of the electron in the upper Hubbard subband. Symmetry analysis of the magnetic structure reveals the possible importance of coupling between the Nd and Cu sublattices, which is reflected in the spin direction of Nd^{3+}, the high Néel temperature (2 K), and a polarization-induced moment above T_N. A simple isotropic exchange model for the magnetic structure at high temperatures with explicit consideration of the coupling between the neodymium and copper sublattices constrains the relative values of the various exchange constants and further amplifies the importance of the interaction between the Nd and Cu sites along the c axis in coupling the two sublattices at the experimentally observed wavevector. This simple model also demonstrates that isotropic exchange interactions are not responsible for the observed spin reorientation transitions at high temperatures.

Acknowledgment. We thank the SERC for financial support of this work and the Institut Laue Langevin and the Rutherford Appleton Laboratory for provision of neutron time. We thank J. K. Cockcroft, A. W. Hewat, and J.-L. Soubeyroux for experimental assistance at ILL, R. M. Ibberson at RAL, and J. M. F. Gunn (RAL), D. W. Murphy, and A. C. W. P. James (AT&T Bell Laboratories) for useful discussions. M.J.R. is a Junior Research Fellow at Merton College, Oxford, England.

Supplementary Material Available: Data for the TGA determination of oxygen concentration in $Nd_{2-x}Ce_xCuO_{4-\delta}$ (Table I), final parameters derived from the Rietveld refinement of $Nd_{1.85}Ce_{0.15}CuO_{4-\delta}$ at 4.2 K (Table II), and exchange interactions involving the Cu(0,0,0) and the Nd(0,0,z) and Nd(0,0,$\bar{z}$) sublattices (Table V) (3 pages). Ordering information is given on any current masthead page.

Contribution from the Department of Chemistry and Biochemistry, Queens College-CUNY, Flushing, New York 11367

Effects of Ligand Planarity and Peripheral Charge on Intercalative Binding of Ru(2,2′-bipyridine)$_2$L^{2+} to Calf Thymus DNA

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Received November 2, 1990

The binding of mixed-ligand complexes of the type Ru(bpy)$_2$L^{2+} (bpy = 2,2′-bipyridine and L = aromatic diimine) to calf thymus DNA has been investigated by absorption, emission, and circular dichroism spectroscopy and equilibrium dialysis binding studies. Resolved spectral features of the ligand L simplify interpretation of the spectra regarding intercalation. When L is 4′,7′-phenanthrolino-5′,6′:2,3-pyrazine (ppz), evidence of intercalative binding is provided by hypochromicity in the visible MLCT band associated with the ppz ligand as well as greatly increased emission. CD spectra of dialyzates indicate enantioselectivity associated with binding. Binding constants are comparable to those measured for Ru(phen)$_3^{2+}$ under similar conditions. For the structurally related ligand 2,3-di-2-pyridylpyrazine (dpp), which is incapable of assuming a completely planar conformation, no evidence of intercalative binding is observed. Overall binding constants for the dpp complex are too low to measure. When a metal site (-PtX_2) is incorporated into the complexed ppz, intercalative binding is still evident with no significant change in binding constant, but an increase in the site size (l parameter of the McGhee and von Hippel equation) was required to fit the data. We interpret this as due to increased disruption of the double-stranded DNA structure upon intercalation of the ppz ligand with the coordinated platinum. For a series of complexes when L is quaterpyridyl (qpy), or its mono- or dimethyl quaternary form, all complexes show evidence of binding strongly to DNA. The binding constants show the following ordering: Me_2qpy^{2+} > qpy ~ $Meqpy^+$. Circular dichrosim spectra of DNA dialyzates indicate that only the Me_2qpy^{2+} complex binds enantioselectively. Hypochromicity is observed for the visible MLCT bands of only the qpy complex, and a hyperchromic effect, accompanied by a red shift, is observed for the Me_2qpy^{2+} complex. This complex is also the only member of the series to show increased fluorescence (also accompanied by a red shift) in the presence of DNA. All of the data suggest a distinctly unique mode of binding for the Me_2qpy^{2+} complex, possibly induced by electrostatic interaction of the charged quaternary methyl groups with a pair of DNA phosphates on either chain of the double-stranded structure.

Introduction

A number of recent studies,[1-14,23,24,28,29] in particular those by Barton et al.,[1-10,13,28,29] have provided evidence for specific modes of binding of selected tris chelates of transition metals with nucleic acids. One of the most intriguing cases is provided by tris chelates

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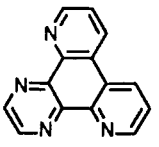
of ruthenium(II) with heterocyclic aromatic fused-ring systems such as 1,10-phenanthroline (phen), which have been shown[1,4,5,8,10,14] to bind enantioselectively to DNA. Enantioselective binding is accomplished via an intercalative interaction of one ligand with base pairs within the major groove of B-form DNA. This mode of binding favors the fit of the Δ isomer due to more favorable steric interactions with atoms forming the surface of the major groove. Hypochromicity of the visible-region metal-to-ligand charge-transfer (MLCT) absorption bands associated with the phen (or other ligands), circular dichroism spectra of DNA dialyzates, increases in fluorescence intensities (and quantum yields for fluorescence) of LMCT transitions, and fluorescence polarization anisotropies have all been presented as evidence of this unique mode of binding to DNA. The Λ isomer also shows enantioselectivity, but binds mainly in the minor groove. Exclusive binding of the Δ isomer of the complex $Ru(DIP)_3^{2+}$ (DIP = 4,7-diphenylphenanthroline) to B-form DNA by an intercalative mode[8,13] provides evidence that the optimum choice of ligand can maximize an enantioselective effect. A recent report, employing linear dichroism and circular dichroism studies, has challenged[12] the conclusions of Barton et al. regarding the favored intercalative binding of the Δ isomer with the major groove of DNA. A model is proposed in which both isomers bind via intercalation within the major groove.

We have been interested[14-19] in the synthesis and study of mixed-ligand complexes of ruthenium(II) with aromatic diimine ligands, primarily with the aim of forming dinuclear complexes with unusual excited-state redox properties. The complexes $Ru(bpy)_2L^{2+}$ where L = 4′,7′-phenanthrolino-5′,6′:2:3-pyrazine (ppz) (**1**) or 2,3-di-2-pyridylpyrazine (ddp) (**2**) and bpy = 2,2′-



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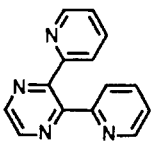

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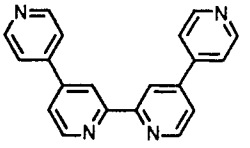
bipyridine have been reported and characterized.[15-18] These form mononuclear and dinuclear complexes where L is either completely planar (**1**) or sterically hindered from planarity (**2**). Except for this feature, the ligands are essentially identical in size and shape. By comparison of the differences in binding to DNA for mixed-ligand complexes of the composition described above, this pair of complexed ligands provides a probe of the influence of ligand conformation on binding.

Another series of complexes of the same general formula is based on[19,20] the ligand quaterpyridyl (qpy) (**3**) and its mono-



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methylated ($Meqpy^+$) (**4**) and dimethylated (Me_2qpy^{2+}) (**5**) forms. These ligands were designed to provide complexes in which a methylviologen-like ligand is coordinated to a photoactivatable metal center. In the studies reported here, the variable charge on the periphery of the coordinated ligand provides a probe of the effect of peripheral charge on binding to DNA of the mixed-ligand complexes containing these ligands.

In addition to the above-mentioned features, each of the mixed ligand complexes with ligands **1–5** shows a spectrally resolved, red-shifted, MLCT transition (or shoulder) involving the unique ligand. Because 2,2′-bipyridine (bpy) has been previously determined[2,24,25] to be at best only minimally efficient at inducing intercalative binding with DNA, this feature allows the study of the effects of binding to DNA as influenced by the specific ligand, which is the only one for each complex presumed to be capable of inducing intercalative binding within the major groove of DNA.

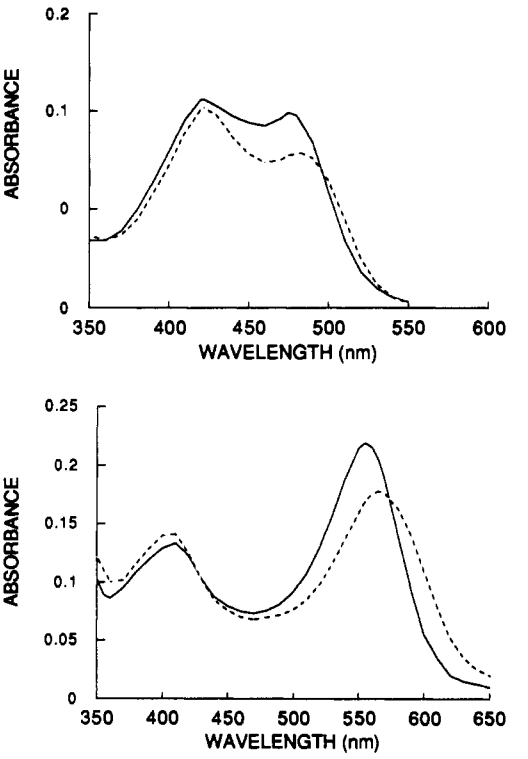

Figure 1. Visible absorption spectra: (top) $Ru(bpy)_2ppz^{2+}$ (9.6 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] (DNA phosphate to ruthenium ratio) = 62; (bottom) $Ru(bpy)_2ppz\cdot PtX_2^{2+}$ (14 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] = 69. All solutions in 5 mM Tris, pH 7.4, 50 mM NaCl.

Experimental Section

Materials. The mixed-ligand complexes of formula $Ru(bpy)_2L^{2+}$, where L = **1–5** were synthesized and purified as described elsewhere.[20] The complex $Ru(bpy)_2(ppz)PtCl_2^{2+}$ was prepared as described elsewhere.[21] Because the chloride ligands are subject to hydrolysis[22] in aqueous solution within the time scales employed here (e.g. equilibrium dialysis experiments), we characterize the platinum center as PtX_2 where X is likely hydroxide. Calf thymus DNA (Sigma Chemical Co.) was dissolved in buffer, ethanol precipitated, redissolved in appropriate buffers as required, and centrifuged to clarify.

Methods. Instrumentation. UV–visible spectra were run on a Perkin-Elmer 320 spectrometer. Fluorescence spectra were run on a Hitachi/Perkin-Elmer MPF-2A spectrofluorimeter. Results were confirmed by using a laser Raman spectrometer previously described,[15-18] employing an RCA C-31034A photomultiplier tube, for spectra with red-shifted peaks (700–800-nm region). Circular dichroism spectra were run an a Jasco 500C CD/ORD instrument. Spectra were manually digitized to facilitate computer graphic display. For CD spectra, the weakest CD signal maximum showed a signal to noise ratio of 5.5/1.

Equilibrium Dialysis. Equilibrium dialysis experiments were run according to the protocols of Barton et al.[2,7,8] in 5 mM Tris, pH 7.4, in the presence of 50 mM NaCl to facilitate comparison of results with previously published values. A 1.0-mL aliquot of calf thymus DNA (1 mg/mL), in the above-described buffer, was sealed in dialysis tubing and dialyzed for 48–72 h at 25 °C versus 2.0 mL of ruthenium complex ion solution. Concentrations were determined by visible spectroscopy. Data were fit to the McGhee and von Hippel equation,[23] which models non-cooperative binding to the helix, in the form adopted by Barton et al.,[2,7,8] by using a microcomputer-based nonlinear least-squares procedure.

Results

Effects of Binding to DNA on Visible MLCT Transitions. Visible spectra for each of the complexes studied, in buffer alone and in the presence of calf thymus DNA, are presented in Figures 1 and 2. All visible spectra are for solutions in 5 mM Tris buffer, pH 7.4, with 50 mM NaCl. For the complex $Ru(bpy)_2(\mathbf{1})^{2+}$, the MLCT band at 475 nm shifts to a final value of 485 nm as the DNA phosphate to ruthenium ratio ([P]/[Ru]) increases. At the same time, this transition shows a decrease in intensity with a maximal value of 20%. This band has been assigned,[15,17] on the

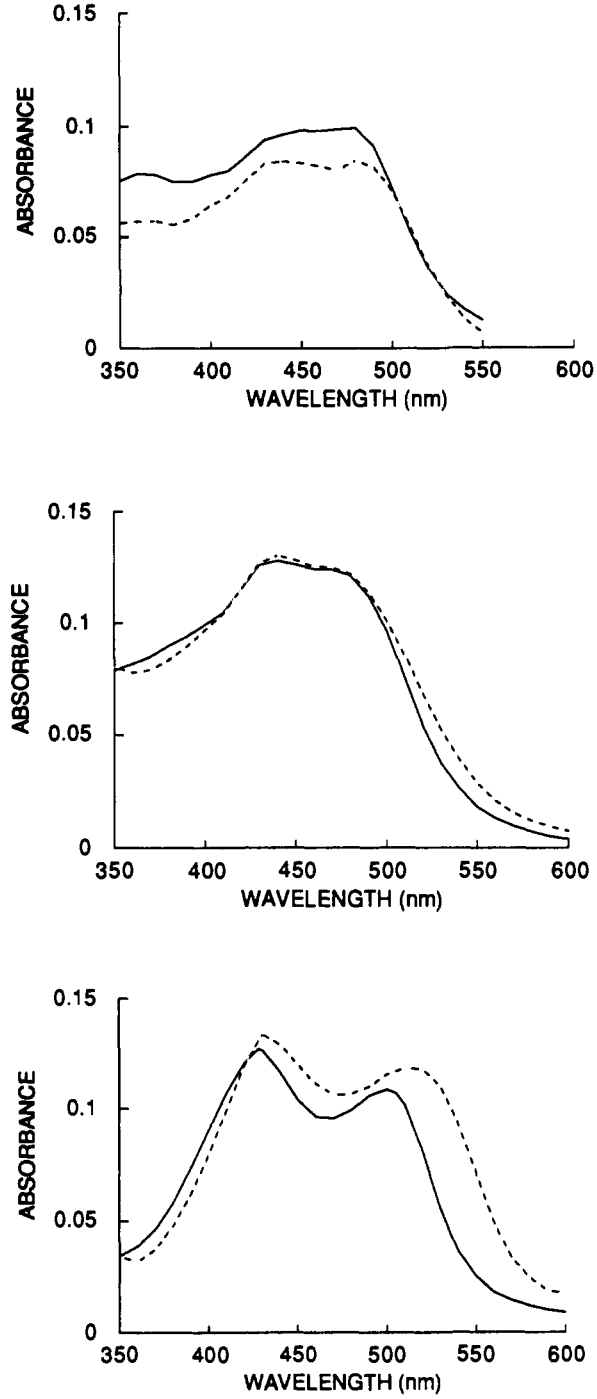

Figure 2. Visible absorption spectra: (top) $Ru(bpy)_2qpy^{2+}$ (4.1 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] (DNA phosphate to ruthenium ratio) = 67; (middle) $Ru(bpy)_2Meqpy^{3+}$ (8.5 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] = 99; (bottom) $Ru(bpy)_2Me_2qpy^{4+}$ (8.2 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] = 73. All solutions in 5 mM Tris, pH 7.4, 50 mM NaCl.

basis of resonance Raman studies, as a charge transfer terminating on the ppz ligand. The MLCT band at 422 nm, which is assigned[15,17] to a transition terminating on bpy, shows no wavelength shift and only modest intensity changes as the [P]/[Ru] ratio increases. Visible spectra of the complex $Ru(bpy)_2(2)^{2+}$ show no change in intensity or wavelength with increasing [P]/[Ru] ratio over the same range of values (spectra not shown).

For the complex in which PtX_2 has been added to the periphery of the ppz ligand, the spectra in Figure 1 are notable for the red shift of the MLCT transition to the ppz ligand (shifted to 556 nm from 475 nm in the ppz complex). This shift is similar in magnitude to that observed[14-18] for binuclear complexes in which a $Ru(bpy)_2^{2+}$ has been added, or in which the ppz is protonated. In the presence of DNA, this band shows a further red shift to 570 nm and a maximum decrease in intensity of 20%. Again, the transition at 408 nm, associated with bpy, shows little change.

Table I. Fluorescence Data[a]

L	λ_{max}, nm without DNA	λ_{max}, nm with DNA	$\Delta\lambda$, nm	I/I_0[b]
ppz	700	662	−28	9.2
dpp	685	685	0	1
ppz·PtX_2	no emission observed			
qpy	665	665	0	1
$Meqpy^+$	655	655	0	1
Me_2qpy^{2+}	723	738	+15	1.8

[a] All wavelengths and intensities are for air-saturated solutions and are uncorrected. [P]/[Ru] ratios in all cases were 60× or greater. See Figure 4 for details. [b] Peak heights at maximum used.

For the complex $Ru(bpy)_2(3)^{2+}$, the spectra in Figure 2 show a larger decrease in intensity near 475 nm in the presence of DNA, with a lesser decrease near 430 nm. In this case, the MLCT transitions to bpy and qpy are not completely resolved, but resonance Raman studies indicate[20] that the transition to qpy is red-shifted compared to the bpy-centered transition. For $Ru(bpy)_2(4)^{2+}$, the shoulder at 475 nm undergoes no intensity decrease (<3%) in the presence of DNA, and no wavelength shift is evident. The complex $Ru(bpy)_2(5)^{2+}$ presents a unique result for the series. The MLCT transition to $qpyMe_2^{2+}$ at 500 nm undergoes a red shift to 520 nm in the presence of DNA, and in addition shows a slight *increase* in intensity (~5%). To our knowledge, this is the first recorded hyperchromic effect for such a complex in the presence of DNA. For the series of complexes based on the qpy (or methylated qpy) ligand, the MLCT band (or region) associated with the qpy ligand alone shows significant changes. The region associated with bpy shows much smaller changes.

Effects of Binding to DNA on Fluoresence Spectra. The fluorescence spectra for the complexes with ligands **1**, **3**, **4**, and **5**, with and without DNA, are shown in Figure 3. All spectra were run at [P]/[Ru] ratios >60. Table I summarizes the maximum wavelengths for emission and I/I_0 values (for [P]/[Ru] ratios >60) for the complexes studied. Only the ppz (**1**) and Me_2qpy^{2+} (**5**) complexes show emission maximum wavelength shifts upon binding to DNA. The ppz complex shows a blue shift of 28 nm (595 cm^{-1}), while the $Meqpy^{2+}$ complex shows a red shift of 15 nm (281 cm^{-1}). Emission intensities are essentially unchanged for the complexes with dpp (**2**), qpy (**3**), and $Meqpy^+$ (**4**) upon binding to DNA. The complex with ppz shows the largest increase among the complexes in this study, and the Me_2qpy^{2+} complex once again shows results unique to the qpy series, with a modest increase in intensity. The complex in which platinum(II) has been added to the coordinated ppz shows no luminescence, either alone in solution or in the presence of DNA.

Equilibrium Dialysis Binding Studies. Equilibrium dialysis experiments provide parameters K_b, the binding constant, and l, the average binding site size, from a fit to the McGhee and von Hippel equation as adapted by Barton et al.[2,7,8]

$$r/C_f = (K_b/2)(1 - 2lr)[(1 - 2lr)/(1 - 2(l - 1)r)]^{l-1}$$

where r is the fraction of DNA phosphate "sites" occupied and C_f is the free solution concentration of ruthenium complex. Comparison of typical best-fit curves and the associated experimental data for the complexes studied are shown in Figure 4. Table II summarizes the average K_b and l values obtained by at least two sets of data for each complex.

The ppz complex gives a binding constant of $(5.5 \pm 0.5) \times 10^3$ (l = 3–4). While the addition of a platinum site (PtX_2) to the ppz periphery does not diminish the binding constant ($(6.5 \pm 0.5) \times 10^3$), this value is obtained only by fitting with a greatly increased parameter for the binding site size, l = 16–18.

For the series of complexes with the ligands based on qpy, the highest K_b is found for $qpyMe_2^{2+}$ ($(2.8 \pm 0.6) \times 10^4$), with qpy

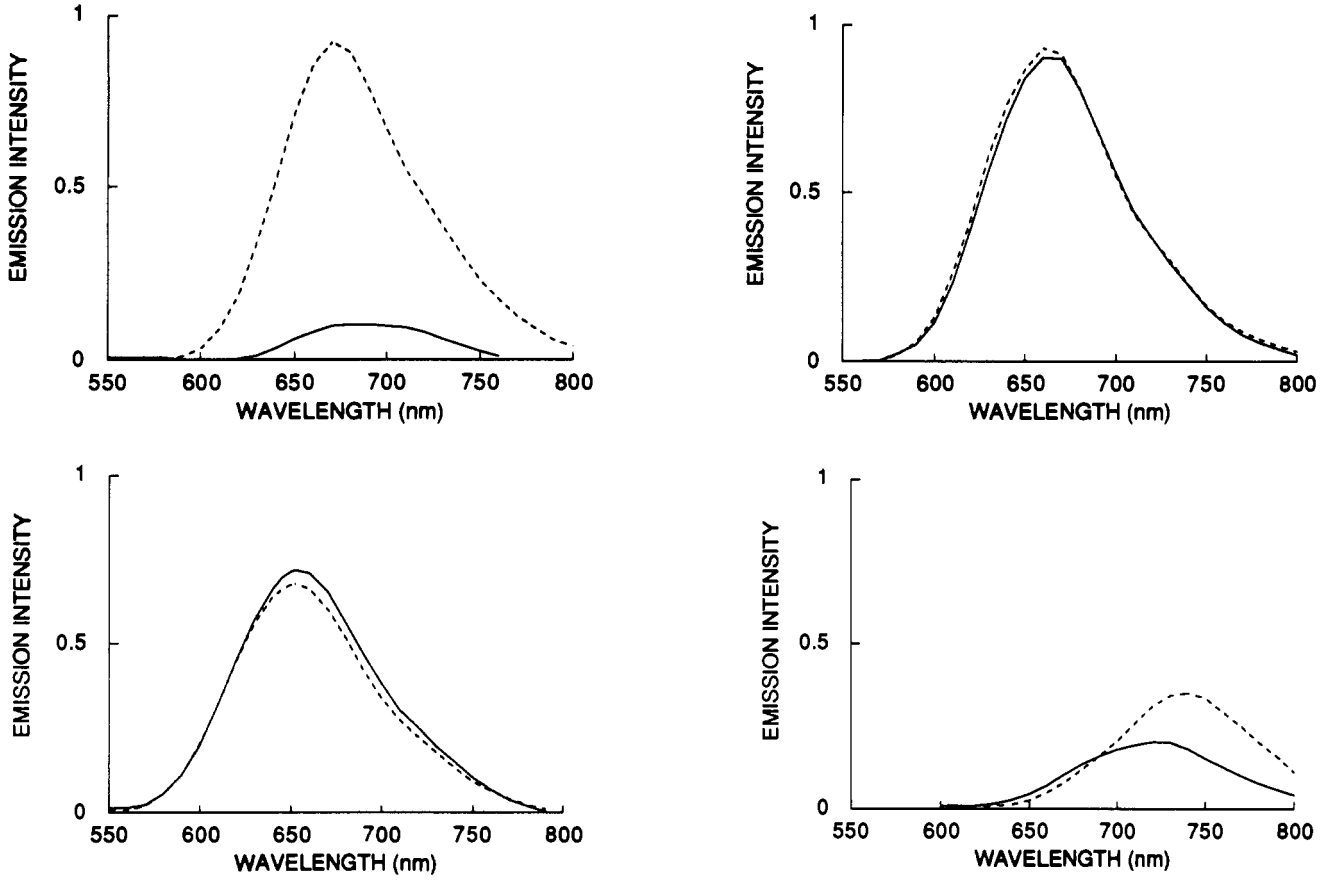

Figure 3. Emission spectra: (top left) $Ru(bpy)_2ppz^{2+}$ (9.6 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] (DNA phosphate to ruthenium ratio) = 62; (top right) $Ru(bpy)_2qpy^{2+}$ (4.1 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] (DNA phosphate to ruthenium ratio) = 67; (bottom left) $Ru(bpy)_2Meqpy^{3+}$ (8.5 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] = 99; (bottom right) $Ru(bpy)_2Me_2qpy^{4+}$ (8.2 μM) alone (—) and in the presence of calf thymus DNA (--), [P]/[Ru] = 73. All solutions in 5 mM Tris, pH 7.4, 50 mM NaCl.

Table II. DNA Binding Parameters[a]

L	K_b	l
ppz	$(5.5 \pm 0.5) \times 10^3$	3–4
ppz·PtX_2	$(6.5 \pm 0.5) \times 10^3$	16–18
qpy	$(1.3 \bullet 0.2) \times 10^4$	2–3
$Meqpy^+$	$(1.4 \pm 0.3) \times 10^4$	2
Me_2qpy^{2+}	$(2.8 \pm 0.6) \times 10^4$	3

[a] Fit to McGhee and von Hippel equation. See Figure 4 and text for details.

($(1.3 \pm 0.2) \times 10^4$) and $qpyMe^+$ ($(1.4 \pm 0.3) \times 10^4$) only about a factor of 2 lower. The binding site size for this series is 2–3.

Enantioselectivity of Binding to DNA. Dialyzates from experiments in which racemic solutions of the complexes studied were dialyzed against 1.6 mM calf thymus DNA for 48 h were subjected to circular dichroism analysis. The presence of a CD signal indicates enrichement of the dialyzate in the isomer that binds less strongly to the DNA, as described in previous studies.[1-10,23,24,27] In Figure 5, the CD spectra of several complexes are displayed in the ultaviolet region, 220–320 nm. Although CD signals are expected for the visible MLCT bands, the ellipticities are more than an order of magnitude smaller.

In Figure 5 (top, solid curve) the dialyzate for the ppz (**1**) complex shows a strong CD signal with a positive peak at 273 nm and a stronger negative peak at 289 nm. This is contrasted with the weaker, but nevertheless clearcut, CD signal for the $qpyMe_2^{2+}$ (**5**) complex dialyzate (dashed curve), which shows a positive peak at 290 nm. This same trace is repeated (now a solid curve) in the bottom of Figure 5 on an expanded scale, and compared to CD curves for the qpy (**3**) and $qpyMe^+$ (**4**) complex dialyzates, which show no discernible signals. Not shown in Figure 5 is the CD spectrum for the dialyzate of the ppz·PtX_2 complex, which resembles that of the ppz complex, with a positive peak at 271 nm and a negative peak of greater magnitude at 287 nm. Also, CD spectra of the dialyzates from the dpp (**2**) complex show no signals.

The CD results indicate that the ppz (**1**) complex and its PtX_2 adduct as well as the $qpyMe_2^{2+}$ (**5**) complex interact enantioselectively with calf thymus DNA.

Discussion

Effect of Ligand Planarity on Intercalative Binding to DNA. Our equilibrium dialysis result show that the complex $Ru(bpy)_2(1)^{2+}$ binds to DNA with a binding constant comparable[2,9] to that of $Ru(phen)_3^{2+}$ under very similar conditions. Furthermore, the combination of fluorescence, circular dichroism, and visible absorption data provides evidence that the major interaction with DNA is likely intercalative in nature and occurs within the major groove of B-form DNA, as has been demonstrated[1-10,24,25] for $Ru(phen)_3^{2+}$. The hypochromicity observed specifically for the MLCT transition, which has been assigned as terminating on the ppz ligand, indicates that this ligand is reasonably assumed to intercalate between base pairs accessible via the major groove. This interaction would then have the effect of pulling the remaining structure, including the two additional bipyridine ligands coordinated to the ruthenium, into the major groove to interact via van der Waals contacts with the atoms of DNA lining the groove. It is noteworthy that this effect is capable of enantioselectivity, as evidenced by the circular dichroism signal of the dialyzate, despite the presence of only two bipyridines as ancillary ligands. It may be recalled that the complex $Ru(bpy)_3^{2+}$ shows little or no enantioselectivity[2] in its binding to DNA.

When PtX_2 is coordinated to the periphery of the ppz ligand, most aspects of the binding are not seriously affected. Hypochromicity is significant for the MLCT transition upon binding

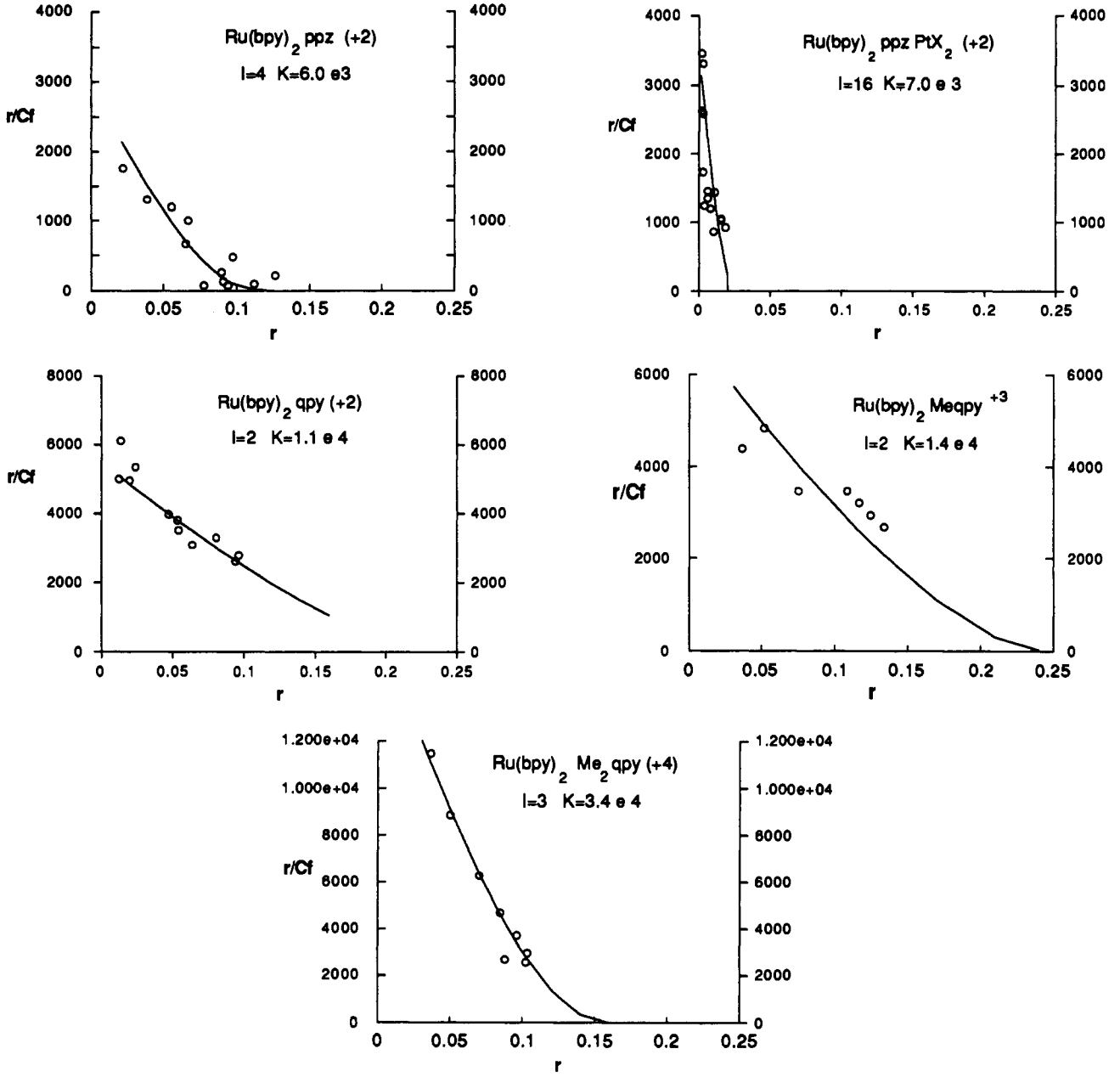

Figure 4. Representative experimental data (O) and best fit (—) to the McGhee and von Hippel equation (see text) for equilibrium dialysis experiments. Values of binding constant (K_b) and site size (l) used to obtain the solid line are included in the figure along with the complex formula.

to DNA, and the binding is still enantioselective. Though the binding constant remains comparable to that of the ppz complex, a reasonable fit to the McGhee and von Hippel equation requires an average site size of 16–18 base pairs, as compared to two to four base pairs for the other complexes studied here. Because of the similarity of the platinum-substituted complex to the unsubstituted one in most other respects, we propose that this drastic increase in average site size is indicative of more significant disruption of the DNA structure as complex binds. The raw data alone show that the fraction of total sites which are occupied by the platinum-containing complex is drastically reduced, as indicated by the intercept allowed by these data with the r axis. This effect may be due to the larger size of the coordinated platinum, which must be accomodated between the base pairs of DNA to effect intercalation. It is interesting to note that one of the earliest examples of intercalation of a transition-metal-containing complex was a terpyridyl complex of platinum(II). The crystal structure[26] of this complex with a dinucleotide shows that the platinum penetrates the region between the base pairs involved in the intercalative binding.

A most interesting result from out study is the lack of evidence for intercalative binding of the dpp (2) complex to DNA. The overall size and shape of the two complexes differs only in the lack of planarity of the dpp ligand. The ppz ligand is of course completely planar. For coordinated dpp, the pyrazine and one of the pyridyl substituents would be closest to coplanar, with the remaining pyridyl substituent rotated out of plane due primarily to the interaction between the 3- and 3′-hydrogens. The crystal structure for a binuclear complex of dpp has been reported[27] to show the pyridyl–pyrazine twist angle is 19.5° when both diimine sites are coordinated to a metal center. It would appear from these comparative results that the ability of such complexes to bind intercalatively is very sensitively influenced by the inability of the intercalating ligand to assume a completely planar conformation.

For the case of the mixed-ligand complex with qpy(3), where the absorption evidence suggests an intercalative mode of binding, the steric constraint to coplanarity of the pyridyl substituents and the coordinated bipyridyl is much less severe.

The above observations can be contrasted to the results for $Ru(DIP)_3^{2+}$, where the interpretation[8,13] has called for an intercalative mode of binding (involving opening up of the DNA structure), despite the reported noncoplanarity of the phenyl substituents with the phenanthroline system due to hydrogens that interact in a fashion very similar to those at the 3′- and 3″-positions

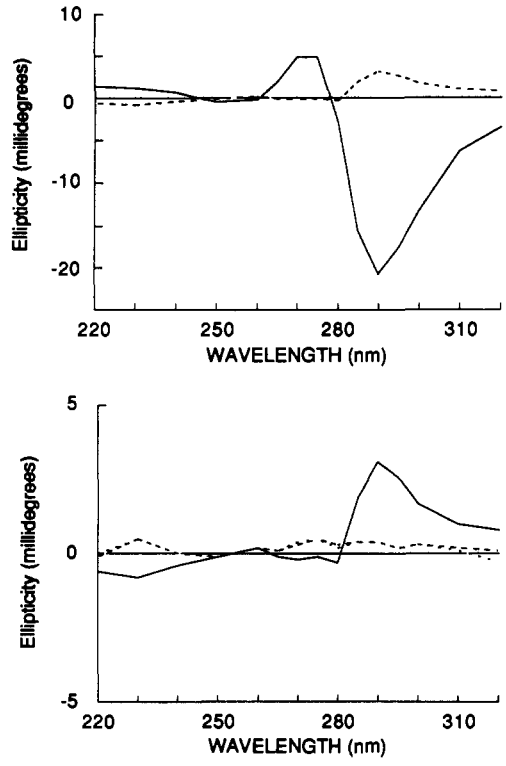

Figure 5. Circular dichroism spectra of 41-h DNA dialyzates versus calf thymus DNA (1.6 mM DNA phosphate): (top) $Ru(bpy)_2ppz^{2+}$ (16 μM) (—) and $Ru(bpy)_2Me_2qpy^{4+}$ (13 μM) (--); (bottom) $Ru(bpy)_2Me_2qpy^{4+}$ (13 μM) (—), $Ru(bpy)_2Meqpy^{3+}$ (11 μM) (--), and $Ru(bpy)_2qpy^{2+}$ (7.1 μM) (···) All solutions in 5 mM Tris, pH 7.4, 50 mM NaCl.

of the dpp ligand. For the DIP ligand, however, the interaction between analogous hydrogens are somewhat less direct and therefore probably less severe. The phenyl rings may be able to approach more closely to coplanarity with the phen system in this case than in the case of ppz. This difference may be enough to allow for the intercalation of the DIP, but not the dpp ligand.

Effect of Ligand Peripheral Charge on Binding to DNA. As evidenced by the absorption, circular dichroism and equilibrium dialysis results, there is an apparent discontinuity in the series of complexes with ligands based on qpy (**3**). While the complex containing qpy shows some hypochromicity associated with the qpy ligand near 475 nm, the $Meqpy^+$ complex shows none. The visible spectrum of the Me_2qpy^{2+} complex shows a red shift for the MLCT transition assigned as terminating on this ligand, and, in contrast to other complexes studied to date, a small *hyper*-chromic effect. This intensification of the MLCT transition is not consistent with intercalation of the Me_2qpy^{2+} ligand, and indeed, suggests some other specific orientation with respect to the aromatic base pairs of DNA.

Likewise, the fluorescence maximum and intensities for the qpy and $Meqpy^+$ complexes are unaffected by binding to DNA, while the Me_2pqy^{2+} complex shows a red shift and intensity increase.

Finally, enanthioselectivity is observed only for the Me_2qpy^{2+} complex, but not for the other two members of the series. The binding constant is about the same for the complexes with qpy or $Mepqy^+$, but increases by about a factor of 2 for the Me_2qpy^{2+} complex. All three are, however, quite large and indicate strong binding to DNA.

The only difference in the members of the series is the increase in positive charge at the periphery of the ligand, as one, then two pyridyl substituents are methylated. Aqueous solubility increases with each methyl substituent added, as the overall charge on the complex ion rises from +2 to +4. This demonstrates that the hydrophilicity of the complexes, specifically the region associated with the qpy ligand, increases along the series with increasing charge. As a result, one might anticipate[2] binding based primarily on an intercalative interaction within the hydrophobic major groove to decrease along the series. The qpy and $Meqpy^+$ complexes show comparable binding constants, as well as modest (or no) hypochromism for the MLCT region associated with these ligands. Space-filling models suggest that, because of the size of these ligands, they would not be accommodated symmetrically within the major groove of DNA. Rather, one of the pyridyl substituents could intercalate, leaving the other facing out of the groove and exposed to solvent. This model would allow that the qpy and $Meqpy^+$ complexes interact in a somewhat similar way, with a pyridyl group partially intercalated, and the other pyridyl, or the methylated pyridyl on $Meqpy^+$, then free to remain in the more hydrophilic environment outside the major groove. In the case of $Meqpy^+$, the positively charged quaternary methyl could also associate with a DNA phosphate. This exposure to solvent could explain the lack of change in fluorescence. For these complexes, the emission arises from a π^* orbital on the L ligand. Because the overall binding constant in fact increases for the Me_2qpy^{2+} complex, we infer that the dominant mode of binding is no longer intercalative, but is based on electrostatic attraction. Space-filling models of the complexes show that the two positive charges on the Me_2qpy^{2+} ligand are the right distance apart (~12 Å) to interact electrostatically with a pair of phosphates, one on each of the strands of DNA in the double-stranded B-form. The result is a binding that bridges the two strands, but is not intercalative in nature. In fact, such models show that the two ancillary bipyridine ligands can only be accommodated facing away from the major groove, with the Me_2qpy^{2+} ligand facing in, bridging the phosphates. This mode of binding would be more rigid, and result in alignment of the Me_2qpy^{2+} parallel with adjacent base pairs within the major groove, but not intercalating with them. The Me_2qpy^{2+} ligand would be more shielded from solvent, providing a rationale for the emission shift and intensity increase, as well as the observed shift in the MLCT band.

Acknowledgment. We thank the PSC-BHE awards program of CUNY and the Biomedical Research Support Grant (NIH) for support of this research.

Registry No. $Ru(bpy)_2ppz^{2+}$, 107495-10-3; $Ru(bpy)_2ppzPt(OH)_2^{2+}$, 133602-32-1; $Ru(bpy)_2qpy^{2+}$, 133578-98-0; $Ru(bpy)_2Meqpy^{3+}$, 133578-99-1; $Ru(bpy)_2Me_2qpy^{4+}$, 133579-00-7; $Ru(bpy)_2dpp^{2+}$, 88635-47-6.