Luminescence of ruthenium(II) polypyridyls: evidence for intercalative binding to Z-DNA

Alan E.Friedman, Challa V.Kumar,[1+], Nicholas J.Turro[1] and Jacqueline K.Barton*
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125 and [1]Columbia University, Department of Chemistry, New York, NY 10027, USA

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ABSTRACT

Photophysical studies have been undertaken to characterize the binding interactions of enantiomers of $Ru(phen)_3^{2+}$, $Ru(DIP)_3^{2+}$, and racemic $Ru(bpy)_2dppz^{2+}$ (where phen = 1,10-phenanthroline, DIP = 4,7-diphenylphenanthroline, and dppz = dipyridophenazine) with Z-form poly d(GC). Parallel enhancements in steady state luminescent intensity and a lengthening of luminescent lifetimes are seen for ruthenium enantiomers with Z-DNA as for B-DNA but with enantioselectivities reversed. Greater enhancements are seen for Δ-isomers with the right-handed helix but for Λ-isomers with the left-handed helix. $Ru(bpy)_2dppz^{2+}$, an avid intercalator in B-DNA, displays no luminescence free in aqueous solution, but luminesces brightly bound to either B- or Z-poly d(GC). Stern-Volmer quenching studies also support the enantioselective preference in binding to B-DNA by Δ-isomers and a reversal with binding to Z-DNA preferentially by the Λ-isomers. Steady state polarization studies indicate a rigid association of the complexes with both B-and Z-DNA on the time-scale of their emission and again with symmetrical enantioselectivities for the left and right-handed helices. Given the well characterized intercalative association of the complexes with B-DNA, the parallel results seen here with Z-DNA point strongly to a comparable intercalative association with the Z-form helix. That molecules may interact with Z-DNA through intercalation has not been demonstrated previously and now requires consideration in describing the range of interactions of small molecules and proteins with Z-DNA.

INTRODUCTION

There has been considerable interest in understanding those factors which govern the sequence-specific recognition of DNA by proteins and smaller natural products (1,2). Our laboratory has focused on shape-selective interactions with nucleic acids through the design of synthetic transition metal complexes which bind DNA with conformational selectivity (3). Among the complexes prepared have been those which bind preferentially to A-DNA (4), Z-DNA (5,6), cruciforms (7) as well as one which appears to target unique tertiary folds in RNA (8). Indeed a high level of site-specificity can be achieved based solely upon considerations of shape. It is likely that such 'indirect readout' (9) plays a substantial role in site recognition by proteins.

If altered non-B-DNA forms are to serve as targets for recognition by proteins and new synthetic designs, it becomes important to explore the range of binding interactions available with the various conformations. In the case of B-DNA, the dominant non-covalent interactions appear to be groove-bound associations stabilized through hydrogen bonding and Van der Waals interactions and intercalative binding, stabilized through π-stacking interactions (3). For most small molecules, in particular for those which are groove bound, the association is in the minor groove. In contrast for DNA-binding proteins, the ensemble of interactions occurs predominantly in the major groove.

By exploiting the versatile and convenient spectroscopic as well as structural properties of polypyridyl complexes of ruthenium, we have been able to characterize the non-covalent binding of tris(phenanthroline)ruthenium(II), $Ru(phen)_3^{2+}$ (Figure 1), and its derivatives with B-DNA (10−12). Ruthenium(II) polypyridyls contain an intense metal to ligand charge transfer (MLCT) transition in the visible region which is perturbed in a manner which depends on the mode of binding to DNA. Furthermore, two enantiomeric forms of this tris-chelated complex may be isolated, and these forms are inert to substitution and racemization. Based upon extensive photophysical characterization (10−12), we found that $Ru(phen)_3^{2+}$ binds to B-DNA in two modes: (i) through intercalation favoring the Δ-isomer and (ii) through a surface-bound association favoring the Λ-isomer. NMR studies (13) with oligonucleotides provided evidence in support of these two enantioselective binding modes and pointed to the surface bound association as occurring in the minor groove of the helix with the intercalation arising instead from the major groove. The enantioselectivity was furthermore demonstrated to depend upon DNA helicity, leading to the

* To whom correspondence should be addressed

+ Present address: Department of Chemistry, University of Connecticutt, Storrs, CT 06269, USA

development of a spectroscopic probe (5) for Z-DNA, Λ-tris(diphenyl-phenanthroline)ruthenium(II), Λ-$Ru(DIP)_3^{2+}$.

Here we report a detailed photophysical characterization of the interaction of enantiomers of $Ru(phen)_3^{2+}$ and $Ru(DIP)_3^{2+}$ (Figure 1) with the synthetic polynucleotide poly d(GC)·d(GC) in both the B-form and the Z-form. Comparable binding interactions on Z-form helices are identified as found with B-DNA, but *with enantioselectivities reversed depending upon the DNA helicity*. Furthermore, described here are photophysical studies of a new DNA probe (14), bis(bipyridine)(dipyridophenazine)ruthenium(II), $Ru(bpy)_2(dppz)^{2+}$, which binds to B-DNA avidly by intercalation and serves as a 'molecular light switch', showing no detectable luminescence in aqueous solution but becoming highly luminescent upon intercalation into a helix. Here as well comparable photophysical results are found with Z-DNA. The complexes used are shown schematically in Figure 1. These results taken together point to a parallel interaction of the ruthenium complexes with Z-DNA as with B-DNA and hence provide evidence for the intercalation of these complexes into a Z-form helix. That molecules may interact with Z-DNA through intercalation has not been demonstrated previously.

EXPERIMENTAL

Buffers and Chemicals

All experiments were carred out at pH 7.0 with distilled deionized water in either B-forming buffer, 50 mM NaCl, 5 mM Tris, or Z-forming buffer, 20 mM NaCl, 2 mM Tris, 4 mM $[Co(NH_3)_6]Cl_3$. For $[Ru(DIP)_3]Cl_2$ solutions, 10% DMSO was added. $K_4Fe(CN)_6$ was purchased from Aldrich Chemicals (Gold Label) and used without further purification.

Ruthenium Complexes

Tris(bipyridine)ruthenium(II) dichloride $[Ru(bpy)_3]Cl_2$, was purchased from Alfa inorganics and used without further purification. Tris(phenanthroline)ruthenium(II) dichloride, $[Ru(phen)_3]Cl_2$, and tris(4,7-diphenylphenanthroline)ruthenium(II) dichloride, $[Ru(DIP)_3]Cl_2$, were synthesized and enantiomers were separated as described previously (10–12). Resolution of $[Ru(phen)_3]^{2+}$ enantiomers gave typically isomeric purities of 93% and 95% for Λ and Δ isomers, respectively. Resolution of $[Ru(DIP)_3]^{2+}$ enantiomers gave typically isomeric purities of 94% and 92% for Λ and Δ isomers, respectively. Bis(bipyridine)dipyridophenazine-ruthenium(II) ditetrafluoroborate $[Ru(bpy)_2(dppz)](BF_4)_2$, was first received as a generous gift from J.-P. Sauvage. Subsequent batches were prepared in our laboratory (15).

Nucleic Acids

Calf thymus DNA was obtained from Sigma Chemical Co. and purified by phenol extraction. The synthetic deoxyribonucleotides polymers were obtained from Pharmacia Biochemicals. All nucleic acid solutions were extensively dialyzed to bring them to the appropriate ionic strengths and to remove small fragments. The Z-conformation was promoted by the addition of cobalt hexammine and monitored by the distinctive negative Cotton effect observed at 290 nm in the circular dichroism spectrum. Under the conditions employed, no Z* formation (16) was evident by circular dichroism.

Spectroscopic Measurements

All absorption spectra were measured with either a Varian Cary 219 spectrophotometer or a Varian DMS 300 spectrophotometer. Circular dichroic measurements were performed on a Jasco J-500 automatic recording spectropolarimeter. All luminescence measurements were conducted on a SLM 8000C recording spectrometer at 20°C in the appropriate buffer systems. Luminescence quantum yields, Φ, were determined by direct comparison to a 10 μM $[Ru(bpy)_3]Cl_2$ solution irradiated at 464 nm. Peak integrals were obtained using the SLM software package. Quenching experiments were conducted by adding 5–20 μM aliquots of a 20 mM ferrocyanide stock solution to 100 μM nucleic acid concentrations in the appropriate buffer with 10 μM metal complex.

Luminescent Lifetime Measurement

Lifetime measurements were performed on an instrument constructed at the Beckman Institute. The excitation source was a XeCl excimer (Lambda Physik LPX-200) pumped dye laser (Lambda Physik FL3002) operating at 480 nm (Coumarin 480). The excitation beam was focused into a 0.5×0.5 cm cuvette. Emitted light was collected (f/15), and focused onto the entrance slit of an ISA double grating (100 mm) monochrometer, and detected with a photomultiplier (Hamamatsu R955). The signal from the PMT was amplified (200 MHz, single-ended) and digitized by a Tektronix RTD 210 transient recorder. Emission decays were averages of 500 laser shots. Decays were fit to single or multiexponential functions using a non-linear least squares minimization. Measurements were also made on a single photon counting unit using a PRA 1000A lamp and Ortec electronics and a TN-1710 MCA interfaced with an HP 87 personal computer with decay traces fit with software developed at Columbia University. Comparable results were obtained on the two instruments.

Steady-State Polarization

Steady-state emission polarization measurements were made on an SLM 8000C spectrophotometer employing Glan-Thompson calcite prism polarizers arranged in a 'T'-shaped geometry. Emission was monitored by employing Corning 2-73 glass filters. The orientation of the polarizers was checked with glycogen and fluoricil solutions. In addition, polarization of $Ru(bpy)_3^{2+}$ in glycerol agreed well with reported values (17). The polarization of $Ru(phen)_3^{2+}$ in glycerol checked well with the maximum expected value (1/7) for a D_3 molecule (17). $Ru(DIP)_3^{2+}$ and $Ru(bpy)_2dppz^{2+}$ have symmetry less than D_3 and therefore can have maximum polarizations greater than 1/7 but less than the theoretical limit of 1/2. Polarizations are given as the ratio of $[(R_v/R_h)-1]/[(R_v/R_h)+1]$: $R_v = I_{vv}/I_{hv}$, and $R_h = I_{vh}/I_{hh}$; where I_{vv} refers to the intensity of vertically polarized emission observed when the sample is irradiated with vertical light, I_{hv} refers to the intensity of horizontally polarized emission observed when the sample is irradiated with vertical light, I_{vh} refers to the intensity of vertical polarized emission observed when the sample is irradiated with horizontally polarized light, and I_{hh} refers to the intensity of horizontally polarized emission observed when the sample is irradiated with horizontally polarized light. All solutions were equilibrated in a thermostated cell holder at 20°C for at least 30 min in order to achieve stable readings. Ruthenium concentrations used were typically 1–10 μM. 20 readings were averaged for a single measurement and the deviation was usually less than 1%.

RESULTS

Luminescence Enhancements

The photophysical properties of the racemic complexes $Ru(phen)_3^{2+}$, and $Ru(DIP)_3^{2+}$, under various experimental conditions have been described previously in the presence and absence of B-DNA.[10,11] Upon binding to DNA, the MLCT excited state is perturbed as revealed by absorption hypochromism, luminescence intensity enhancement and an increase in excited state lifetime. Table I shows luminescence quantum yields for ruthenium enantiomers in the presence and absence of B-and Z-DNA. The luminescence of the complexes is enhanced appreciably with DNA binding, both for the B and Z forms. This luminescence enhancement provides a good measure of DNA binding; $Ru(bpy)_3^{2+}$ (Figure 1), with an identical charge, shows no detectable increase in luminescence under the conditions examined, indicating the absence of either a surface or intercalative binding by $Ru(bpy)_3^{2+}$ (10).

One may notice that the luminescence enhancements for both racemic $Ru(phen)_3^{2+}$ and $Ru(DIP)_3^{2+}$ are slightly greater with Z-DNA than with the B-form at identical ruthenium/nucleotide ratios (e.g. compare entries 6 to 9 and 13 to 16) . One contribution to this increase may be the somewhat lower ionic strength of the medium used to promote Z-formations, but in fact luminescence results for calf thymus DNA, which does not substantially convert to the Z-form, under similar buffer conditions shows a smaller increase than with Z-form poly d(GC) (entries 17−20). The presence of cobalt hexammine furthermore should, if anything,

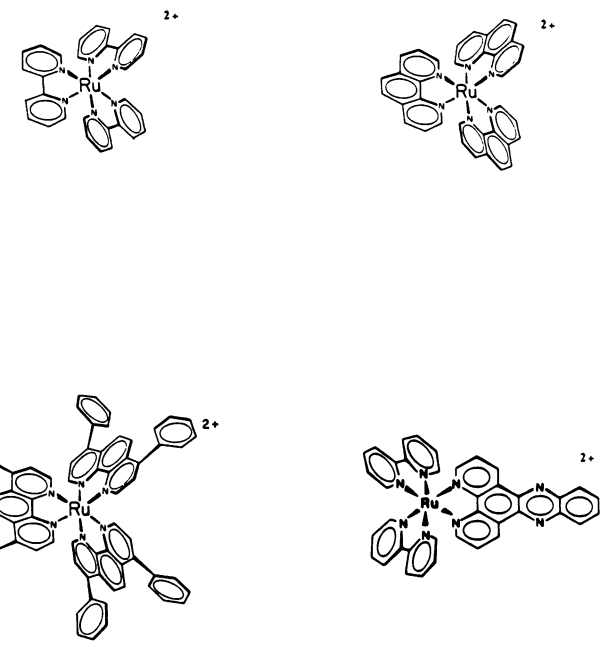

Figure 1. Spectroscopic Probes of Nucleic Acids, from top, left (clockwise): $Ru(bpy)_3^{2+}$, $Ru(phen)_3^{2+}$, $Ru(bpy)_2dppz^{2+}$, and $Ru(DIP)_3^{2+}$.

Table 1. Luminescence characteristics of ruthenium isomers in the presence of B- or Z-form DNA.

Complex[a]	Buffer[b]	DNA[c]	λ_{ex} (nm)	λ_{em} (nm)	Luminescence Φ[d]	Luminescence Enhancement with DNA[e]	τ[f] (nsec)
1. rac/Δ/Λ-$Ru(bpy)_3^{2+}$	B-forming	none	464	610	0.04		630
2. rac/Δ/Λ-$Ru(bpy)_3^{2+}$	B-forming	B-poly dGC	464	610	0.04	0.00	630
3. rac/Δ/Λ-$Ru(phen)_3^{2+}$	B-forming	none	464	615	0.052		525
4. Δ-$Ru(phen)_3^{2+}$	B-forming	B-poly dGC	464	615	0.091	1.46	
5. Λ-$Ru(phen)_3^{2+}$	B-forming	B-poly dGC	464	615	0.062	1.20	
6. rac-$Ru(phen)_3^{2+}$	B-forming	B-poly dGC	464	615	0.070	1.35	
7. Δ-$Ru(phen)_3^{2+}$	Z-forming	B-poly dGC	464	615	0.065	1.26	
8. Λ-$Ru(phen)_3^{2+}$	Z-forming	B-poly dGC	464	615	0.103	2.00	
9. rac-$Ru(phen)_3^{2+}$	Z-forming	B-poly dGC	464	615	0.076	1.47	
10. rac/Δ/Λ-$Ru(DIP)_3^{2+}$	B-forming	none	482	630	0.062		922
11. Δ-$Ru(DIP)_3^{2+}$	B-forming	B-poly dGC	482	630	0.144	2.32	618/1547
12. Λ-$Ru(DIP)_3^{2+}$	B-forming	B-poly dGC	482	630	0.065	1.05	934
13. rac-$Ru(DIP)_3^{2+}$	B-forming	B-poly dGC	482	630	0.101	1.63	
14. Δ-$Ru(DIP)_3^{2+}$	Z-forming	B-poly dGC	482	630	0.063	1.02	865
15. Λ-$Ru(DIP)_3^{2+}$	Z-forming	B-poly dGC	482	630	0.152	2.44	545/1632
16. rac-$Ru(DIP)_3^{2+}$	Z-forming	B-poly dGC	482	630	0.109	1.76	
17. Δ-$Ru(DIP)_3^{2+}$	B-forming	CT DNA	482	630	0.142	2.29	560/1998
18. Λ-$Ru(DIP)_3^{2+}$	B-forming	CT DNA	482	630	0.066	1.06	883
19. Δ-$Ru(DIP)_3^{2+}$	Z-forming	CT DNA	482	630	0.130	2.10	440/1874
20. Λ-$Ru(DIP)_3^{2+}$	Z-forming	CT DNA	482	630	0.063	1.02	852
21. $Ru(bpy)_2dppz^{2+}$	B-forming	none	482	no detectable emission			
22. $Ru(bpy)_2dppz^{2+}$	B-forming	B-poly dGC	482	628	0.020	$>10^4$	75/258
23. $Ru(bpy)_2dppz^{2+}$	Z-forming	Z-poly dGC	482	628	0.021	$>10^4$	

[a]All measurements were conducted at 25°C with 10 μM metal complex.
[b]B-forming buffer consisted of 50.0 mM NaCl, 5.0 mM Tris, pH = 7.0, Z-forming buffer consisted of 20.0 mM NaCl, 2.0 mM Tris, 4 μM $Co(NH_3)_6^{3+}$, pH = 7.0.For $Ru(DIP)_3^{2+}$ solutions also contained 10% DMSO.
[c]The concentration of DNA used was 100 μM nucleotides.
[d]The luminescence spectra were measured using an SLM 8000C spectrofluorimeter. Φ were determined using $[Ru(bpy)_3]Cl_2$ as a standard. Measurements taken show deviations of 8%.
[e]Integral ratios of luminescence for a given metal complex in the presence of DNA to that in the absence of DNA.
[f]Emission lifetimes were determined by deconvolution of the biexponential decay traces as described in experimental. Where two values are given a biexponential decay was observed. For single values, a single exponential could be used to describe the decay. Values have an uncertainty of 10%.

quench the luminescence, not increase it. Given the increased luminescence with Z-DNA compared to B-DNA, then either a greater quantum yield is found for the Z-bound species or binding to this form is favored over the B-form at equal added binding ratios. Some concern for the homogeneity of solutions was also given owing to the poor solubility of $Ru(DIP)_3^{2+}$ in aqueous solution and the propensity of Z-DNA to aggregate (16). If solutions are centrifuged for short time or millepore filtered, the same results were obtained.

Perhaps more striking is the comparison seen in enantioselectivities with B- versus Z-DNA. While both Δ-$Ru(phen)_3^{2+}$ and Δ-$Ru(DIP)_3^{2+}$ show greater enhancements than their Λ-counterparts with B-form poly d(GC), the opposite is seen with Z-DNA. With the B-form, Δ-$Ru(phen)_3^{2+}$ shows an enhancement of 1.46 (entry 4) compared to a value of 1.20 (entry 5) for the Λ-isomer, whereas with the Z-form, the Δ-isomer shows an enhancement of only 1.26 (entry 7) compared to a value of 2.00 (entry 8) for the Λ-isomer with Z-DNA. For complexes of the DIP ligand, with the B-form, Δ-$Ru(DIP)_3^{2+}$ shows an enhancement of 2.32 (entry 11) compared to a value of 1.05 (entry 12) for the Λ-isomer, whereas with the Z-form, the Δ-isomer shows an enhancement of 1.02 (entry 14) compared to a value of 2.44 (entry 15) for the Λ-isomer with Z-DNA. With Z-form poly d(GC), therefore, it is consistently the Λ-isomer that is preferred. The level of enantioselectivity does, however, differ somewhat with Z-DNA compared with B-DNA.

Luminescence is apparent also on binding $Ru(bpy)_2dppz^{2+}$ to Z-DNA. Photophysical studies of $Ru(bpy)_2dppz^{2+}$ in the presence of DNA in H_2O/D_2O mixtures or in aprotic solvents have indicated the remarkable luminescence enhancement seen with binding to B-DNA to result from protection of the phenazine ring from protonation (15). The dppz-localized charge transfer state appears to be efficiently quenched with protonation of the phenazine nitrogen atoms; intercalation of the dppz ligand limits accessibility of protons to the phenazine ring. Binding to the Z-form helix also appears to inhibit the excited state quenching, indeed even to a greater extent than with B-DNA. The quantum yields for luminescence of $Ru(bpy)_2dppz^{2+}$ are 0.020 and 0.021 (entries 22 and 23) with B- and Z-poly d(GC) respectively; for comparison the quantum yield for luminescence of $Ru(bpy)_2dppz^{2+}$ in isopropanol is 0.02.

Excited State Lifetimes

Binding of the ruthenium complexes to DNA in a steady state experiment is characterized not only by an increase in the quantum yield but also by a lengthening of the luminescent lifetime τ. For example, when Δ-$Ru(DIP)_3^{2+}$ (10 μM) is bound to B-form poly d(GC) (100 μM) the single component lifetime of 922 ns for the free species (entry 10) is transformed to a biexponential decay with a long lived component of lifetime 1.55 μs and a shorter component of 620 ns (entry 11). Based upon extensive photophysical experiments, the longer lived component results from binding and has been assigned to the intercalated form (10–12) while the short component has been assigned to either a surface bound or a free species. Λ-$Ru(DIP)_3^{2+}$ (10 μM) bound to B-form poly d(GC) (100 μM) displays different behavior in this experiment; only one lifetime is observed of 934 ns (entry 12). This lifetime is virtually indistinguishable from the lifetime of the free complex. These results are expected when compared to the results for the $Ru(phen)_3^{2+}$ system (11).

Parallel results *but with opposite enantioselectivity* are seen with Z-DNA. For example, when Δ-$Ru(DIP)_3^{2+}$ (10 mM) is bound to Z-form poly d(GC) (100 μM) the lifetime remains approximately the same at 865 ns (entry 14). The slight shortening of the lifetime may be due to quenching by $Co(NH_3)_6^{3+}$ required for the formation of Z-DNA. The Λ-enantiomer in contrast binds appreciably to this form, and a longer lifetime component is observed. For Λ-$Ru(DIP)_3^{2+}$ (10 μM) bound to Z-form poly d(GC) (100 μM) the lifetimes obtained are 1.63 μs and 545 ns (entry 15).

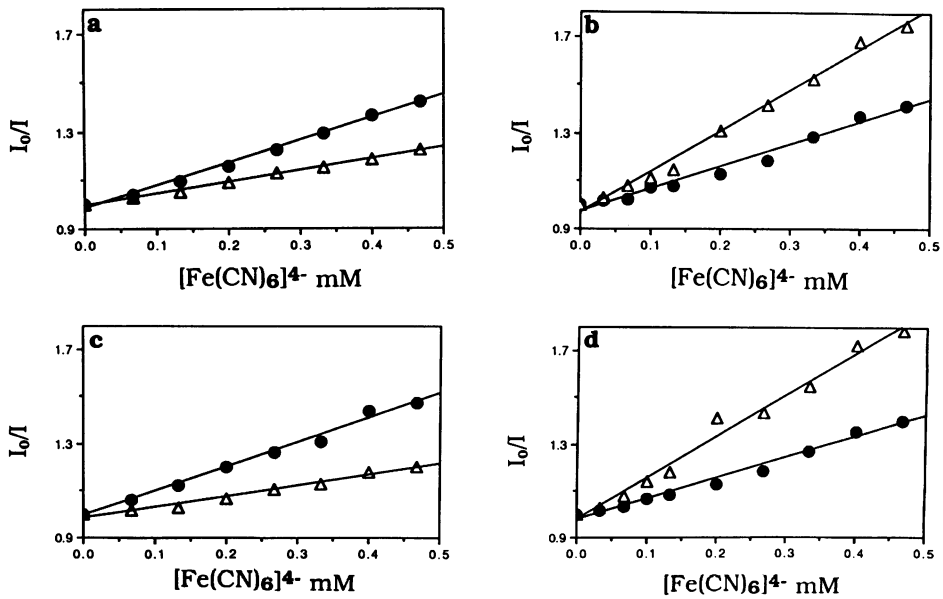

Figure 2. Stern-Volmer Luminescence Quenching of Δ ($\triangle$) and Λ ($\bigcirc$) isomers of ruthenium complexes (10 μM) in the presence of poly d(GC)·d(GC) (100 μM) in either the B-form (**a** and **c**) or Z-form (**b** and **d**). In panels **a** and **b** Stern-Volmer plots are given for $Ru(phen)_3^{2+}$ isomers and in **c** and **d**, for $Ru(DIP)_3^{2+}$. Notice the reversal of enantiomeric discrimination for both ruthenium complexes between the right-handed B and left-handed Z-forms.

Luminescence Quenching by $Fe(CN)_6^{4-}$

Another means to gauge relative binding to the DNA polyanion is through luminescence quenching studies (11). Anionic quenchers of the ruthenium emission, such as $Fe(CN)_6^{4-}$, poorly quench complexes which are closely bound to the DNA polyanion but very efficiently quench the emission of ruthenium complexes which are free in solution due to ion pairing. Figure 2 shows Stern Volmer plots for the quenching of ruthenium enantiomers by $Fe(CN)_6^{4-}$ in the presence of B- or Z-form poly d(GC). In these plots, sufficiently low quencher concentrations are utilized to yield linear dependences on quencher concentration; at higher ferrocyanide concentrations, curvature is observed. In these plots the steeper slope, k_{sv}, reflects more efficient quenching (less protection).

Not surprisingly with B-form DNA, enantioselective quenching is observed; higher quenching constants are observed with the Λ-isomers reflecting their greater accessibility to the anionic quencher. The Δ-isomers, bound more tightly to the DNA polyanion, are better protected from quencher. The Stern-Volmer quenching constant (k_{sv}) for the quenching of Δ-$Ru(phen)_3^{2+}$ is $3.8\times10^3\ M^{-1}$, lower than the k_{sv} for the free complex under similar conditions ($4.9\times10^3\ M^{-1}$); the D isomer is bound more tightly into the DNA while the L isomer is largely surface bound. For comparison the luminescence quenching of $Ru(DIP)_3^{2+}$ is also shown in the presence of B-form poly d(GC) under the same conditions. Very similar results are observed. The L enantiomer binds with less affinity and is therefore more easily quenched ($k_{sv} = 8.4\times10^3\ M^{-1}$). The D enantiomer is more tightly bound and so more difficult to quench ($k_{sv} = 3.7\times10^3\ M^{-1}$). The DIP complex seems to bind with higher affinity than the analogous phen complex (when the D form is used); this likely reflects a combination of better intercalative abilities of the ligand as well as increased hydrophobicity.

The luminescent quenching of the enantiomers of $Ru(phen)_3^{2+}$ and $Ru(DIP)_3^{2+}$ with B-form DNA is again directly opposed to the enantioselectivities observed by these transition metal complexes in the presence of the left-handed Z-DNA. Figure 2B represents the quenching of the isomers in the presence of Z-form poly d(GC) (100 μM). Here it is apparent that Δ-$Ru(phen)_3^{2+}$ binds more weakly to the left-handed helix than does the Λ-enantiomer. The Stern-Volmer quenching constant k_{sv} for the D complex in the presence of Z-DNA is $13.1\times10^3\ M^{-1}$, lower than the value for the free complex but higher than the the k_{sv} for the Λ-isomer, $8.4\times10^3\ M^{-1}$. The Λ-isomer binds more tightly to the left-handed helix. These results again compare well to the analogous experiments with the enantiomers of $Ru(DIP)_3^{2+}$. The k_{sv} for the Δ-isomer is $13.4\times10^3\ M^{-1}$, slightly higher than the analogous $Ru(phen)_3^{2+}$ complex. The Λ-isomer exhibits a k_{sv} of $7.9\times10^3\ M^{-1}$.

Luminescence Polarization

Polarized luminescence experiments are valuable in assessing the mode of binding of the complex on the helix through measurement of rotational dynamics. After excitation with polarized light, in order for polarization to be preserved in the emitted light, the complex must be rigidly oriented on the time scale of the emission. In free solution rotational motion causes rapid loss of polarization within nanoseconds. Since the ruthenium complexes bound to DNA display excited state lifetimes in the microsecond range, maintenance of polarized emission indeed must reflect a highly oriented, rigidly held species, as would be expected upon intercalation. It should be noted from earlier studies involving differential quenching of polarized emission, that in the case of B-DNA, the surface bound mode contributes little or no polarized emission (10,11).

Figure 3 shows the results of polarization measurements for

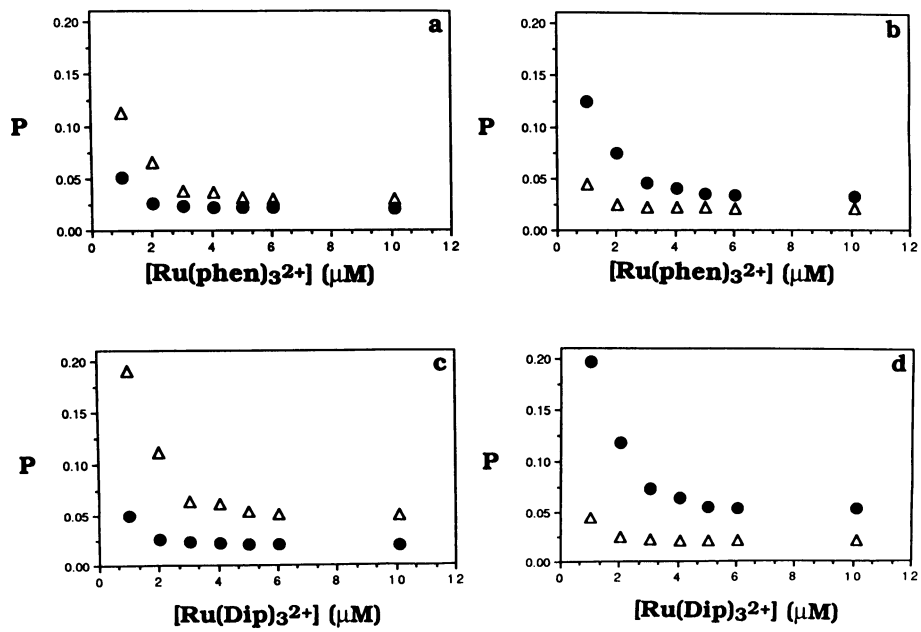

Figure 3. Steady state luminescence polarizations of Δ (△) and Λ (○) isomers of ruthenium complexes as a function of their concentration in the presence of poly d(GC)·d(GC) (100 μM) in either the B-form (**a** and **c**) or Z-form (**b** and **d**). In panels **a** and **b** polarization titrations are given for $Ru(phen)_3^{2+}$ isomers and in **c** and **d**, for $Ru(DIP)_3^{2+}$. Notice here also the reversal of enantiomeric discrimination for both ruthenium complexes between the right-handed B- and left-handed Z-forms.

enantiomers of $Ru(phen)_3^{2+}$ and $Ru(DIP)_3^{2+}$ as a function of increasing concentration of ruthenium in the presence of a fixed amount of B- and Z-form poly d(GC). The observed polarization for either free complex in buffer was found as expected to be very low (0.003 ± .0002); these values agreed well with those previously reported in H_2O and in glycerol (17). When these complexes were allowed to interact with DNA, however, significantly higher polarizations were observed, and indeed depending on how well the shape of the complex matched that of the DNA helix as shown for the other probe methods discussed above.

Graphically represented in Figure 3A is the effect of increasing concentration on the polarization for the Δ-isomer of $Ru(phen)_3^{2+}$ in the presence of 100 μM B-form DNA. At low concentrations (1 μM) of the metal complex, the highest luminescence polarization for this complex (0.12) is observed. At high DNA:ruthenium ratios the greatest population of the ruthenium complex is in the bound form. As the concentration of the complex is increased and the amount of free ruthenium increases, there is a significant decrease in the observed polarization. This decrease levels off as the polarization reaches the value of 0.03 at 10:1 DNA (base pair): metal ratio. Indeed, the highest polarization values obtained agree well with theoretical limits for these polarizations (17). Importantly, when the analogous sample is prepared with the Λ-isomer much less luminescence polarization is observed; the highest polarizations observed were 0.05. Upon increasing concentration, the value decreased to a limiting value of 0.022. Again comparable results are found with isomers of $Ru(DIP)_3^{2+}$. Here, however, the overall molecular symmetry of $Ru(DIP)_3^{2+}$ is lower than the analogous phen complex, owing to the non-planarity of the phenyl rings, thus allowing for higher polarization. With Δ-$Ru(DIP)_3^{2+}$ at low metal concentrations the limiting polarization approaches 0.27 but, interestingly, with Λ-$Ru(DIP)_3^{2+}$, at low concentrations the polarization is still only 0.05 (like $Ru(phen)_3^{2+}$). Intercalative binding by the DIP complexes is highly enantioselective for B-DNA.

Comparable results are again obtained with ruthenium enantiomers in the presence of the left-handed Z-form DNA. The finding of polarizations here establish a previously unsuspected rigid interaction of the complexes with the Z-form helix.

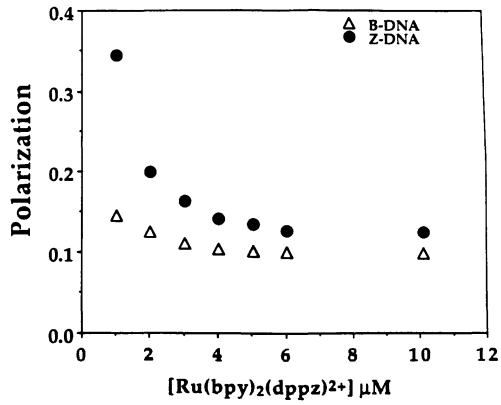

Figure 4. Steady state luminescence polarization of $Ru(bpy)_2(dppz)^{2+}$ in the presence of poly d(GC)·d(GC) (100 μM) in either the B- (△) or Z- (○) form as a function of ruthenium concentration.

Furthermore, the chiral discrimination seen with the Z-form is opposite that with right-handed B-DNA. The Λ-isomer binds with higher affinity to the Z-form as evidenced by the higher luminescence polarization at 100:1, DNA:metal of 0.13 and 0.21 for $Ru(phen)_3^{2+}$ and $Ru(DIP)_3^{2+}$, respectively, compared to values of 0.05 for the analogous Δ-isomers.

To characterize further the binding to the Z-DNA helix, the steady state polarization of $Ru(bpy)_2(dppz)^{2+}$, our most avid intercalator, was examined. Figure 4 displays the variation in polarization with increasing concentration of the complex in the presence of both forms of DNA. $Ru(bpy)_2(dppz)^{2+}$ in the presence of Z-form DNA is seen to produce the highest steady state luminescence polarization thus far; the excited state lifetime of this bound complex is reduced compared to the phenanthroline and DIP complexes, however. Nonetheless, this complex, with the extensive planar surface of the dppz ligand, must therefore also be quite rigidly held on Z-DNA.

Lastly, for all these complexes it is important to compare the limiting polarizations at high DNA/metal ratios (corresponding to complete binding) between B-form and Z-form. With B-poly d(GC) these polarization values extrapolate to 0.14, 0.27, and 0.16, for Δ-$Ru(phen)_3^{2+}$, Δ-$Ru(DIP)_3^{2+}$, and $Ru(bpy)_2(dppz)^{2+}$, respectively compared to 0.17, 0.28, and 0.45 for Λ-$Ru(phen)_3^{2+}$, Λ-$Ru(DIP)_3^{2+}$, and $Ru(bpy)_2(dppz)^{2+}$, respectively with Z-poly d(GC). Thus consistently, across this series of complexes, a higher polarization value for the fully bound form is seen with Z-DNA compared to B-DNA. This trend argues strongly that the interaction with the Z-form helix is more static than with B-DNA, indeed that the Z-form helix itself may be more rigid than its B-form counterpart. This observation supports the finding of increased rigidity of the base-pairs in Z-DNA compared to a B-form helix by NMR (18).

DISCUSSION

The results described clearly indicate the binding of the ruthenium complexes to both B- and Z-DNAs and provide a means of elucidating their binding modes. The spectroscopic parameters employed, enhanced emission, emission lifetimes, Stern-Volmer quenching, and emission polarization, all lead to a self-consistent set of conclusions concerning the mode and efficiency of binding of these complexes to B- and Z-DNA. Spectroscopic perturbations resulting from binding to the Z-form parallel those found with B-DNA. Comparable enhancements in luminescent intensity and lifetimes are seen as a result of DNA binding with comparable protection of the bound ruthenium complexes from anionic solution quenchers. Moreover the rigid mode of binding of the complexes is revealed in polarized emission studies.

The results show also that, in general, the ruthenium complexes bind somewhat more avidly to the Z-form than to the B-form polymer. Thus greater enhancements in luminescence are seen on binding to Z-poly d(GC) compared to B-DNA. However, this result may be explained either due to an increased binding affinity or as the result of an increased quantum efficiency for the Z-DNA-bound species. The luminescence quenching experiments support the greater binding affinity to the Z-conformation; lower Stern-Volmer quenching constants are apparent with Z-DNA compared to B-DNA, consistent with the higher binding to the Z-conformation.

Importantly symmetrical enantiomeric discrimination is observed for binding by the chiral complexes to the left-handed helix as compared to the right-handed helix. *The chiral*

discrimination is seen to depend upon the matching of the symmetry of the metal complex to that of the DNA helix. In the case of B-DNA, the enantiomeric preference for the Δ-isomer was shown earlier (3,10−12) to depend upon an intercalative mode of binding, with the non-intercalated ligands of the Δ-isomer disposed in an orientation along the right-handed helix; for the Λ-isomer intercalated into a right-handed helix, steric repulsion between the non-intercalated ligands and the right-handed phosphate backbone can arise. That a reversal of discrimination is apparent with Z-DNA, the left-handed helix, indicates that a similar basis for discrimination may exist. Binding to the left-handed helix would yield similar steric constraints, but with the opposite enantiomer being favored. It should be noted, however, that because of the lengthened Z-form helix, a smaller enantioselective preference ought to be observed, and indeed with Z-DNA compared to B-DNA, a lower absolute enantioselectivity between isomers is actually found.

All the data taken together therefore support an intercalative interaction of these ruthenium complexes with Z-DNA. Criteria have been established (19) to identify an interaction in B-DNA as intercalative in the absence of a crystallographic determination of structure. These include (i) experiments which evaluate structural changes in the DNA helix; (ii) experiments that indicate an electronic interaction with the DNA bases; (iii) experiments that demonstrate molecular orientation or rigidity; and (iv) considerations of molecular structure. In the case of binding of the ruthenium complexes to B-DNA, all these criteria have been satisfied. Besides the photophysical results described here, conventional helix unwinding experiments have been used to establish DNA structural changes (10−12). In the case of Z-DNA, all criteria except, importantly, that which establishes a structural change in the DNA have been satisfied. A comparable unwinding experiment in Z-DNA with sufficient sensitivity is difficult to achieve. However, all spectroscopic assays with Z-DNA parallel (with opposite chirality) those with B-DNA, suggesting that a similar mode of association must be present. Furthermore in the case of $Ru(bpy)_2dppz^{2+}$, extremely avid intercalative binding to the B-form helix is observed, and again a similar avidity in binding to Z-DNA exists. *These experiments therefore in total point strongly to an intercalative mode of association with Z-DNA.* The structural details of such an intercalative interaction may vary substantially however with Z-DNA compared to a B-form helix.

Why does the binding of these metallointercalators to Z-DNA differ so substantially from that by flat aromatic heterocyclic intercalators such as ethidium? In the case of ethidium, a cooperative transition back to the B-form is observed (20). In the case of the metallointercalators, it appears instead that binding is preferentially stabilized in the Z-form. Furthermore no cooperative transitions to an intermediate common structure occur with the ruthenium complexes, since clearly opposite enantioselectivities are seen with B-DNA compared to Z-DNA. While complementary, then, the bound mode with Z-DNA is distinct from that with B-DNA. This behaviour with the ruthenium complexes also stands in contrast to that found with ethidium. Perhaps the explanation rests in the different orientation of the different intercalators on the helix. Intercalation of ethidium occurs from the minor groove of the helix (21). In contrast, with B-DNA it appears that the metallointercalators associate from the major groove (3,13,22,23). Such a 'major groove intercalator' ought to be easily accomodated in the Z-helix, whereas binding from the minor groove would be precluded, indeed likely to promote a conversion to the B-form given the narrowness and depth of the minor groove (24) in Z-DNA. It is noteworthy in this context that chromomycin A_3, which binds DNA in the major groove, also shows no tendency to promote transitions to B-DNA from the Z-form (25).

In summary then it appears that these metallointercalators may associate with Z-DNA through an intercalative interaction. It would certainly be valuable to characterize structurally this interaction in some detail. Likely the binding involves only a partial insertion of one of the heterocyclic ligands between the base pairs. Perhaps Van der Waals interactions of the non-intercalated ligands against the surface of the Z-helix add some stability. Our results indicate that the complex is more rigidly held in the Z-form helix compared to B-DNA, perhaps the result of the intrinsically greater rigidity (18) of Z-DNA compared to B-DNA. This intercalative interaction ought to be considered in describing the range of interactions of small molecules and proteins with Z-DNA.

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