$Ru(phen)_2dppz^{2+}$ Luminescence: Dependence on DNA Sequences and Groove-Binding Agents

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Emission of Δ-$Ru(phen)_2dppz^{2+}$ bound to nucleic acid polymers of different sequence has been investigated by time-resolved luminescence spectroscopy and the effect of major and minor groove DNA binding agents on the luminescence profile of the complex evaluated. In the presence of a 1:1 mixture of poly d(AT) and poly d(GC), the excited-state decay of Δ-$Ru(phen)_2dppz^{2+}$ can be described by a linear combination of the decay profiles in the presence of poly d(AT) and poly d(GC) independently. This analysis indicates that ∼85% of the complexes are bound to poly d(AT) and that the metallointercalator preferentially occupies AT sites in mixed-sequence polymers such as calf thymus or T4 DNA. When *rac*-$Ru(phen)_2dppz^{2+}$ bound to [d(5′-GAGTGCACTC-3′)$_2$] is titrated with the major groove intercalator Δ-α-[Rh[(*R,R*)-Me_2trien]phi]$^{3+}$, the ruthenium emission yield decreases while the absorbance of the $\pi-\pi^*$ transition centered on the dppz ligand increases, until saturation behavior is observed at a 1:1 Rh/duplex ratio. These titrations indicate that $Ru(phen)_2dppz^{2+}$ is displaced from the major groove by the rhodium complex. In contrast, for *rac*-$Ru(phen)_2dppz^{2+}$ bound to poly d(AT), addition of the minor groove binding agent distamycin produces an increase in ruthenium emission which saturates at ∼1 distamycin/5 bp, consistent with the double helix being able to accommodate major and minor groove binders simultaneously. Distamycin has no effect on the emission of $Ru(phen)_2dppz^{2+}$ emission bound to poly d(GC). These photophysical studies establish a sequence preference in binding to DNA by $Ru(phen)_2dppz^{2+}$ as well as providing support for the original assignment by NMR of ruthenium intercalation from the major groove side of the DNA helix.

Introduction

Octahedral transition metal complexes which intercalate into nucleic acids have become useful probes of DNA and RNA.[1] Phenanthrenequinone diimine (phi) complexes of rhodium(III) bind to DNA by intercalation of the phi ligand from the major groove and have been applied in site-specific DNA recognition and oxidation chemistry.[1,2] Metal complexes containing dppz (dppz = dipyridophenazine) as a ligand also bind to DNA with high affinity,[3−11] and many have served as luminescent probes.[3−8] This luminescence signature is remarkably sensitive to local environment and depends upon nucleic-acid sequence and structure.[3−5] The wider applicability of dppz complexes as nucleic acid probes requires a firm understanding of the structural basis for their photophysical characteristics.

The lack of apparent DNA sequence-specificity for $Ru(phen)_2dppz^{2+}$ makes a detailed structural determination of its interactions with duplex DNA using NMR spectroscopy more difficult. Early ^{1}H NMR spectroscopic experiments[11] of $Ru(phen)_2dppz^{2+}$ with DNA showed only broad resonances, due not only to the lack of site-specificity but also to the intermediate exchange kinetics at ambient temperature and to the abundance of overlapping resonances in the aromatic region. Selective

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deuteration of the coordinating ligands of $Ru(phen)_2dppz^{2+}$, variations in temperature to alter the exchange kinetics, and restricting the examination to a DNA hexamer were then applied in carrying out two-dimensional (2D) ^{1}H-NMR experiments on Δ-$Ru(phen)_2dppz^{2+}$ bound to $d(GTCGAC)_2$.[12,13] This approach yielded NOESY data which were consistent with intercalation of Δ-$Ru(phen)_2dppz^{2+}$ into the 6 base pair (bp) oligonucleotide from the major groove side of the B-form DNA duplex with a family of stacking orientations.

Recently, photophysical studies of Λ- and Δ-$Ru(phen)_2dppz^{2+}$ bound to calf thymus (CT) DNA, T4 DNA, and several synthetic DNA polymers were carried out. On the basis of the differences in emission characteristics among these DNAs, it was concluded that $Ru(phen)_2dppz^{2+}$ isomers bound by intercalation from the minor groove of the DNA helix.[14]

Here, we reinvestigate the binding of Δ-$Ru(phen)_2dppz^{2+}$ to poly d(AT), poly d(GC), and calf thymus (CT) DNA by time-resolved luminescence spectroscopy and describe experiments in which well-characterized major and minor groove DNA binding agents compete with $Ru(phen)_2dppz^{2+}$ for DNA binding sites. These experiments illuminate the sequence dependence of the $Ru(phen)_2dppz^{2+}$ emission and support the original assignment[12] of ruthenium intercalation from the major groove side of duplex DNA.

Experimental Section

Materials. $[Ru(phen)_2dppz]Cl_2$ and Δ-α-[Rh[(*R*,*R*)-Me_2trien]phi]-Cl_3 ([Rh(MT)phi]Cl_3; Me_2trien = 2,9-diamino-4,7-diazadecane) were prepared as described previously.[13,15] Resolution of *rac*-$Ru(phen)_2$-$dppz^{2+}$ was achieved by ion-exchange chromatography using antimonyl tartrate (Sigma) as the chiral eluent.[13] Distamycin was obtained from Sigma, dissolved in minimal ethanol, diluted into buffer (5 mM Tris, 50 mM NaCl, pH 7.8), and then quantitated by ultraviolet−visible (UV−vis) absorbance ($\epsilon_{302\ nm} = 3.7 \times 10^4\ M^{-1}\ cm^{-1}$; Sigma). To avoid degradation of distamycin, fresh stock solutions were prepared by dissolving solid prior to each experiment. Oligonucleotides were prepared on an ABI 394 automated DNA synthesizer, purified by reversed phase HPLC (triethylammonium acetate (100 mM)/acetonitrile mobile phase; C_{18} stationary phase), and converted to the sodium salt as described.[13] CT DNA, poly d(AT), and poly d(GC) were obtained from Pharmacia and exchanged into buffer (5 mM Tris, 50 mM NaCl, pH 8.5).

Instrumentation. Steady-state emission data were obtained on an SLM 8000 spectrofluorimeter, and absorption data were obtained on a Cary 2200 spectrophotometer. Time-resolved luminescence data were obtained as described previously[7] with excitation at 480 nm and observation at 617 nm.

Methods. Stock solutions of metal complex and DNA were quantitated by UV−vis: $\epsilon_{440\ nm}(Ru(phen)_2dppz^{2+}) = 2.1 \times 10^4\ M^{-1}\ cm^{-1}$; $\epsilon_{373\ nm}(Rh(MT)phi^{3+}) = 1.35 \times 10^4\ M^{-1}\ cm^{-1}$ (pH 7.0); $\epsilon_{260\ nm}$-(poly d(AT)) = $6.6 \times 10^3\ M^{-1}$ (nucleotide; nuc) cm^{-1}; $\epsilon_{260\ nm}$(CT) = $6.6 \times 10^3\ M^{-1}$ (nuc) cm^{-1}; $\epsilon_{254\ nm}$(poly d(GC)) = $8.4 \times 10^3\ M^{-1}$ (nuc) cm^{-1}. Oligonucleotide duplex solutions were prepared by annealing the self-complementary strand by heating a solution of strand to 90 °C and cooling to room temperature over ~2 h. Where appropriate, luminescence samples were prepared by diluting concentrated (~15 mM nuc) DNA solutions into dilute (~10 μM) metal complex solutions. Distamycin titrations were typically carried out by adding concentrated (~0.5 mM) distamycin to solutions of ruthenium bound to DNA, although separate samples prepared in reverse order gave identical results.

To obtain luminescence lifetimes (τ's), time-resolved emission data were fit to

$$y(t) = 100[C1\ \exp^{(-t/\tau 1)} + (1 - C1)\ \exp^{(-t/\tau 2)}]$$

by a nonlinear least-squares method with convolution of the instrument response function using in-house (Beckman Institute Laser Resource Center) software as described previously.[7] For competition experiments involving DNA sequence, luminescence data were fit according to the multiexponential function

$$y(t) = (100/(C1 + C2 + C3 + C4))[C1\ \exp^{(-t/\tau 1)} + C2\ \exp^{(-t/\tau 2)}] + C3\ \exp^{(-t/\tau 3)} + C4\ \exp^{(-t/\tau 4)}])$$

consisting of a linear combination of the decay profiles with poly d(AT) and poly d(GC), separately. The preexponential factors (C1−4) were floating parameters and allowed to converge, and the lifetimes ($\tau 1$ = 123 ns; $\tau 2$ = 709 ns; $\tau 3$ = 44 ns; $\tau 4$ = 288 ns) were held constant. In the results obtained, the percent contribution of the 123 and 709 ns lifetimes reflects the fraction of complex bound to poly d(AT) and the percent contribution of the 44 and 288 ns lifetimes reflects the fraction bound to poly d(GC) (Table 1). Error in lifetimes and percent contributions are estimated to be ±10%.

For titrations followed by UV−vis, a double beam spectrophotometer was employed. The sample solution contained the 10 bp duplex (10 μM), and $Ru(phen)_2dppz^{2+}$ (10 μM) and the reference solution contained only the 10 bp duplex. Over the course of the titration, small aliquots of Rh(MT)phi^{3+} (1.5 mM) were added to both reference and sample solutions. Under these conditions, the absorbances of the DNA and the rhodium are subtracted.

Results and Discussion

Luminescence of $Ru(phen)_2dppz^{2+}$ Bound to AT and GC Sites. The luminescence lifetimes for photoexcited Δ-Ru-$(phen)_2dppz^{2+}$ bound to a series of nucleic acid polymers are shown in Table 1. For each of the DNA sequences investigated, the emission follows a biexponential decay.[3,7] As has been noted,[5,7,14] variations, primarily in the distribution of lifetimes, are observed as a function of loading. Despite the high sensitivity of ruthenium emission to its local environment, in the presence of CT DNA and poly d(AT), the luminescence decays are found to be remarkably similar in both the magnitude and relative distribution of lifetimes. Bound to poly d(GC), however, the excited-state lifetimes are shorter and the distribution of lifetimes is strongly shifted in favor of the longer lived species, to give a significantly different profile. These data are fully consistent with those reported earlier,[3c,14] and over the range of 0.8−4 mJ/pulse, the data are independent of excitation power. Such a lack of a power dependence to the excited-state decay argues against the distribution of metal complex across the polymer giving rise to the biexponential decay as proposed[14] since at high laser powers one would expect to observe self-quenching even at low loadings.

In order to test the sequence preference of Δ-$Ru(phen)_2dppz^{2+}$ directly in a photophysical experiment, we measured the luminescence decay of the complex in the presence of a 1:1 mixture of poly d(AT) and poly d(GC). These measurements were carried out at low loadings of Ru/DNA to maximize the effect of sequence and to minimize variations in emission that are associated with high loadings. Here, in the 1:1 mixture of poly d(AT) and poly d(GC), the luminescence decay can be described by a linear combination of the decay profiles in the presence of poly d(AT) and poly d(GC) independently. This analysis indicates that, despite a high affinity for both DNA

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polymers, ~85% of the complexes are bound preferentially to poly d(AT).

Table 1. Luminescence Decay Parameters for $Ru(phen)_2dppz^{2+}$ Bound to DNA Polymers[a]

nucleic acid	luminescence lifetimes[b] τ1 ns (%)	τ2 ns (%)
calf thymus[c]	150 (80)	800 (20)
poly d(AT)[c]	123 (82)	709 (18)
poly d(GC)[c]	44 (22)	288 (78)
poly d(GC) + poly d(AT)[d]	126 (79)	599 (21)
poly d(GC) + poly d(AT)[d,e]	44 (3)	288 (12)
	123[i] (70)	709[j] (15)
[d(5′-GAGTGCACTC-3′)$_2$][f]	128 (66)	545 (34)
[d(5′-GAGTGCACTC-3′)$_2$] + Rh(MT)phi^{3+} [g]	85 (75)	446 (25)
poly d(AT) + distamycin[h]	120 (65)	671 (35)

[a] Data obtained as described previously[7] with excitation at 480 nm and observation at 617 nm. [b] Data were fit to $y(t) = 100[C1\ \exp^{(-t/\tau1)} + (1 - C1)\ \exp^{(-t/\tau2)}]$ by a nonlinear least-squares method with convolution of the instrument response function using in-house software (BILRC) as described previously (Arkin, M. R.; Stemp, E. D. A.; Turro, C.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 2267−2274). Error in lifetimes and percent contributions are estimated to be ±10%. [c] Δ-$Ru(phen)_2dppz^{2+}$ (10 μM) bound to DNA (1 mM nucleotides (nuc); Ru:bp = 1:50) in buffer (5 mM Tris, 50 mM NaCl, pH 8.5). Lifetimes are consistent with those reported previously; see ref 5a. [d] Δ-$Ru(phen)_2dppz^{2+}$ (10 μM) in the presence of 1:1 poly d(AT) and poly d(GC) (1 mM nuc; Ru:bp = 1:50) in buffer. Identical results are obtained at 2 mM nuc (Ru:bp = 1/100). [e] Data fit to multiexponential function consisting of a linear combination of decay profiles for Δ-$Ru(phen)_2dppz^{2+}$ bound to poly d(AT) and poly d(GC), separately. The preexponential factors were allowed to float in the minimization routine, while the lifetimes were fixed (see Experimental Section). [f] *rac*-$Ru(phen)_2dppz^{2+}$ (10 μM) in the presence decamer (10 μM duplex) in buffer (10 mM phosphate, 20 mM NaCl, pH 7.0). [g] $Rh(MT)phi^{3+}$ = Δ-α-[Rh[(*R*,*R*)-Me_2trien]phi]$^{3+}$ (10 μM). [h] *rac*-$Ru(phen)_2dppz^{2+}$ (10 μM) bound to poly d(AT) (1 mM nucleotides; Ru:bp = 1:50) in buffer (5 mM Tris, 50 mM NaCl, pH 7.8) in the presence of distamycin (100 μM). [i] τ3. [j] τ4.

The recent assignment of $Ru(phen)_2dppz^{2+}$ intercalation from the minor groove side of duplex DNA was based primarily on comparative luminescence studies[14] for the complex bound to CT (42% GC)[16] and T4 DNA (34% GC), a mixed-sequence DNA like CT but one in which 5-hydroxymethylcytosine residues are 100% glucosylated in the major groove.[17] Luminescence titrations and excited-state decay kinetics for $Ru(phen)_2dppz^{2+}$ were essentially the same for both DNAs. These results were interpreted as evidence for intercalation of the complex from the minor groove side of the duplex, since the steric obstruction provided by the sugar units should have prevented binding and caused a significant reduction in the emission yield of the complex bound to T4 DNA compared to CT. Given the finding here of preferential binding of $Ru(phen)_2dppz^{2+}$ to AT sites, the small differences observed between CT and T4 DNA are not surprising.

We can apply these data in estimating site occupation within both CT and T4 DNA. For $Ru(phen)_2dppz^{2+}$ bound to CT, the luminescence lifetimes are slightly longer than with poly d(AT). The decay profile may reflect the heterogeneity of AT-containing sites within the mixed-sequence CT DNA. The ratio of equilibrium constants for $Ru(phen)_2dppz^{2+}$ binding to poly d(AT) and poly d(GC) ($K_{AT/GC}$) in the presence of both polymers can be expressed as $K_{AT/GC}$ = ([Ru·AT])([GC])/([Ru·GC])([AT]). Assuming a 3 bp binding site for ruthenium, data from the 1:1 poly d(AT)/poly d(GC) competition experiment yields $K_{AT/GC} = 5.9$. Using this constant, the ratio of ruthenium binding to AT vs GC sites ([Ru·AT]/[Ru·GC]) in CT (42% GC) and T4 (34% GC) DNA are calculated to be 8.2 and 11.5, respectively, or 89% AT occupation in CT and 92% AT occupation in T4. Hence, at low loadings of metal to DNA, it is fully understandable that the emission profiles for these sequences are so similar.

A similarity in emission for $Ru(phen)_2dppz^{2+}$ bound to CT and T4 DNA would therefore be expected whether the complex were in the major groove or the minor groove. On both DNAs, the luminescence appears to emanate substantially from intercalation within AT sites. Differences in luminescence yield for Δ-$Ru(phen)_2dppz^{2+}$ bound to the two DNAs are observed only at high metal to DNA ratios, where the emission is actually somewhat higher with T4.[14] If the complex were associating from the minor groove, these emission yields should not differ at any loading. At high loadings of ruthenium on T4, the higher emission intensity might result from an increase in protection of the complex from solvent quenching by glucosylated sites in the major groove or follow from an increase in the rigidity[1,7] of the helix.

The emission profile for ruthenium bound to poly d(IC) (I = inosine) has also been cited in support of intercalation of the complex from the minor groove since the IC base pair lacks the amino group in the minor groove.[14] Bound to poly d(IC), the biexponential decay reported[14] resembles more closely that for ruthenium bound to poly d(AT) than to poly d(GC); as with poly d(AT), emission arises primarily from the shorter excited-state decay component. Many factors determine the relative distribution of binding orientations which give rise to the biexponential decay; certainly the dipole characteristics of the different base pairs must affect the relative stability of different stacking orientations, irrespective of whether stacking originates from the major or minor groove side. On the basis of steric considerations, one would expect that eliminating the amino group from the minor groove should actually facilitate complete intercalation and an increase in excited state lifetime, yet with poly d(IC), a side-on mode with a shorter excited-state lifetime is favored. Hence, little support for the minor groove assignment can be gleaned from the observation made with poly d(IC).

Competitive Binding in the Major Groove. We decided to explore the groove assignment for intercalation of $Ru(phen)_2dppz^{2+}$ in more detail through competition experiments with DNA-binding molecules where the groove orientation has been well-established. In the case of the interactions of small molecules with DNA, there are in fact only a few examples of species which bind DNA from the major groove side.[1,18,19]

Phi complexes of rhodium have been shown using a variety of techniques to intercalate in double helical DNA from the major groove side. In particular, 2D NMR studies have established that $Rh(MT)phi^{3+}$ (Figure 1) binds site-specifically ($K_B \sim 10^8\ M^{-1}$) in the center of a 10 bp duplex [d(5′-GAG***TGCA***CTC-3′)$_2$] from the major groove side, with only 1 equiv of the rhodium complex bound to the duplex even in the

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(19) Bright luminescence is found for dppz complexes of ruthenium bound to poly dT·poly dA·poly dT.[3c]

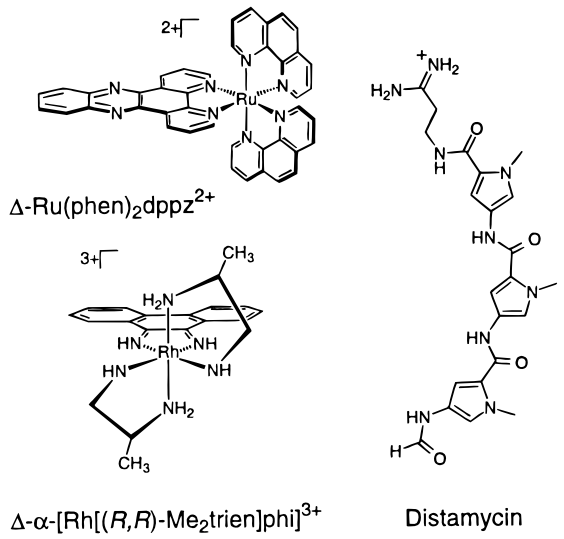

Figure 1. Schematic illustrations of DNA binding agents Δ-Ru(phen)$_2$dppz^{2+}, Δ-α-[Rh[(*R*,*R*)-Me$_2$trien]phi]$^{3+}$ (Rh(MT)phi^{3+}), and distamycin.

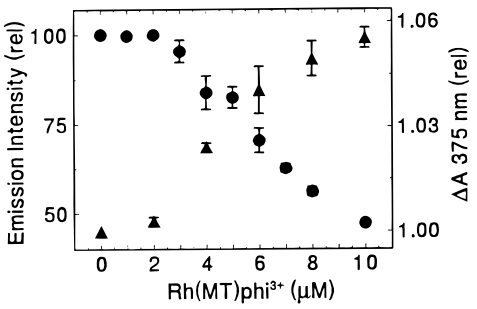

Figure 2. Steady-state emission intensity (●) and relative absorbance change at 375 nm (▲) for Ru(phen)$_2$dppz^{2+} (10 μM) bound to [d(5′-GAGTGCACTC-3′)$_2$] (10 μM duplex; 10 mM phosphate, 20 mM NaCl, pH 7) in the presence of increasing concentrations of Δ-α-[Rh[(*R*,*R*)-Me$_2$trien]phi]$^{3+}$ (Rh(MT)phi^{3+}). The reduction in emission intensity and the increase in absorbance of the $\pi-\pi^*$ transition for the intercalating dppz ligand are consistent with displacement of Ru(phen)$_2$dppz^{2+} by Rh(MT)phi^{3+}, which binds by intercalation in the center of the decamer from the major groove side.[22] Error bars represent standard deviations for at least two trials. Emission intensity and absorbance at 375 nm are normalized to the values at [Rh(MT)phi^{3+}] = 0 μM, and absorbance changes are corrected for DNA and Rh(MT)phi^{3+} interference.

presence of excess Rh(MT)phi^{3+}.[20] The sequence selectivity of this intercalation depends upon hydrogen-bonding interactions between the axial amines of the complex and guanines above and below the intercalation site, as well as methyl−methyl interactions between the methyl groups in Rh(MT)phi^{3+} and thymines at the base position once removed from the intercalated base step.

Figure 2 shows the effect of increasing Rh(MT)phi^{3+} concentration on the emission yield (squares) of Ru(phen)$_2$dppz^{2+} bound to [d(5′-GAGTGCACTC-3′)$_2$]. It should be noted that, on the basis of luminescence titrations, *rac*-Ru(phen)$_2$dppz^{2+} binds well to [d(5′-GAGTGCACTC-3′)$_2$] with saturation in luminescence at ∼3 complexes/duplex, consistent with a 3−4 bp binding site size for the racemic complex. With increasing concentrations of rhodium, the emission yield decreases, which is consistent with displacement of the lumiphore from the DNA since the ruthenium complex emits only when intercalated and protected from water. Such a loss in intensity cannot be a simple charge effect since titrations with the ruthenium(II) complex indicate that at least 2 additional complexes can be accommodated on the duplex. The observed loss of emission intensity also does not arise as a result of quenching by electron transfer. Unlike Rh(phi)$_2$bpy^{3+}, which promotes ∼70% quenching of ruthenium luminescence on CT DNA at low loading (1.5 equiv), Rh(MT)phi^{3+}, at the same loading, shows no detectable quenching of ruthenium emission. In general, phi complexes of rhodium(III) containing saturated amine ligands do not quench the ruthenium excited state in DNA or micelles.[21,22]

We also followed this titration by UV−vis spectroscopy. Intercalative binding of dppz complexes to DNA is accompanied by significant hypochromism and a red shift in the $\pi-\pi^*$ transition centered on the phenazine portion of the ligand.[3−10] As expected for a displacement effect, the absorbance at 375 nm increases with increasing rhodium concentration and begins to saturate at 1:1 rhodium/duplex (Figure 2). Given that the increase in absorbance mirrors the decrease in ruthenium emission, both absorption and emission spectroscopies indicate that Rh(MT)phi^{3+} displaces Ru(phen)$_2$dppz^{2+} from the duplex.

This displacement of ruthenium by rhodium contrasts the cooperative clustering of ruthenium with Rh(phi)$_2$L^{3+} (L = bpy, phen) complexes which has been suggested.[23] In fact, owing to the smaller, aliphatic nature of the ancillary ligands of Rh(MT)phi^{3+} compared to phen or phi, cooperative clustering of Rh(MT)phi^{3+} with Ru(phen)$_2$dppz^{2+} might be expected to be far less likely here. Clearly, the decrease in luminescence combined with the loss of hypochromicity is opposite to what would accompany binding cooperativity on the helix. Instead the loss of emission must reflect an anticooperative interaction.

Addition of Rh(MT)phi^{3+} does not completely eliminate the ruthenium emission, however. The decrease in emission begins to level off after reaching a 1:1 rhodium/duplex ratio, with slightly more than 50% of the ruthenium emission depleted. Compared to the decay profile in the absence of the rhodium complex, the lifetimes of the biexponential decay in the presence of rhodium are reduced slightly and the distribution is shifted in favor of the shorter lifetime (Table 1). Calculation of a steady-state emission yield from the luminescence decay parameters indicates a decrease in emission of only 35%, and therefore the reduction cannot be accounted for solely on the basis of excited-state lifetime. Since the only binding sites present with rhodium bound are near the ends of the duplex, the shorter lifetimes observed could indicate redistribution of ruthenium from the center of the decamer outward in addition to some displacement into solution as observed by UV−vis.

These data are consistent with binding of Ru(phen)$_2$dppz^{2+} from the major groove side of the double helix and its competitive displacement by Rh(MT)phi^{3+}. On the basis of this result alone, however, this conclusion is not definitive, since intercalation in general can provide a probe of both DNA grooves. Nonetheless, if the ruthenium complex were bound in the minor groove, and displaced because of rhodium intercalation from the other side of the helix, one might have expected only the base step where rhodium intercalated to be

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(21) Arkin, M. R.; Stemp, E. D. A.; Turro, C.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 2267−2274.

(22) In quenching experiments with Rh(phi)$_2$bpy^{3+}, the concern that this rhodium complex might displace the ruthenium donor even at low loadings of metal on DNA was ruled by out ultrafast transient absorption experiments which showed no evidence for free ruthenium or any D_2O effect in the dynamics of ground state recovery with Rh(phi)$_2$bpy^{3+}.[4]

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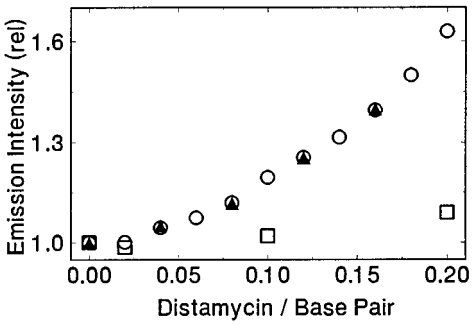

Figure 3. Relative emission intensity for $Ru(phen)_2dppz^{2+}$ bound to poly d(AT) (○, ▲) and poly d(GC) (□) in the presence of increasing concentrations of distamycin. On poly d(AT), the changes in ruthenium emission saturate at ∼1 distamycin/∼5 bp, consistent with the helix accommodating major and minor groove binders simultaneously. At concentrations of distamycin > 0.2/bp on poly d(AT), the ruthenium lumincescence yield changes with time and the intensity vs [distamycin] curves diverge. Error in emission intensities are estimated to be ±10%. Conditions are as follows: (○) 10 μM Ru, 1 mM nucleotides (nuc) poly d(AT); (▲) 20 μM Ru, 0.5 mM nuc poly d(AT); (□) 10 μM Ru, 1 mM nuc poly d(GC) (in 5 mM Tris, 50 mM NaCl, pH 7.8).

somewhat blocked. Instead we observe a larger region of the duplex to be affected.

Competitive Binding in the Minor Groove. We also probed the groove association of $Ru(phen)_2dppz^{2+}$ in titrations with distamycin (Figure 1). Distamycin binds well to poly d(AT) ($K_B \sim 10^7$ M^{-1}) but not to poly d(GC) ($K_B \sim 10^4$ M^{-1}).[24,25] As expected, the emission yield of ruthenium bound to poly d(GC) is not significantly affected by the presence of distamycin (Figure 3). Bound to poly d(AT), however, the $Ru(phen)_2dppz^{2+}$ luminescence yield is actually increased upon addition of distamycin (Figure 3). This increase in the ruthenium luminescence saturates at ∼1 distamycin/5 bp, consistent with the ligand binding to the duplex as a monomer and saturating the minor groove.[26]

That the ruthenium luminescence increases rather than being unaffected by distamycin association likely reflects a stiffening of the duplex on binding distamycin.[24,25] As indicated in Table 1, the increase in quantum yield with addition of distamycin to $Ru(phen)_2dppz^{2+}$ bound to poly d(AT) is derived from a redistribution of the lifetimes in the biexponential decay in favor of the longer lifetime (Table 1). Such an effect on the relative proportion of lifetimes is also observed at high metal complex to DNA ratios,[1,5,7,14] which has also been ascribed to duplex stiffening.[1,7] One might consider that addition of distamycin only concentrates the metal complexes in sections of the minor groove which are uncovered by distamycin. Such a consideration is ruled out, however, since titrations at two ruthenium: bp ratios which differ by a factor of 4 are superimposable until reaching saturation.[27] It is noteworthy also that the luminescence data for ruthenium bound to poly d(AT) in the presence of distamycin resemble data for ruthenium in the presence of poly dA·poly dT, which is a more rigid polymer.[25]

It is also possible to load ruthenium onto the helix when the minor groove is saturated with distamycin. When 20 μM $Ru(phen)_2dppz^{2+}$ is added to poly d(AT) (250 μM bp) containing saturating concentrations of distamycin (50 μM), bright ruthenium luminescence is observed.

These competitive binding titrations in the minor groove therefore also support intercalation of $Ru(phen)_2dppz^{2+}$ from the major groove side. If ruthenium were bound in the minor groove, one would have expected a decrease in luminescence as the metal was displaced with increasing distamycin concentrations. Thus these results are certainly consistent with the helix being able to accommodate major and minor groove-binding agents simultaneously.

Groove Preference for $Ru(phen)_2dppz^{2+}$ and Other Metallointercalators. A variety of results have suggested that metallointercalators may in general associate with double helical DNA from the major groove.[12,13,20,28−30] These observations include the high-resolution NMR structure[20] of $Rh(MT)phi^{3+}$ bound to a DNA decamer and chemical cleavage experiments[28] with $Rh(MGP)_2phi^{3+}$ (MGP = 4-(guanidylmethyl)-1,10-phenanthroline) and $Rh(en)_2phi^{3+}$ (en = ethylenediamine) on synthetic oligonucleotides in the absence and presence of covalent modifications in the major groove. Of particular note is the crystal structure of (terpyridyl)(2-hydroxyethanethiolato)platinum(II) intercalated in $d(CpG)_2$;[29] in this case the metallointercalator is primarily planar, and the complex is oriented within the base step with the metal center on the major groove side. This structure, albeit only of an intercalated dinucleotide, suggests that factors other than steric bulk are important in determining groove preference for intercalation. Indeed, as with organic intercalators,[31] dipole and induced-dipole orientations of the aromatic heterocyclic moiety which intercalates are likely to govern the stacking geometry. Certainly, those factors which dominate groove selectivity for metallointercalators need still to be established.

It is noteworthy that NMR experiments have recently indicated that the metallointercalator $Ru(phen)_2dpq^{2+}$ (dpq = dipyrido[2,2-*d*:2′,3′-*f*]quinoxaline) may bind to a 6 bp duplex by intercalation from the minor groove.[32] Interestingly, the chemical shift perturbations and nuclear Overhauser effects observed with intercalation of this compound differ significantly from those described for $Ru(phen)_2dppz^{2+}$.[12,13] While it is clear that one binding mode for $Ru(phen)_2dpq^{2+}$ involves association from the minor groove, it is not certain from the data presented

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(26) In contrast distamycin displaces ethidium. See: Baguley, B. C. *Mol. Cell Biochem.* **1982**, *43*, 167.

(27) At concentrations of distamycin > 0.2/bp on poly d(AT), the ruthenium lumincescence yield changes with time and the intensity vs [distamycin] curves diverge.

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(30) Also noteworthy are early NMR experiments with $Ru(phen)_3^{2+}$ isomers bound to a DNA hexamer which consistently showed perturbations in the major groove for binding favored by the Δ-isomer in addition to perturbations in the minor groove attributed to surface binding favored by the Λ-isomer. See: Rehmann, J. P.; Barton, J. K. *Biochemistry* **1991**, 29, 1701, 1710.

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that this mode is intercalative. If this minor groove association is indeed intercalative, the comparison to $Ru(phen)_2dppz^{2+}$ must be a consequence of the change in electronic structure between the dppz and dpq ligands. NMR studies of Δ-$Ru(phen)_2dppz^{2+}$ bound to the hexamer duplex $d(GTCGAC)_2$ clearly show NOE crosspeaks of bound adenine proton resonances in the major groove and the upfield-shifted 4′,7′ protons of intercalated dppz.[12]

The data we present here are fully consistent with intercalation of $Ru(phen)_2dppz^{2+}$ from the major groove of DNA, as originally described by NMR.[12,13] These data are also helpful in reconciling a photophysical study[14] which suggested minor groove association. The primary observation put forth in support of minor groove association was the absence of a substantive change in luminescence for T4 DNA compared to CT DNA. Our results here regarding sequence preferences for $Ru(phen)_2dppz^{2+}$ make such observations understandable. Another suggestion for minor groove association was made on the basis of the different excited-state lifetimes found for ruthenium bound to inosine-containing polymers compared to poly d(GC). In general the excited-state lifetimes for $Ru(phen)_2dppz^{2+}$ is seen to be sensitive to an array of factors, many of which are not yet quantitatively understood. Last, Tuite et al.[14] have argued that the high luminescence yield of $Ru(phen)_2dppz^{2+}$ bound to triple helical DNA indicates minor groove association since they consider that the third strand must block major groove intercalation. But here too, little difference in emission should be expected between the double and triple helix with binding of ruthenium from the minor groove. Instead we propose that the third DNA strand provides a more substantial platform for intercalative stacking from the major groove (and third strand) side, yielding an increase in protection from quenching by water and thus an increased excited-state lifetime.[33]

Since photophysical studies provide only an indirect description of structure, their conclusions regarding structural models must be viewed with some caution. High-resolution structural information must depend upon NMR and crystallographic studies. Importantly, the photophysical experiments described here and previously are now reconciled with NMR data.

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(33) Preferential intercalation at base triples has been found with $Rh(phen)_2phi^{3+}$. See: Chow, C. S.; Behlen, L. S.; Uhlenbeck, O. C.; Barton, J. K. *Biochemistry* **1992**, *31*, 972−982.