Synthesis and DNA-Binding Properties of $[Ru(NH_3)_4dppz]^{2+}$

Rajesh B. Nair, Emily S. Teng, Shalawn L. Kirkland, and Catherine J. Murphy*

Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina 29208

Received April 14, 1997

Introduction

Ruthenium(II) polypyridyl complexes have attracted wide interest due to their optical properties. Polypyridyl ruthenium(II) complexes have been studied as light absorbers, photoluminescent sensors, and intramolecular energy and electron transfer agents.[1] Promising candidates for luminescent probes in aqueous solution are dipyridophenazine (dppz) complexes of ruthenium(II), because they are nonemissive in water but emit brightly in nonaqueous solvent or in aquated polymers like DNA.[2−5] $[Ru(bpy)_2dppz]^{2+}$ and $[Ru(phen)_2dppz]^{2+}$ exhibit a molecular "light switch" effect by intercalative binding to DNA. These complexes show no photoluminescence in aqueous solution at ambient temperatures but display intense photoluminescence upon binding to DNA.[2−5] This quenching of luminescence in aqueous media is mainly due to the interaction of the phenazine nitrogens of the ligand with the water via hydrogen-bonding or excited-state proton transfer.[5,6] Upon intercalative binding to DNA, the phenazine nitrogens are protected from water and hence luminescence is observed.

Due to the limited number of mono(dipyridophenazine) complexes of ruthenium(II), information on the influence of the ancillary ligands on the optical properties of these complexes is lacking. Studies of this kind could also provide us with more insight into the "light switch" effect for these complexes. In this Note we report the synthesis and characterization of $[Ru(NH_3)_4dppz](PF_6)_2$, Figure 1, and also explore the interactions of the complex with DNA.

Experimental Section

Instrumentation and Materials. 1,10-Phenanthroline, $RuCl_3 \cdot xH_2O$, hydrazine hydrate, NH_4PF_6, purified zinc, mercury, ethanol, methanol, ether, nitromethane, acetonitrile, propylene carbonate, cyclohexanone, acetone, dimethylformamide, dimethylacetamide, and dimethyl sulfoxide were obtained from Aldrich, and $Na_2HPO_4 \cdot 7H_2O$ and $NaH_2PO_4 \cdot H_2O$ were obtained from Mallinckrodt. All reagents were of the highest purity available. Calf thymus DNA was obtained from Sigma and was purified by phenol/chloroform extraction.

Elemental analyses were performed by National Chemical Consulting, Inc., Tenafly, NJ. Absorption spectra of the complex in various solvents were obtained at room temperature on a Perkin-Elmer Lambda 14 UV−visible spectrophotometer.

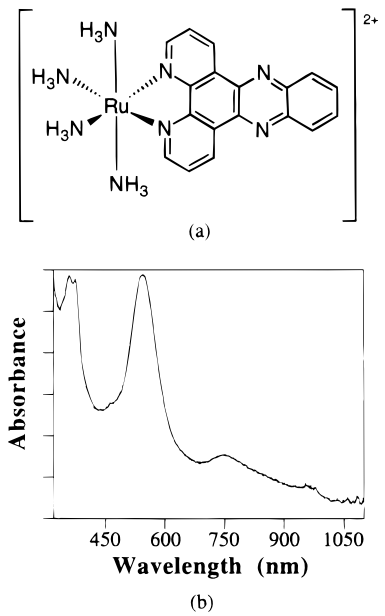


Figure 1. (a) $[Ru(NH_3)_4dppz]^{2+}$. (b) Absorption spectrum of $[Ru(NH_3)_4dppz]^{2+}$ in water.

Synthesis and Characterization. Dipyridophenazine was synthesized according to a literature method.[7] The complex $[Ru(NH_3)_5(OH_2)](PF_6)_2$ was synthesized according to standard procedures.[8−10]

$[Ru(NH_3)_4dppz](PF_6)_2$. $[Ru(NH_3)_4dppz](PF_6)_2$ was synthesized via a modification of the literature method for $[Ru(NH_3)_4bpy](PF_6)_2$.[10] A 0.28 g amount of $[Ru(NH_3)_5(OH_2)](PF_6)_2$ (0.57 mmol) was dissolved in 20 mL of acetone, forming an orange solution of the $[Ru(NH_3)_5(CH_3COCH_3)]^{2+}$ ion. To this was added 0.16 g of dipyridophenazine (0.57 mmol), and the solution was allowed to stir for 12 h under nitrogen. The crude purple $[Ru(NH_3)_4dppz](PF_6)_2$ product was filtered into 6 vol of ether, and the resulting solution was filtered to obtain dark purple semicrystalline $[Ru(NH_3)_4dppz](PF_6)_2$ (0.18 g, 0.24 mmol, 42% yield). Anal. Calcd for $[Ru(NH_3)_4dppz](PF_6)_2$, molecular mass 741.42 amu: C, 29.16; H, 2.99; N, 15.11. Found: C, 29.47; H, 3.32; N, 16.06. ^{1}H NMR ($(CD_3)_2SO$) δ 9.6 (2H, d), 9.23 (2H, dd), 8.44 (2H, dd), 8.1 (2H, dd), and 8.0 (2H, dd). The electronic absorption spectrum of the complex showed the characteristic high-energy double-humped peak due to the ligand dipyridophenazine (∼370 nm) and a broad low-energy band presumably due to the metal to ligand charge transfer (MLCT), Figure 1b; $\epsilon_{544} = 2600\ M^{-1}\ cm^{-1}$ in water.

Binding Studies with Calf Thymus DNA. Binding constants were determined by absorption titration of $[Ru(NH_3)_4dppz](PF_6)_2$ with calf thymus DNA at room temperature, in 5 mM phosphate buffer at pH 7.2, at a complex concentration of 58.0 μM and calf thymus DNA added from 0 to 10.4 μM. Similar titrations were performed with 8.2 μM $[Ru(phen)_2dppz]Br_2$ from 0 to 21.0 μM DNA. The dilution of metal complex concentrations at the end of the titrations was negligible. Fits of experimental absorption titrations were performed with Mathematica v3.

Thermal denaturation studies were performed in 5 mM, pH 7.2, phosphate buffer containing 198.9 μM calf thymus DNA with 19.89

* To whom correspondence should be addressed. Phone: (803) 777-3628. Fax: (803) 777-9521. E-mail: murphy@psc.sc.edu.

(1) (a) Juris, A.; Balzani, V.; Barigelletti, F.; Campagna, S.; Belsar, P.; von Zelewsky, A. *Coord. Chem. Rev.* **1988**, *84*, 85. (b) Demas, J. N.; DeGraff, B. A. *Anal. Chem.* **1991**, *63*, 829.
(2) Friedman, A. E.; Chambron, J. C.; Sauvage, J. P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960.
(3) Hartshorn R. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 5919.
(4) Murphy, C. J.; Barton, J. K. *Methods Enzymol.* **1993**, *226*, 576.
(5) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809.
(6) Turro, C.; Bossmann, S. H.; Jenkins, Y.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1995**, *117*, 9026.
(7) (a) Yamada, M.; Tanaka, Y.; Yoshimoto, Y. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 1006; (b) Dickeson, J. E.; Summers, L. A. *Aust. J. Chem.* **1970**, *23*, 1023.
(8) Allen, A. D.; Senoff, C. V. *Chem. Commun.* **1965**, *621*.
(9) Allen, A. D.; Bottomley, F.; Harris, R. O.; Reinsalu, V. P.; Senoff, C. V. *J. Am. Chem. Soc.* **1967**, *89*, 5595.
(10) Curtis, J. C.; Sullivan, B. P.; Meyer, T. J. *Inorg. Chem.* **1983**, *22*, 224.

S0020-1669(97)00432-1 CCC: $15.00
Published on Web 01/12/1998

Table 1. Absorption Maxima of $[Ru(NH_3)_4dppz]^{2+}$ Compared to $[Ru(NH_3)_4bpy]^{2+}$ in Various Solvents

solvent	DN[a] (kcal/mol)	λ_{max} (nm) $[Ru(NH_3)_4dppz]^{2+}$	λ_{max} (nm) $[Ru(NH_3)_4bpy]^{2+}$ [a]
nitromethane	2.7	554	511
acetonitrile	14.1	550	523
propylene carbonate	15.1	560	532
acetone	17.1	550	534
dimethylformamide	26.6	550	554
dimethylacetamide	27.8	550	558
dimethyl sulfoxide	29.8	550	561

[a] The donor number, DN, is a measure of the electron-pair donating ability of the solvent. DN values and values of λ_{max} for $[Ru(NH_3)_4bpy]^{2+}$ were taken from ref 10.

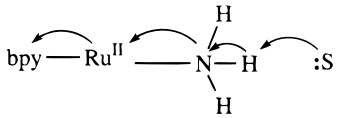
μM $[Ru(NH_3)_4dppz]^{2+}$ and also with 19.89 μM $[Ru(phen)_2dppz]^{2+}$.

Dialysis experiments were performed with Sigma cellulose tubing (molecular weight cutoff of 12 000) in phosphate buffer at total concentrations of 7.8 μM for both metal complex and DNA (nucleotides).

Results and Discussion

The absorption spectrum of $[Ru(NH_3)_4dppz](PF_6)_2$ upon dissolution in various nonaqueous solvents showed fine structure at ~370 nm which is characteristic of the $\pi-\pi^*$ transition of the dppz ligand and a broad peak at ~544 nm which we assign as a MLCT band (Figure 1). The small ~750 nm peak is unassigned at present, but other workers have noted multiple Ru(dπ)−Lπ^* transitions ($\epsilon \sim 10^3$ M^{-1} cm^{-1}) for other $[Ru(NH_3)_4L]^{2+}$ complexes.[11]

The MLCT transition energies of the related complexes $[Ru(NH_3)_4bpy]^{2+}$ (Table 1) and $[Ru(NH_3)_4phen]^{2+}$ exhibit large solvent effects, causing significant visible color changes, which have been attributed to interactions between coordinated NH_3 and electron-donating solvents:[10,12]



In this model, stronger donor solvents push electron density on Ru(II), causing the Ru−bpy MLCT to shift to lower energy in these solvents. However, in $[Ru(NH_3)_4dppz]^{2+}$, the absorption maximum of the MLCT band did not shift much as a function of solvent (Table 1). The electron-withdrawing character of the phenazine portion of the dppz ligand could be responsible for this shutdown of solvent effects by withdrawing electron density from the N−H bond and rendering it less susceptible to solvent. We note that the absorption maxima for the dppz complex in all solvents are similar to the ones for the bpy complex when the bpy complex is dissolved in good donor solvents. Thus, the effect of a good electronic "push" from solvent is apparently similar to that of a good electronic "pull" from ligand. We have observed a similar attenuation of MLCT band solvatochromism for $[Ru(CN)_4dppz]^{2-}$ compared to its bpy and phen counterparts.[13] $[Ru(NH_3)_4dppz]^{2+}$ did not show steady-state emission in any of the solvents at room temperature, like its bpy and phen counterparts; this is a "light switch" that is permanently "off".

The absorption spectrum of $[Ru(NH_3)_4dppz](PF_6)_2$ upon titration with calf thymus DNA, Figure 2, did not show any wavelength shift in the charge transfer band. No photoluminescence is observed upon addition of DNA either. However, the addition of DNA clearly yielded an absorbance hypochromism of 13.6%, which is possibly associated with intercalative binding of the complex to the helix. The binding constant of $[Ru(NH_3)_4dppz]^{2+}$ for DNA, K, was determined to be 1.24×10^5 M^{-1} using eq 1,[14] where ϵ_a is the extinction

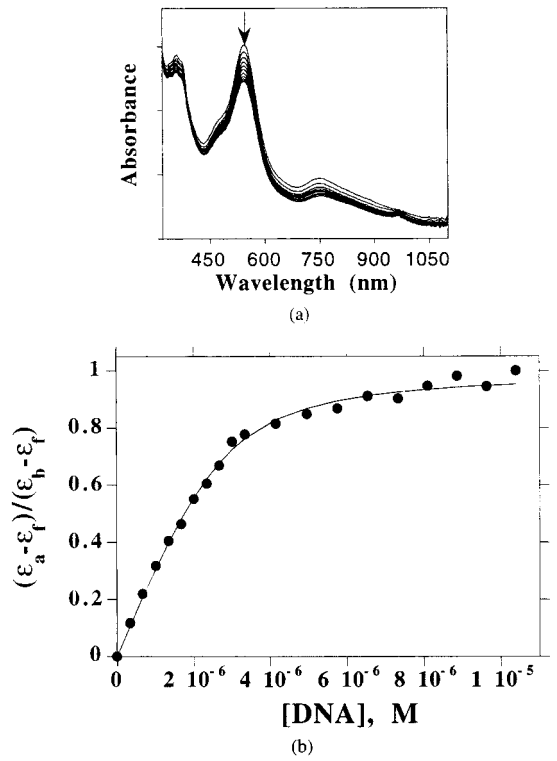


Figure 2. (a) Absorption spectra of $[Ru(NH_3)_4dppz]^{2+}$ (58 μM), in 5 mM phosphate buffer at pH 7.2, in the absence and presence of increasing amounts of DNA (0−10.4 μM). (b) Plot of $(\epsilon_a - \epsilon_f)/(\epsilon_b - \epsilon_f)$ vs [DNA] for $[Ru(NH_3)_4dppz]^{2+}$. The best fit line, superimposed on the data, according to eq 1 yields $K = 1.24 \times 10^5$ M^{-1} and $s = 0.02$.

$$(\epsilon_a - \epsilon_f)/(\epsilon_b - \epsilon_f) = (b - (b^2 - 2K^2C_t[\mathrm{DNA}]/s)^{1/2})/2KC_t \quad (1a)$$

$$b = 1 + KC_t + K[\mathrm{DNA}]/2s \quad (1b)$$

coefficient observed for the MLCT absorption band at a given DNA concentration, ϵ_f is the extinction coefficient of the complex free in solution, ϵ_b is the extinction coefficient of the complex when fully bound to DNA (it is assumed that when further addition of DNA does not change the absorbance, all complex is bound and ϵ_b can be calculated from Beer's Law), K is the equilibrium binding constant, C_t is the total metal complex concentration, [DNA] is the DNA concentration in nucleotides, and s is the binding site size. Equation 1 has been applied to absorption and emission titration data for noncoop-

(11) Ruminski, R. R.; Freiheit, D.; Serveiss, D.; Snyder, B.; Johnson, J. E. B. *Inorg. Chim. Acta* **1994**, *224*, 27.
(12) Mines, G. A.; Roberts, J. A.; Hupp, J. T. *Inorg. Chem.* **1992**, *31*, 125.
(13) Murphy, C. J.; Drane, W. D. *Proc. SPIE* **1995**, *2388*, 266. $[Ru(CN)_4dppz]^{2-}$ appears to be a "light switch" that is permanently "on", in the green: Keller, C. E.; Plessinger, W. D.; Pollard, C.; Murphy, C. J. Manuscript in preparation. For comparison, the MLCT band maximum for $[Ru(phen)_2dppz]^{2+}$ does not shift as a function of solvent, but its emission in nonaqueous solvents is somewhat solvatochromic: Nair, R. B.; Cullum, B. M.; Murphy, C. J. *Inorg. Chem.* **1997**, *36*, 962.
(14) Smith, S. R.; Neyhart, G. A.; Karlsbeck, W. A.; Thorp, H. H. *New J. Chem.* **1994**, *18*, 397. This model was adapted from Bard et al.: Carter, M. T.; Rodrigues, M.; Bard, A. J. *J. Am. Chem. Soc.* **1989**, *111*, 8901.

erative metallointercalator binding to calf thymus DNA.[14] The value of K that we obtained in the absorption titration is similar to what we obtained by equilibrium dialysis (1.8×10^5 M^{-1}). For comparison, we found the binding constant of $[Ru(phen)_2dppz]^{2+}$ to calf thymus DNA to be 5.1×10^6 M^{-1} (by absorption titration and fitting to eq 1; $s = 0.6$),[15] suggesting that the intercalative binding of the dipyridophenazine ligand in $[Ru(phen)_2dppz]^{2+}$ is stronger than in $[Ru(NH_3)_4dppz]^{2+}$. The binding constant of $[Ru(phen)_2dppz]^{2+}$ to DNA determined by us is comparable to the values found by others ($K = 6 \times 10^7$ M^{-1})[16] and ($(1-3) \times 10^6$ M^{-1}),[17] and is also similar to those found for other dicationic dppz complexes such as $[Ru(bpy)_2dppz]^{2+}$ ($>10^6$ M^{-1})[2] and $[Ru(terpy)(dppz)OH_2]^{2+}$ (7×10^5 M^{-1}).[18]

Intercalation should promote base stacking in DNA and hence should lead to an increase in the melting temperature of DNA (corresponding to the transition from double-stranded to single-stranded nucleic acid). Thermal denaturation studies of calf thymus DNA with the metal complexes showed ΔT_m values of +9.1 °C for $[Ru(phen)_2dppz]^{2+}$ and +5.2 °C for $[Ru(NH_3)_4dppz]^{2+}$ compared to calf thymus DNA alone, consistent with the notion that the intercalative binding of $[Ru(phen)_2dppz]^{2+}$ is stronger than that of $[Ru(NH_3)_4dppz]^{2+}$. Other metallointercalators give ΔT_m values of 10−14 °C,[18,19] while $[Ru(NH_3)_5Cl]^{2+}$, a divalent Ru−ammine complex which obviously cannot intercalate, gives ΔT_m of 1−2 °C for binding to calf thymus DNA.[20] The binding constant of 10^5 M^{-1} and the ΔT_m of 5.2 °C for the DNA−$[Ru(NH_3)_4dppz]^{2+}$ adduct are on the border between simple electrostatic association with the helix and intercalation.[18]

Our observation that $[Ru(NH_3)_4dppz]^{2+}$ binds far less well to DNA than $[Ru(phen)_2dppz]^{2+}$ is surprising in the context of other work in this area. An NMR study of $[Rh(NH_3)_4phi]^{3+}$ and $[Rh(phen)_2phi]^{3+}$ binding to an oligonucleotide demonstrated that the intercalating phi ligand was inserted further into the base stack in the ammine complex, likely due to less steric clashing and favorable hydrogen bonding of the ammonia ligands to the DNA.[21] Similarly, a comparison of the bimetallic complexes $[Ru(NH_3)_4]_2(dpb)^{4+}$ and $[Ru(bpy)_2]_2(dpb)^{4+}$ revealed that the ammine complex bound far better to DNA (via insertion of the intercalating dpb ligand) than the bpy analog.[22] Again steric hindrance of the bpy ligands and favorable hydrogen-bonding interactions of the ammonia ligands with the DNA were invoked to explain these results.[22] In our case the presence of the ammine ligands is detrimental to DNA binding. Also peculiar is the small binding site size for $[Ru(NH_3)_4dppz]^{2+}$.[15]

It is possible that the NH_3 "face" of $[Ru(NH_3)_4dppz]^{2+}$ competes with the dppz "face" for DNA binding. Thus the 40-fold lower binding affinity of $[Ru(NH_3)_4dppz]^{2+}$ compared to $[Ru(phen)_2dppz]^{2+}$ (from both equilibrium dialysis and absorption titration experiments) may be due to direct hydrogen bonding between the NH_3 ligands to the oxygens and nitrogens of bases as well as to neighboring phosphate groups of the DNA, similar to the interactions that have been observed in the crystal structure of $[Ru(NH_3)_6]^{3+}$ with d(CGCGCG).[23] Thus a portion of the population of $[Ru(NH_3)_4dppz]^{2+}$ molecules may not intercalate at all, and the absorption titration may in part be probing dppz stacking with other dppz ligands from nearby complexes bound on the surface of the DNA.[15,17] However, $[Ru(phen)_2dppz]^{2+}$ binding to calf thymus DNA has been shown to be entropically driven, presumably by release of counterions, changes in hydration, and the hydrophobic interaction of intercalation.[17] An additional point to consider, then, is that $[Ru(NH_3)_4dppz]^{2+}$ may intercalate into DNA like the bis(phen) complex, but its smaller size and good ancillary hydrogen-bonding groups cause fewer counterions and solvent molecules to be displaced upon binding, lowering the equilibrium constant.

Acknowledgment. We thank the Department of Energy and the National Science Foundation for financial support. E.S.T. thanks the South Carolina EPSCoR program for a summer undergraduate research fellowship. We also thank the anonymous referees for valuable comments.

IC970432J

(15) The values for the binding site size we obtain are $s < 1$, which has also been observed in refs 14 and 17 under certain conditions; it is not clear what this physically means. Forcing s to be larger than 1 makes our fits far worse. Other workers have interpreted s values of less than unity to mean that the intercalator molecules are stacking with each other on the DNA surface; see ref 17.

(16) Hiort, C.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc.* **1993**, *115*, 3448.
(17) Haq, I.; Lincoln, P.; Suh, D.; Norden, B.; Chowdhry, B. Z.; Chaires, J. B. *J. Am. Chem. Soc.* **1995**, *117*, 4788.
(18) Neyhart, G. A.; Grover, N.; Smith, S. R.; Kalsbeck, W. A.; Fairley, T. A.; Cory, M.; Thorp, H. H. *J. Am. Chem. Soc.* **1993**, *115*, 4423.
(19) Cusumano, M.; Giannetto, A. *J. Inorg. Biochem.* **1997**, *65*, 137.
(20) Tselpi-Kalouli, E.; Katsaros, N. *J. Inorg. Biochem.* **1989**, *37*, 271. This paper also reports that $[Ru(NH_3)_6]^{3+}$ raises the melting temperature of calf thymus DNA by about 7 °C, but the difference in charge of the complex has a large influence on these values.
(21) Collins, J. G.; Shields, T. P.; Barton, J. K. *J. Am. Chem. Soc.* **1994**, *116*, 9840.
(22) Carlson, D. L.; Huchital, D. H.; Mantilla, E. J.; Sheardy, R. D.; Murphy, W. R., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 6424.
(23) Ho, P. S.; Fredrick, C. A.; Saal, D.; Wang, A. H.-J.; Rich, A. *J. Biomol. Struct. Dyn.* **1987**, *4*, 521. We have been unable to find a binding constant for $[Ru(NH_3)_6]^{2+}$ to DNA in the literature.