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

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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

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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,

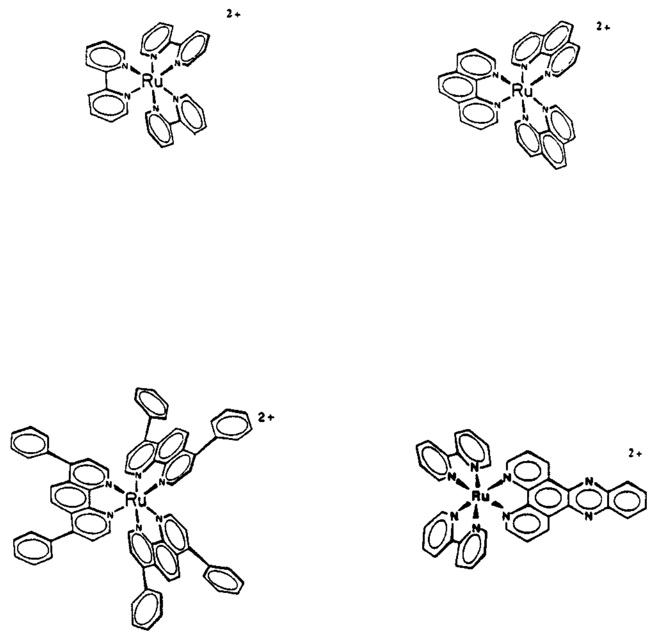

Figure 1. Spectroscopic Probes of Nucleic Acids, from top, left (clockwise): $Ru(bpy)_3^{2+}$, $Ru(phen)_3^{2+}$, $Ru(bpy)_2dppz^{2+}$, and $Ru(DIP)_3^{2+}$.

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%.

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 $3.8 \times 10^3\ M^{-1}$, lower than the k_{sv} for the free complex under similar conditions ($4.9 \times 10^3\ M^{-1}$); 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 ($k_{sv} = 8.4 \times 10^3\ M^{-1}$). The D enantiomer is more tightly bound and so more difficult to quench ($k_{sv} = 3.7 \times 10^3\ M^{-1}$). 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 $13.1 \times 10^3\ M^{-1}$, lower than the value for the free complex but higher than the the k_{sv} for the Λ-isomer, $8.4 \times 10^3\ M^{-1}$. 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 $13.4 \times 10^3\ M^{-1}$, slightly higher than the analogous $Ru(phen)_3^{2+}$ complex. The Λ-isomer exhibits a k_{sv} of $7.9 \times 10^3\ M^{-1}$.

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

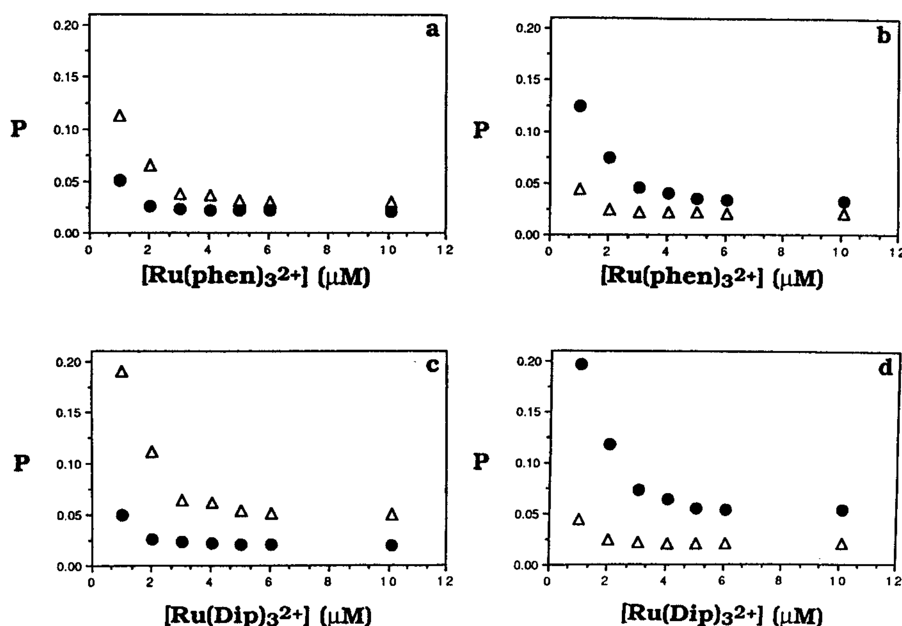

Figure 3. Steady state luminescence polarizations of Δ (△) and Λ (○) isomers of ruthenium complexes as a function of their concentration in the presence of poly d(GC)·d(GC) (100 μM) in either the B-form (**a** and **c**) or Z-form (**b** and **d**). In panels **a** and **b** polarization titrations are 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.

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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