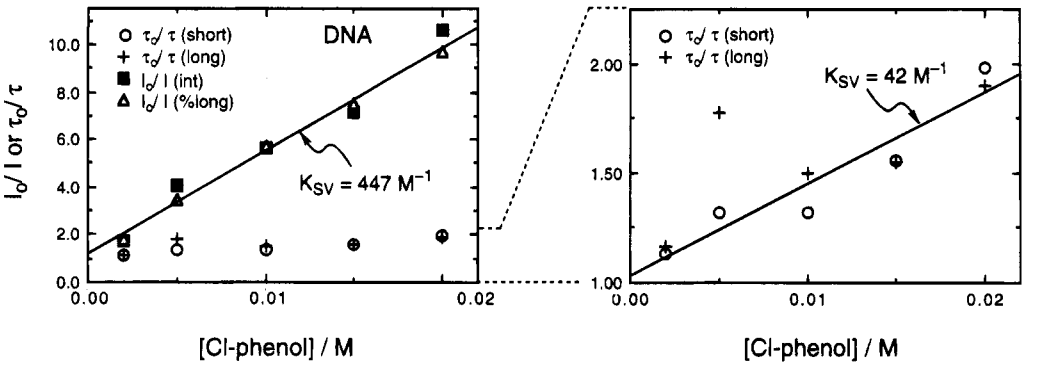

Figure 5. Stern–Volmer plots for the quenching of the short and long lifetime component and the emission intensity of 8 μM *Ru(phen)$_2$(dppz)$^{2+}$ by *o*-chlorophenol in the presence of 80 μM calf-thymus DNA (in 5 mM trizma buffer, 50 mM NaCl). The left panel shows the difference in the slopes between the intensity (see text) and lifetime quenching, whereas the right panel is an expanison of the left and shows the quenching of both lifetime components.

character than HQ,[39] was utilized, the results differ significantly from those observed with the hydrophilic HQ. Both lifetime components are quenched, however the emission intensity decreases much faster with Cl-phenol concentration than either lifetime component indicating significant static quenching. The Stern–Volmer plots for both lifetime components and the emission intensity of a racemic *Ru(phen)$_2$(dppz)$^{2+}$ in the presence of DNA are shown in Figure 5. The quenching rate constants for each lifetime component for Δ- and Λ-*Ru(phen)$_2$-(dppz)$^{2+}$ and their racemic mixture are listed in Table 3. The quenching of the short lifetime component of racemic solutions by Cl-phenol bound to DNA takes place with the same rate constant (k_q^{sh} = 4.5 × 10^8 M^{-1} s^{-1}) as in acetonitrile (k_q^{ac} = 4.5 × 10^8 M^{-1} s^{-1}). The long component is quenched with a bimolecular rate constant five times slower, k_q^{lo} = 8.2 × 10^7 M^{-1} s^{-1}.

Thus, with Cl-phenol as quencher, the short lifetime component is quenched with a bimolecular rate constant comparable to that in acetonitrile. These findings clearly indicate that the *Ru(phen)$_2$(dppz)$^{2+}$ species giving rise to the short lifetime component, at least for the most part, is kinetically as susceptible to quenching as molecules in the bulk solution. Nevertheless, the short component of *Ru(phen)$_2$(dppz)$^{2+}$ has some dynamic contribution from a species bound to DNA, since the complex is non-luminescent in the bulk aqueous phase and since the lifetime of the short lifetime component varies as a function of DNA concentration in the absence of quencher. This DNA concentration dependence of the observed emission lifetime of *Ru(phen)$_2$(dppz)$^{2+}$ has been recently reported in detail.[7,11]

The quenching of the MLCT excited state lifetimes of Ru-(phen)$_2$(dppz)$^{2+}$ by HQ is three times slower in the presence of DNA compared to acetonitrile. Since HQ is expected to reside predominantly in the aqueous phase, this result indicates that the two species giving rise to the short and long lifetime components are bound to DNA and are protected from the aqueous medium. Similar results have been observed for this complex with Fe(CN)$_6$,[6-9] which is also expected to reside in the aqueous phase and not interact with the negatively-charged double helix. Since neither lifetime component was quenched by Fe(CN)$_6^{4-}$, we assigned each component to an intercalative binding mode.[8]

The quenching rate constants of both the short and long lifetime components of *Ru(phen)$_2$(dppz)$^{2+}$ bound to DNA are nearly equal for HQ as the proton donor, although they are both four times lower than in acetonitrile. The quenching of the short lifetime component of the DNA-bound complex by Cl-phenol is comparable to that in acetonitrile, whereas the long component of the decay is attenuated by a factor of 5 from that of the short component. The difference between the two quenchers is consistent with HQ being predominantly in the aqueous phase, while Cl-phenol is able to approach and to interact with the hydrophobic regions of DNA. The overall pattern of Cl-phenol quenching rates is consistent with the assignment of the long lifetime component to an intercalated complex, for which the dppz nitrogens are somewhat protected from quenching, and with a second form of *Ru(phen)$_2$(dppz)$^{2+}$ bound to DNA which gives rise to the short component.

In the present study there is strong evidence that indeed there are two different geometries with markedly different exposure of the dppz nitrogens to the solvent and with different hydrophobicity in their immediate surrounding. Although Nordén agrees that there are two emitting species, he clearly states that they do not differ in binding geometry.[11] One of the differences proposed by Nordén between the two is the DNA bases surrounding the Ru(phen)$_2$(dppz)$^{2+}$ molecules.[11] It is known, however, that the two emitting species are observed for each enantiomer in poly(dG)·poly(dC) and poly(dA)·poly-(dT), where the base sequence is constant.[8,40] In the present work, the marked difference in reactivity between the two emitting species indicates that there is a large difference in the exposure of the dppz nitrogens. This is not expected to be the case if the only difference between the two emitting species is the base sequence that surrounds the molecule. The other difference proposed by Nordén between the emitting species is due to clustering or stacking of Ru complexes, where the long lifetime is due to an intercalated Ru(phen)$_2$(dppz)$^{2+}$ surrounded by other Ru complexes and the short lifetime to an isolated intercalated Ru(phen)$_2$(dppz)$^{2+}$. The results presented in this paper are not consistent with this picture, since there is static quenching only of the long component by the hydrophobic quencher. If the short component was indeed due to an isolated molecule, it would also be subjected to static quenching. Recent NMR studies of Δ-Ru(phen)$_2$dppz^{2+} bound to an oligonucleotide are consistent with the presence of one symmetric and a family of asymmetric intercalative binding geometries for the metal complex bound to the duplex.[40b]

The values of the quenching rate constants of the MLCT excited state of Δ-Ru(phen)$_2$(dppz)$^{2+}$ by HQ and Cl-phenol are

(39) The solubility of Cl-phenol in water is 11 g/dm^3 (Banerjee, S.; Yalkowsky, S. H.; Valvani, S. C. *Environ. Sci. Technol.* **1980**, *14*, 1227), whereas that of HQ is ~50 g/dm^3. The latter was measured by a previously described method (Keith, L. H.; Walters, D. B. *National Toxicology Program's Chemical Solubility Compendium*; Lewis Publishers: Chelsea, MI; 1992, pp 1–2).

(40) (a) Turro, C.; Arkin, M. R.; Stemp, E.; Turro, N. J.; Barton, J. K. Unpublished results. (b) Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.* Submitted for publication.

similar to those of the racemic solution (Table 3). This finding is in agreement with the predominance of the emission from the Δ enantiomer in racemic mixtures, owing to its greater emissive quantum yield.

(b) Quenching of the Emission Intensity. As is evident from Figure 4, the slope of the Stern−Volmer plot for the quenching of the emission intensity of *Ru(phen)$_2$(dppz)$^{2+}$ for HQ as a proton donor parallels that for the quenching of the long lifetime component. This is the expected behavior for dominant dynamic quenching where the integrated intensity of the long component contributes to approximately 76% of the emission intensity (from integrated pre-exponential factors). This suggests that the species which give rise to both the short and long lifetime components are protected from the bulk solvent, where HQ is expected to reside. As discussed above, the plot of I_0/I has a much steeper slope than those of either lifetime component when Cl-phenol is utilized as the quencher (Figure 5). The slope of the Stern−Volmer plot derived from the emission intensity data (447 M^{-1}) is approximately ten times greater than those from the lifetime data (42 M^{-1}). Such a variation in Stern−Volmer constants is indicative of static quenching. This can be interpreted to occur if a hydrophobic quencher binds to DNA near intercalated Ru(phen)$_2$(dppz)$^{2+}$ molecules, thereby rapidly quenching the emission of those probes on a time scale that is short compared to the excited state lifetime and leading to a decrease in intensity without a measurable change in lifetime.

Owing to the binding of Ru(phen)$_2$(dppz)$^{2+}$ to the hydrophobic region of DNA, the decrease in emission intensity may also be due to displacement of the complex into the aqueous phase by Cl-phenol rather than static quenching. However, addition of 20 mM toluene to a solution with the same concentrations of Ru(II) complex and DNA leads to an I_0/I value of 1.41. A similar result was observed upon addition of *p*-dimethoxybenzene to Ru(phen)$_2$(dppz)$^{2+}$ bound to DNA, which contains a more polar structure. The same concentration of Cl-phenol produces an I_0/I value of 10.3 (Figure 5). It may therefore be concluded that the loss in intensity of bound *Ru(phen)$_2$(dppz)$^{2+}$ is largely due to static quenching effected when quencher molecules bind next to or near the probe molecules in the major groove, and only to a small extent to the displacement of the probe from the double helix by the hydrophobic quencher molecules.

The static quenching of Cl-phenol is accompanied by a large decrease in the contribution of the long lifetime component relative to that of the short component in the emission decay. This observation is consistent with static quenching of the intercalated *Ru(phen)$_2$(dppz)$^{2+}$ molecules, which give rise to the long component. Under the simplifying assumption that the short component is not quenched in a static manner, then the relative amplitudes of the short and long component may be utilized to calculate the relative intensity quenching. This method assumes that the percent contribution of the long component is related to that of the short component, which remains constant throughout the quenching experiment. The I_0/I value is then given by

$$\frac{I_0}{I} = \frac{(\%\ \tau_{sh})}{(\%\ \tau_{sh}^0)} \frac{(\%\ \tau_{lo}^0)}{(\%\ \tau_{lo})} \qquad (1)$$

where and (% τ_{sh}) and (% τ_{lo}) are the percent contributions to the short and long lifetime components at a given quencher concentration, respectively, and the absence of quencher is denoted by the superscript "0". The I_0/I values calculated in this manner are plotted in Figure 5 (labeled I_0/I(% long) in the graph), along with those obtained from the integration of the emission intensities (labeled I_0/I(int) in the graph). The slope of the I_0/I vs Cl-phenol is 436 M^{-1}, which is very close the 447 M^{-1} obtained from the emission intensity data.

It may be concluded from the similarity between the I_0/I points and the slopes obtained by each method that the calculation from the percent contribution to the biexponential decay is a valid manner to determine I_0/I in cases where the quencher also binds to the host. However, eq 1 is no longer valid if the quencher binding constant is similar to that of the probe, such that the quencher displaces probe molecules as it binds to DNA, since in those cases the relative intensity of the short component would no longer be expected to be constant as quencher is added. This is important in systems where the quencher binds to DNA (or other host) and absorbs light at the excitation wavelength, which is often the case when the quencher is a metal complex.

Since the emission of *Ru(phen)$_2$(dppz)$^{2+}$ in water is only observed when the complex is bound to DNA, the emission intensity, I_0, should be proportional to the concentration of the bound complex, [Ru−DNA]$_i$. The static quenching in the presence of Cl-phenol is much greater than the dynamic quenching, and therefore it may be assumed that there is no emission from a bound *Ru(phen)$_2$(dppz)$^{2+}$ complex that has a quencher bound in its vicinity (Ru−DNA−Q). A similar result was observed by Jaycox with positively charged viologen quenchers, which are also expected to bind to DNA and quench the Ru complex via electron transfer in a static manner.[41] The quenchers utilized in the latter study were viologens of the type R−(py)$_2$−R^{2+} (R = −CH$_3$ (**1**), −(CH$_2$)$_2$CH$_3$) (**2**)), R−(py)$_2$−R′$^{2+}$ (R = −CH$_3$, R′= −(CH$_2$)$_{11}$CH$_3$ (**3**)), and CH$_3$−(py)$_2$−(CH$_2$)$_6$−(py)$_2$−CH$_3^{4+}$ (**4**), exhibiting different charges and hydrophobicities, as well as Co(phen)$_3^{3+}$ (**5**), where (py)$_2$ = 4,4′-bipyridinium. In all cases the emission intensity in the presence of quenchers which bind to DNA, *I*, is then proportional to ([Ru−DNA]$_i$ − [Ru−DNA−Q]), where [Ru−DNA−Q] = K_Q[Ru−DNA]$_i$[Q]$_0$/(1 + K_Q[Q]$_0$). Utilizing these expressions we obtain eq 2, where K_Q is the binding constant of

$$I_0/I = 1 + K_Q[Q]_0 \qquad (2)$$

the quencher to DNA. This equation is similar to that previously derived for static and dynamic quenching in micelles, which reduces to eq 2 when the static component is much larger than the dynamic quenching.[42] It should be noted that eq 2 is only valid for cases where the probe is not displaced by the quencher ($K_p \gg K_Q$, where K_p is the binding constant of the probe), and the quencher is in excess. These conditions are typically satisfied by Ru(phen)$_2$(dppz)$^{2+}$ since its binding constant to calf thymus DNA is large, $K_p \sim 6 \times 10^7$ M^{-1}.

The I_0/I plots of Ru(phen)$_2$(dppz)$^{2+}$ bound to DNA with Cl-phenol as a quencher (Figure 5) have an average slope of 440 M^{-1}, which according to eq 2 corresponds to the binding constant of the quencher. Similarly, the positively charged quenchers **1**−**5** yield slopes of I_0/I vs [Q]$_0$ consistent with eq 2, where K_Q decreases with the charge of the quencher.[42] For **4** the K_Q obtained with eq 3 was 9200 M^{-1}, whereas for **5** it was 7200 M^{-1}. The latter binding constant is similar to that measured by NMR for Rh(phen)$_3^{3+}$ with calf thymus DNA of 5000 ± 2000 M^{-1}.[1d] Quenchers **1** and **2** had a slope of 400 M^{-1}, and the more hydrophobic **3** yields a slightly stronger binding constant of 700 M^{-1}.

(41) Jaycox, G. D.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Polymer* **1991**, *32*, 634.

(42) Timpe, H.-J.; Israel, G.; Becker, H. G. O.; Gould, I. R.; Turro, N. J. *Chem. Phys. Lett.* **1983**, *99*, 275.

Conclusions

The two emission lifetimes and their relative contributions of racemic and Δ- and Λ-*Ru(phen)$_2$(dppz)$^{2+}$ bound to DNA indicate that in the racemic mixture the emission is due to the Δ enantiomer owing to its greater emission quantum yield.

The quenching of the MLCT excited state of Ru(phen)$_2$(dppz)$^{2+}$ by proton donors in the presence of DNA has provided insight regarding the binding modes of the complex. It is clear from the emission lifetime quenching experiments that *Ru(phen)$_2$(dppz)$^{2+}$ is bound to DNA at two distinct orientations with different exposures of the dppz nitrogens to the aqueous environment and to the quenchers. There is a strongly-bound species, which gives rise to the long lifetime component, whose quenching by the hydrophobic Cl-phenol is slower than that in the absence of DNA. The nitrogens of the dppz ligand in molecules in this particular binding mode are protected from the environment, as expected for a fully intercalated ligand, which is located in a hydrophobic area of the double helix. In the second intercalation geometry, identified with the short lifetime component, the dppz nitrogen(s) are more accessible to hydrophobic quencher molecules, such as Cl-phenol. However, in both binding modes the dppz nitrogens are protected from quencher molecules which remain predominantly in the aqueous phase and do not interact with DNA, such as the hydrophilic HQ.

The quenching of the emission intensity reveals that the static quenching is associated only with the fully intercalated ligand, thus indicating that the hydrophobic quencher must bind in the same microenvironment as Ru(phen)$_2$(dppz)$^{2+}$. The decrease in emission intensity upon addition of quencher may be utilized to obtain the binding constant of quenchers to DNA for cases where the binding constant of the probe is much larger than that of the quencher.

Our findings are inconsistent with the presence of only one binding geometry, and can only be explained if in addition to the intercalated Ru(phen)$_2$(dppz)$^{2+}$ there is a second type of emissive complex bound to the double helix for which the dppz ligand is more exposed to the solvent. The quenching of the emission intensity of Ru(phen)$_2$(dppz)$^{2+}$ in the presence of DNA by Cl-phenol and HQ shows that there are at least two geometry of binding of the complex on calf thymus DNA, which differ significantly on their exposure to the solvent. Furthermore, the intercalated complex, which gives rise to the long lifetime component, is located in a hydrophobic region of the double helix, since it is statically quenched by the more hydrophobic Cl-phenol. The original proposal of a second intercalated structure with a more exposed dppz moiety is consistent with the results.

Acknowledgment. C.T. thanks the Jane Coffin Childs Memorial Fund for Medical Research for a fellowship. This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research. Further support has been provided by the Deutsche Forschungsgemeinschaft (S.H.B.) and the Parsons Foundation (Y.J.). N.J.T. thanks the National Science Foundation and J.K.B. the National Institutes of Health for their generous support of this research.

JA951972N