Photophysical Evidence That Δ- and Λ-[Ru(phen)$_2$(dppz)]$^{2+}$ Intercalate DNA from the Minor Groove

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The potential of substitution-inert metal complexes as photochemical structure- and stereoselective probes of nucleic acid structure has been explored extensively over the past decade.[1,2] Interest in [Ru(bpy)$_2$(dppz)]$^{2+}$ and [Ru(phen)$_2$(dppz)]$^{2+}$ [3,4] (bpy = 2,2′-bipyridyl, phen = 1,10-phenanthroline, dppz = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine) has arisen because although their emission is quenched by water, they emit in nonaqueous solvent,[4,5] in micellar media,[5] and also when bound to nucleic acids.[6−8] Furthermore, unlike [Ru(phen)$_3$]$^{2+}$ for which the exact binding mode remains a matter of debate,[1] [Ru(bpy)$_2$(dppz)]$^{2+}$ and [Ru(phen)$_2$(dppz)]$^{2+}$ undoubtedly bind with the extended dppz ligand intercalated, as shown by linear dichroism,[8,9a] viscometry,[10] unwinding,[6] and resonance Raman[11] studies. Furthermore, the similarity of the [Ru(phen)$_2$(dppz)]$^{2+}$ binding geometry to that of actinomycin D suggested that the metal complex is also bound from the DNA minor groove.[9a]

The emission decays of Δ- or Λ-[Ru(phen)$_2$(dppz)]$^{2+}$ bound to nucleic acids[8] are biexponential, and both the lifetimes and their relative contributions vary with binding ratio and DNA sequence (decays of the racemate may also be fitted with biexponential functions[6,7] but are probably more complex). Accumulated evidence indicates that intercalation protects the excited state from a deactivating solvent protonation process, thus inducing emission. However, the physical origin of the biexponential decays remains unresolved. Barton and co-workers suggest that intercalation occurs from the major groove with two orientations of the dppz ligand in the intercalation pocket such that in one orientation the ligand is more accessible to groove solvent and has a shorter lifetime.[7,12] In contrast, since we found a higher fraction of the shorter lifetime at lower [Ru]/[Nu] ratios, we instead suggested that the two lifetimes may be a dye distribution effect in which isolated complexes are more accessible to solvent and therefore have shorter lifetimes than contiguously bound ligands.[8]

Given that two very different binding models have been proposed, we have extended our photophysical studies of Δ- and Λ-[Ru(phen)$_2$(dppz)]$^{2+}$ to elucidate their binding to DNA and polynucleotides of different conformation and composition.

Since we were unable to obtain satisfactory information from NMR about the binding of [Ru(phen)$_2$(dppz)]$^{2+}$ due to its intermediate exchange rate,[13] we have instead employed T4-DNA to investigate its binding site. T-even phages contain 5-hydroxymethylcytosine for cytosine, and T4-DNA (wild-type) is 100% glycosylated at the cytosine 5-CH$_2$-OH position[14] in the major groove. Hence, the glucose residues are expected to present a significant steric obstruction to intercalation from that groove. Hindered interaction with T4-DNA has previously been used to support major groove binding of [Ru(phen)$_3$]$^{2+}$ [15a] and [copper(2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline)$_2$]$^+$,[15b] and no effect of glycosylation used to support minor groove binding of RecA protein[15c] and the antibiotic saframycin A.[15d] Figure 1 shows emission titrations[16] of Δ- and Λ-[Ru(phen)$_2$(dppz)]$^{2+}$ with CT-DNA (42% GC) and T4-DNA (36% GC). Each enantiomer emits strongly with both nucleic acids, and the emission quantum yields for the Λ-enantiomer are lower than those for the Δ-enantiomer.[8] With the Δ-enantiomer, the saturation points are similar with the two nucleic acids, n = 2.5 bp (base pairs) with CT-DNA and n = 2 bp with T4-DNA;[17] therefore, we can eliminate the possibility that the complex binds just at non-glycosylated sites in the major groove of T4-DNA. Saturation points with the Λ-enantiomer are not so clear-cut,[8] but the curves change slope at similar [Ru]/[Nu] ratios as those of the Δ-enantiomer. These titrations strongly indicate that [Ru(phen)$_2$(dppz)]$^{2+}$ interacts with the minor rather than with the major groove, the latter location having been suggested by NMR studies with a hexamer.[18] If the non-intercalated part of the metal complex is confined in the minor groove, it is thus difficult to envisage two distinct orientations of [Ru(phen)$_2$(dppz)]$^{2+}$ in the intercalation pocket as suggested by Barton and co-workers.[7]

To investigate further, we examined the emission lifetimes of [Ru(phen)$_2$(dppz)]$^{2+}$ with a variety of polynucleotides at a single mixing ratio (Table 1).[19] Given the small binding site size of this rather bulky molecule, it was possible that the two lifetimes with CT-DNA[8] originated from intercalation in both grooves. Together with the steady-state data, the observation of two lifetimes having similar values and relative contributions with T4-DNA and CT-DNA excludes this possibility. From the observation of two lifetimes with poly(dA)·poly(dT) and poly(dG)·poly(dC), we can eliminate the possibility that the two lifetimes[8] with [poly(dA-dT)]$_2$ and [poly(dG-dC)]$_2$ are due to

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(16) Fluorescence lifetimes were measured with a SPEX Fluorolog-τ2 phase shift instrument. Selected data were also confirmed by single-photon counting with an Edinburgh Instruments FL900 instrument (λ_{ex} = 337 nm and λ_{em} = 620 nm). Synthesis of the homochiral complexes is described in ref 8. Calf thymus and T4 DNA (Sigma) and polynucleotides (Pharmacia) were used as provided. Lyophilized DNA samples were dissolved and stored (4 °C) in 5 mM phosphate (pH 6.9) buffer, except poly(dG)·poly(dC) which was dissolved at alkaline pH and dialyzed to pH 6.9. Poly(dT)·poly(dA)·poly(dT) was prepared as described elsewhere (ref 9b).
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(19) Single-exponential O$_2$-dependent decays were observed in methanol or acetonitrile. Samples were not degassed since neither lifetime was significantly O$_2$-dependent for either enantiomer with [poly(dA-dT)]$_2$.

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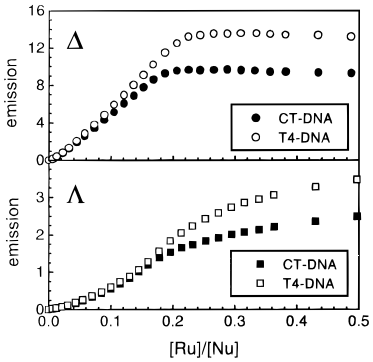

Figure 1. Emission titration of Δ- and Λ-$[Ru(phen)_2(dppz)]^{2+}$ with T4-DNA and CT-DNA. The complex was added to DNA (7.5 μM) in 5 mM phosphate (pH 6.9) buffer and equilibrated for 10 min (λ_{ex} = 480 nm, λ_{em} = 618 nm, 30 °C).

Table 1. Emission Lifetimes for Δ- and Λ-$[Ru(phen)_2(dppz)]^{2+}$ [a]

	Δ				Λ			
nucleic acid[b]	τ_1 (ns)[c]	α_1[d]	τ_2 (ns)[c]	α_2[d]	τ_1 (ns)[c]	α_1[d]	τ_2 (ns)[c]	α_2[d]
CT-DNA[e]	794	26	134	74	262	6	37	94
T4-DNA[e]	856	23	123	77	244	7	36	93
$[poly(dG\text{-}dC)]_2$	248	78	67	22	165	4	41	96
$[poly(dA\text{-}dT)]_2$	737	26	135	74	327	10	36	90
$[poly(dI\text{-}dC)]_2$	912	25	160	75	542	12	35	88
poly(dG)·poly(dC)	156	39	54	61	367	42	74	58
poly(dA)·poly(dT)	796	59	168	41	181	10	43	90
poly(rA)·poly(rU)	627	49	75	51	169	27	36	73
poly(dT*dA·dT)	655	92	324	8	443	73	225	27

[a] Phosphate (pH 6.9) buffer (5 mM); 25 °C, λ_{ex} = 440 nm; λ_{em} > 540 nm. [b] Unless otherwise stated [Nu] = 1000 μM; [Ru] = 20 μM (*i.e.*, 1 Ru/25 base pairs). [c] Lifetime uncertainties are ±10% for τ_1 and ±15% for τ_2. [d] Normalized pre-exponential factors, errors are ±10%. [e] [Nu] = 650 μM; [Ru] = 13 μM (*i.e.*, 1 Ru/25 base pairs). [f] [Nu] = 1500 μM; [Ru] = 20 μM (*i.e.*, 1 Ru/25 base triplets).

intercalation at alternate base pair steps. In general, the two lifetimes do not appear to be explicitly related to base content although there is a tendency for longer lifetimes with AT-containing compared to GC-containing polynucleotides. However, the lifetimes lengthened when the exocyclic amino group of guanine was removed to give $[poly(dI\text{-}dC)]_2$. This group protrudes into the minor groove and blocks GC minor groove binding of drugs such as DAPI and Hoechst.[20] Since the $[poly(dI\text{-}dC)]_2$ minor groove resembles that of $[poly(dA\text{-}dT)]_2$, the observation that the lifetimes with the two polynucleotides are rather similar further supports our contention that the metal complexes intercalate from the minor groove.

However, DNA structure, as well as sequence, may also influence the lifetimes.[7] A notable result is with poly(dG)·poly(dC) which adopts an unusual, ostensibly A-form,[21] conformation. The shortest lifetimes for the Δ-enantiomer are found with this polynucleotide while the lifetimes for the Λ-enantiomer are rather long. This suggests that a wider minor groove may allow more facile intercalation of the Λ-complex with increased protection while making the Δ-complex more accessible to solvent. Also both enantiomers emit when bound to a duplex A-form RNA, *viz*. poly(rA)·poly(rU), although with this polynucleotide the Λ-enantiomer emits less than the Δ-enantiomer, as seen also with the B-form polynucleotides. Nevertheless, the observation of intercalative binding to this polynucleotide and to RNA[7b] also supports minor groove binding of $[Ru(phen)_2(dppz)]^{2+}$. Finally, with triplex poly(dT*dA·dT) the short lifetimes in particular are greatly increased compared to other polynucleotides, presumably due to better protection afforded by overlap with base triplets than base pairs.[7b,9]

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Table 2. Emission Lifetimes for Δ- and Λ-$[Ru(phen)_2(dppz)]^{2+}$ with $[poly(dA\text{-}dT)]_2$[a]

[Ru]/[Nu][b]	[Nu]/[Ru]	Δ				Λ			
		τ_1 (ns)[c]	α_1[d]	τ_2 (ns)[c]	α_2[d]	τ_1 (ns)[c]	α_1[d]	τ_2 (ns)[c]	α_2[d]
0.50	2.0	464	57	68	43	243	47	53	53
0.25	4.0	700	67	143	33	298	57	54	43
0.12	8.0	791	62	144	38	331	35	42	65
0.08	12.5	756	56	129	44	352	25	44	75
0.04	25.0	739	42	132	58	325	18	38	82
0.02	50.0	737	26	135	74	327	10	36	90
0.01	100.0	727	18	137	82	329	9	36	91

[a] Phosphate (pH 6.9) buffer (5 mM); 25 °C; λ_{ex} = 440 nm; λ_{em} > 540 nm. [b] [Ru] = 20 μM. [c] Lifetime uncertainties are ±10% for τ_1 and ±15% for τ_2. [d] Normalized pre-exponential factors, uncertainties are ±10%.

We have previously suggested on the basis of emission data with CT-DNA[8] that the two lifetimes may arise from changes of dye distribution with binding ratio. For either enantiomer with CT-DNA, both the lifetimes and their contributions were found to change significantly during the course of a titration of $[Ru(phen)_2(dppz)]^{2+}$ into DNA.[8] In Table 2, we present similar data for Δ- and Λ-$[Ru(phen)_2(dppz)]^{2+}$ with $[poly(dA\text{-}dT)]_2$. In this case, while the contributions of the two lifetimes vary with binding ratio, their magnitudes do not change greatly. When intercalation sites are saturated, another binding mode may occur, as evidenced by absorption and linear dichroism experiments,[8] and indeed the lifetimes at high [Ru]/[Nu] (≥0.25) were lower than those observed when the complex was solely intercalated. The observation that the contribution of the long lifetime increases with increasing [Ru]/[Nu] ratio is consistent with a model in which the two lifetimes originate from dye distribution effects,[8] since complexes bound contiguously should be better protected from solvent (and solvent-localized quenchers) than those in isolated sites. Alternatively, the effect may be mediated by DNA structure rather than involving dye−dye contacts. If binding of a $[Ru(phen)_2(dppz)]^{2+}$ complex perturbs DNA, then subsequently added molecules may bind to altered DNA conformations and exhibit different emission properties, such as a longer lifetime if the altered structure provides better protection from solvent. The possibility that added $[Ru(phen)_2(dppz)]^{2+}$ binds by aggregating with intercalated dye seems unlikely at [Ru]/[Nu] ratios that are significantly below saturation, since using linear dichroism (LD), we observe only one binding geometry with DNA.[8] However, for [Ru]/[Nu] > 0.2, it is likely that the second binding mode observed by LD and absorption spectroscopies is of this type, and it can be seen from Table 2 that the photophysical properties also change above the saturation point. The fact that we never observe a single lifetime suggests that there may be an element of cooperativity to the binding, but the outstanding question is then why such a high proportion of the short lifetime remains even at saturation. We cannot at this point provide a detailed mechanism for this effect, and studies under varying conditions with different polynucleotides are currently in progress to investigate further the origin of two lifetimes.

In conclusion, our photophysical data indicate (a) that $[Ru(phen)_2(dppz)]^{2+}$ probably intercalates from the minor groove, (b) that biexponential emission decays accompany binding of either enantiomer to any nucleic acid sequence, and (c) that there is a binding ratio dependence of the relative amplitudes of the two lifetimes with $[poly(dA\text{-}dT)]_2$ which is consistent with the two lifetimes being a dye distribution effect. This evidence demonstrates that the interaction of $[Ru(phen)_2(dppz)]^{2+}$ with nucleic acids is more complicated than binding of a simple intercalator.

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