Ruthenium(II) Complexes of Redox-Related, Modified Dipyridophenazine Ligands: Synthesis, Characterization, and DNA Interaction

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The synthesis, spectral characterization, and electrochemical properties of $[Ru(phen)_2(qdppz)]^{2+}$, which incorporates a quinone-fused dipyridophenazine ligand (naphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine-5,18-dione, qdppz), are described in detail. Chemical or electrochemical reduction of $[Ru(phen)_2(qdppz)]^{2+}$ leads to the generation of $[Ru(phen)_2(hqdppz)]^{2+}$—a complex containing the hydroquinone form (hqdppz = 5,18-dihydroxynaphtho[2,3-*a*]-dipyrido[3,2-*h*:2′,3′-*f*]phenazine) of qdppz. Absorption and viscometric titration, thermal denaturation, topoisomerase assay, and differential-pulse voltammetric studies reveal that $[Ru(phen)_2(qdppz)]^{2+}$ is an avid binder of calf-thymus DNA due to a strong intercalation by the ruthenium-bound qdppz, while $[Ru(phen)_2(hqdppz)]^{2+}$ binds to DNA less strongly than the parent "quinone"-containing complex. DNA-photocleavage efficiencies of these complexes also follow a similar trend in that the MLCT-excited state of $[Ru(phen)_2(qdppz)]^{2+}$ is more effective than that of $[Ru(phen)_2(hqdppz)]^{2+}$ in cleaving the supercoiled plasmid pBR 322 DNA ($\lambda_{exc} = 440 \pm 5$ nm), as revealed by the results of agarose gel electrophoresis experiments. The photochemical behaviors of both the quinone- and hydroquinone-appended ruthenium(II) complexes in the presence of DNA not only provide valuable insights into their modes of binding with the duplex but also lead to detailed investigations of their luminescence properties in nonaqueous, aqueous, and aqueous micellar media. On the basis of the results obtained, (i) a photoinduced electron transfer from the MLCT state to the quinone acceptor in $Ru(phen)_2(qdppz)]^{2+}$ and (ii) quenching of the excited states due to proton transfer from water to the dipyridophenazine ligand in both complexes are invoked to rationalize the apparent lack of emission of these redox-related complexes in the DNA medium.

Introduction

Metal complexes of phenanthroline (phen) and other structurally related ligands, such as dipyridophenazine (dppz), are increasingly being employed in studies with DNA.[1−17] An important advantage of using these complexes in such studies is that their ligands and metals can be conveniently varied to suit individual applications. During our investigation on dppz-based complexes,[16] it occurred to us that further derivatization of this ligand with suitable electron-donating/withdrawing groups might not only accentuate DNA-binding and -photocleavage efficiencies of the ensuing complexes but also facilitate the study of other interesting associated functional aspects. Recently, we synthesized two new ligands based on the dppz framework: (i) naphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine-5,18-dione (qddpz) and (ii) 6,7-dicyanodipyrido[2,2-*d*:2′,3′-*f*]-quinoxaline (dicnq). Preliminary characterization and novel luminescence properties of mixed-ligand ruthenium(II) complexes containing these ligands have been reported by us.[17] qdppz incorporates the electroactive quinone subunit in its architecture, and the mixed-ligand complex $[Ru(phen)_2(qdppz)]^{2+}$ can be reversibly reduced to form $[Ru(phen)_2(hqdppz)]^{2+}$ (hqdppz = 5,18-dihydroxynaphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine, the hydroquinone form of qdppz). The $2e^-/2H^+$ redox couple $[Ru(phen)_2(qdppz)]^{2+}/[Ru(phen)_2(hqdppz)]^{2+}$ (see Figure 1) has been demonstrated to be an "electro−photo switch" device,[17] but details of the syntheses, characterization, and, moreover, DNA interactions of these complexes have not yet been reported. This paper, in addition to providing details of the syntheses and characterization of $[Ru(phen)_2(qdppz)]^{2+}$ and $[Ru(phen)_2(hqdppz)]^{2+}$, compares the DNA-binding and -photocleavage properties of these two redox-related complexes. Similar studies carried out with a series of ruthenium(II) complexes of the other modified dppz-based ligand, viz., dicnq, are reported in the succeeding paper.

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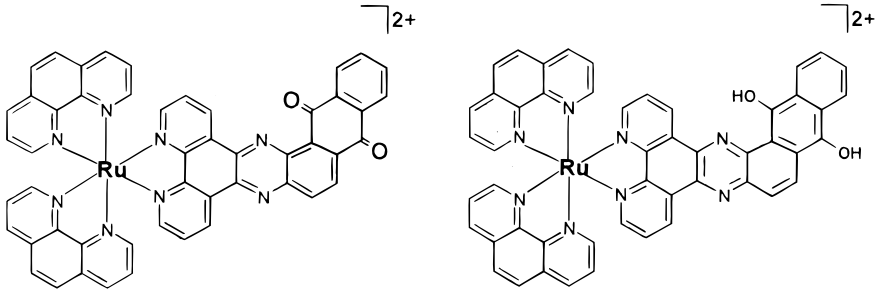

Figure 1. Structures of the two redox-related ruthenium(II) complexes investigated in this study.

Experimental Section

A. Materials. 1. General Details. All the common chemicals and solvents utilized in this study were obtained in their highest available purity from either BDH (Mumbai, India) or Ranbaxy (Mumbai, India). Ruthenium trichloride (hydrate), tetrabutylammonium hexafluorophosphate, and tetrabutylammonium chloride were obtained from Aldrich, calf-thymus DNA (CT DNA), bovine serum albumin (BSA), and dithiothreitol (DTT) were obtained from Sigma, and agarose (molecular biology grade) and ethidium bromide were purchased from Bio-Rad. Supercoiled pBR 322 DNA (CsCl purified) and topoisomerase I (wheat germ) were obtained from Bangalore Genie (Bangalore, India) and were used as received. All solvents utilized for spectroscopic and electrochemical work were rigorously purified before use according to standard procedures.[18] Deionized, triply distilled water was used for preparing various buffers.

2. Syntheses. 1,10-Phenanthroline-5,6-dione,[19] $[Ru(phen)_3]Cl_2$,[20] $[Ru(phen)_2Cl_2$,[21] and $[Ru(phen)_2(dppz)]Cl_2$[22] were synthesized by following the reported procedures. The syntheses of qdppz and the ruthenium(II) complexes are described below.

qdppz (Naphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine-5,18-dione). This compound was prepared by a slight modification of the procedure of Lopez et al.[23] 1,2-Diaminoanthraquinone (0. 22 g, 1 mmol) was added to a 250 mL ethanolic solution of phenanthroline-5,6-dione (0.21 g, 1 mmol), and the resulting mixture was refluxed for 5 h. Evaporation of the solvent gave a greenish-brown residue, which was taken up in 100 mL of $CHCl_3$, and the solution was warmed to ca. 50 °C during 0.5 h. The resulting mixture was cooled and filtered, and a yellow precipitate was obtained upon addition of diethyl ether to the filtrate. The solid was filtered off, washed with diethyl ether, and vacuum-dried. Yield: 70%. Anal. Found: C, 73.02; H, 3.02; N, 12.35. Calcd for $C_{26}H_{12}N_4O_2$: C, 73.43; H, 2.98; N, 12.51. FAB-MS: *m*/*z* 413 (M^+). IR (KBr pellet): 1670, 1585, 1462 cm^{-1}. UV−visible (CH_2Cl_2), λ_{max}, nm (log ϵ): 410 (4.13), 394 (4.13), 281 (4.58), 259 (4.62). ^1H NMR (200 MHz, $CDCl_3$, 298 K): δ 9.85 (d, 1H), 9.65 (d, 1H), 9.35 (d, 2H), 8.71 (dd, 2H), 8.32 (q, 2H), 7.88 (m, 4H). $E_{1/2}$ (DMF, 0.1 M (TBA)PF_6, V vs SCE): −0.46, −1.48.

$[Ru(phen)_2(qdppz)](PF_6)_2$ (Bis(1,10-phenanthroline)(naphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine-5,18-dione)ruthenium(II) Hexafluorophosphate). $Ru(phen)_2Cl_2$ (0.53 g, 1 mmol) and qdppz (0.45 g, 1.1 mmol) were refluxed in ethylene glycol (50 mL) for 12 h. The resulting solution was cooled to the room temperature, after which 20 mL of water was added and the solution was filtered. Addition of solid NH_4PF_6 to the filtrate precipitated crude $[Ru(phen)_2(qdppz)](PF_6)_2$, which was purified by repeated recrystallizations from an acetone/diethyl ether mixture. Yield: 80%. Anal. Found: C, 50.86; H, 2.43; N, 9.22. Calcd for $C_{50}H_{32}N_8O_4P_2F_{12}Ru$: C, 50.46; H, 2.49; N, 9.34. FAB-MS: *m*/*z* 1019 ($[M - PF_6]^+$), 874 ($[M - 2PF_6]^+$). IR (KBr pellet): 1670, 1589, 1427, 837 cm^{-1}. UV−visible (CH_3CN), λ_{max}, nm (log ϵ): 440 (4.29), 388 (4.32), 278 (4.92), 263 (5.09). ^1H NMR (200 MHz, DMSO-d_6, 298 K): δ 9.5 (br, 2H), 8.81 (m, 6H), 8.41 (s, 4H), 8.32 (d, 2H), 8.23 (d, 4H), 8.10 (d, 4H), 7.82 (m, 6H). ^{13}C NMR (200 MHz, CD_3CN/10% D_2O, 298 K, major peaks): δ 126.5, 128.6, 131.6, 137.5, 148.4, 153.7, 155.4, 182.8, 183.5. $E_{1/2}$ (CH_3CN, 0.1 M (TBA)PF_6, V vs SCE): +1.36. $E_{1/2}$ (DMF, 0.1 M (TBA)PF_6, V vs SCE): −0.37, −1.27, −1.49.

$[Ru(phen)_2(qdppz)]Cl_2$ (Bis(1,10-phenanthroline)(naphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine-5,18-dione)ruthenium(II) Chloride). The hexafluorophosphate salt obtained above was dissolved in a minimum amount of acetone, and a saturated solution of tetrabutylammonium chloride in acetone was added dropwise until precipitation was complete. The water-soluble chloride salt was filtered off, washed thoroughly with acetone, and vacuum-dried. Recovery was about 90% of the theoretical yield. IR (KBr pellet): 1666, 1413 cm^{-1}. UV−visible (H_2O), λ_{max}, nm (log ϵ): 439 (4.26), 400 (4.28), 278 (4.89), 263 (5.10). $E_{1/2}$ (5 mM Tris, pH 7.1, 50 mM NaCl, V vs SCE): −0. 16.

$[Ru(phen)_2(hqdppz)]^{2+}$ (Bis(1,10-phenanthroline)(5,18-dihydroxynaphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine)ruthenium(II)). (a) Electrochemical Method. $[Ru(phen)_2(qdppz)](PF_6)_2$ (1×10^{-4} M) was subjected to exhaustive electrolysis under an N_2 atmosphere in aqueous CH_3CN (4−5% H_2O) containing 0.1 M (TBA)PF_6 at −0.5 V until the current value reached ca. <1% of the initial value (∼20 min). Comparison of the total Coulombs passed during the electrolysis with the Coulombs passed during the electrolysis of 1,4-benzoquinone (1×10^{-4} M) revealed that reduction of the $[Ru(phen)_2(qdppz)]^{2+}$ ion involved 1.9 ± 0.1 electrons. Aliquots of the solution containing the reduced species thus obtained were extracted under anaerobic conditions, and the UV−visible as well as fluorescence spectra were recorded using airtight cuvettes. Reversibility of the redox cycle was checked by repeating the reduction and reoxidation (+1.1 V) reactions for the same solution three or four times and by measuring the UV−visible and fluorescence spectra of the resulting sample each time.

(b) Chemical Method. The chloride or the hexafluorophosphate salt of this complex was obtained by $Na_2S_2O_4$ reduction of $[Ru(phen)_2(qdppz)]Cl_2$ or $[Ru(phen)_2(qdppz)](PF_6)_2$, respectively, in slightly basic (pH ≈8.0, NaOH) aqueous or aqueous CH_3CN (CH_3CN/H_2O or CD_3CN/D_2O, 10:1 v/v) solutions.

Both $[Ru(phen)_2(hqdppz)]Cl_2$ and $[Ru(phen)_2(hqdppz)](PF_6)_2$ were found to be air-sensitive species; hence, their spectroscopic measurements (UV−visible (1×10^{-5} M), fluorescence (1×10^{-5}), and ^1H (≈2×10^{-3} M) and ^{13}C NMR (≈7×10^{-3} M)) were conveniently performed, strictly under an atmosphere of nitrogen, for their in situ preparations (inside the cuvette, NMR tube, etc.) obtained by the addition of ca. 3−4 molar equiv of $Na_2S_2O_4$ to previously deaerated solutions of $[Ru(phen)_2(qdppz)]^{2+}$. In each case, the solution was kept aside in the dark for ca. 45−60 min with occasional shaking prior to the spectral measurements. UV−visible (CH_3CN/10%H_2O), λ_{max}, nm (log ϵ): 442 (4.28), 348 (4.22), 300 (sh, 4.74), 263 (5.06). ^1H NMR (200 MHz, CD_3CN/10% D_2O, 298 K): δ 8.72 (m, 6H), 8.34 (s, 6H),

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8.33 (s, 2H), 8.12 (m, 4H), 7.80 (m, 8H). ^{13}C NMR (200 MHz, CD_3CN/10% D_2O, 298 K, major peaks): δ 128.1, 129.7, 132.7, 138.9, 149.8, 155.1, 156.5, 157.6.

B. Methods. 1. Spectroscopy and Electrochemistry. UV−visible and infrared spectra were recorded with a Shimadzu model UV-160 A (coupled with a model TCC-240 A temperature controller) and a JASCO model 5300 FT-IR spectrophotometer, respectively. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker NR-FT 200 spectrometer. Tetramethylsilane was used as an internal standard. The emission spectra were recorded with a JASCO model FP-777 spectrofluorometer using $[Ru(phen)_3]^{2+}$ in CH_3CN as the standard.[24] While hexafluorophosphate salts of the complexes were employed for the luminescence measurements in nonaqueous solvents (rigorously dried $CHCl_3$, CH_2Cl_2, dichloroethane, and CH_3CN) and aqueous CH_3CN (10% H_2O) solutions, the corresponding chloride salts were used for measurements in aqueous, aqueous buffered (buffer A: 5 mM Tris, pH 7.1, 50 mM NaCl), micellar (SDS, 0.1 M), and CT DNA (up to 200 μM) solutions. In all cases, solutions containing 10 μM concentrations of the complex were excited at 440 nm and the emission was monitored between 500 and 650 nm. FAB mass spectra were recorded with a JEOL SX-102/DA-600 mass spectrometer.

Cyclic and differential-pulse voltammetric experiments were performed on an electrochemical work station comprising a Princeton Applied Research (PAR) model 174A polarographic analyzer/PAR model 173 potentiostat, a PAR model 175 universal programmer, and an X-Y recorder (Digital Electronics, Series 2000), as described previously.[25,26] A glassy carbon working electrode, a Pt-wire counter electrode, and a saturated calomel reference electrode (SCE) were employed. Exhaustive bulk-electrolysis experiments were carried out using a BAS-27 potentiostat and Pt-mesh, Pt-flag, and SCE working, counter, and reference electrodes, respectively.

2. DNA-Binding and -Photocleavage Studies. The concentration of CT DNA was measured by using its known extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}).[27] Buffer A was used for absorption titration experiments and luminescence measurements, buffer B (1 mM phosphate, pH 7.0, 2 mM NaCl) was used for thermal denaturation and differential-pulse voltammetric experiments, and buffer C (1.5 mM Na_2HPO_4, 0.5 mM NaH_2PO_4, 0.25 mM Na_2EDTA, pH 7.0) was used for the viscometric titrations. The chloride salts of the complexes were used in studies with DNA.

DNA melting experiments were carried out by monitoring the absorption (260 nm) of CT DNA (160 μM) at various temperatures in the absence and in the presence (0−10 μM) of the complex. The melting temperature (T_m) and the curve width σ_T (=temperature range where 10%−90% of the absorption increase occurred) were calculated as previously described.[28] Absorption titration experiments were performed by maintaining a constant metal complex concentration (10 μM) and varying the nucleic acid concentration (2−40 μM), as described earlier.[16] Differential-pulse voltammetric experiments (highly polished glassy carbon working electrode, Pt-wire counter electrode, and SCE reference electrode) were performed for 0.1 mM complex in the absence and in the presence of increasing amounts (0−3 mM) of CT DNA. Current−voltage curves were recorded after each successive addition of DNA solution and equilibration (ca. 10 min).

Viscometric titrations were performed with a Canhon-Ubblehode viscometer at 25 ± 1 °C. Titrations were performed for $[Ru(phen)_3]^{2+}$, $[Ru(phen)_2(qdppz)]^{2+}$, and ethidium bromide (EtBr) (3−40 μM) and for $Na_2S_2O_4$ (9−120 μM) in the presence and absence of $[Ru(phen)_2(hqdppz)]^{2+}$ (3−40 μM). Each compound was introduced into the degassed DNA solution (300 μM in base pairs) present in the viscometer using a Hamilton syringe fitted with a glass extender. Mixing of the drug and DNA was done by bubbling with nitrogen. Flow times were measured, using a digital stopwatch, at least three times and were accepted if they agreed within 0.1 s. Reduced specific viscosity was calculated according to Cohen and Eisenberg.[29] Plots of η/η_0 (η and η_0 are the reduced specific viscosities of DNA in the presence and absence of the drug) versus [drug]/[DNA] were constructed using the Microcal Origin program. Plots of η/η_0 versus [EtBr]/[DNA] and $[[Ru(phen)_3]^{2+}]$/[DNA] were found to be similar to those reported in the literature.[29,30]

For the gel electrophoresis experiments, supercoiled pBR 322 DNA (100 μM in nucleotides) in Tris−HCl buffer (pH 8.0) was treated with 10 μmol samples of the metal complexes and the mixtures were incubated for 1 h in the dark. The samples were then analyzed by 0.8% agarose gel electrophoresis (Tris−acetic acid−EDTA buffer, pH 8.0) at 40 V for 5 h. The gel was stained with 1 μg/mL ethidium bromide for 0.5 h, after which it was analyzed using the UVP gel documentation system and was also directly photographed and developed as described previously.[16,31−33] Irradiation experiments were carried out by keeping the preincubated (dark, 1 h) samples inside the sample chamber of a JASCO model FP-777 spectrofluorimeter for 25 min (λ_{exc} = 440 ± 5 nm; slit width = 5 nm).

In the experiments with topoisomerase, samples of 0. 5 μg of pBR 322 in 20 μL of the assay buffer (50 mM Tris, 50 mg of NaCl, 1 M EDTA, 1 mM DTT and 100 μg of nuclease-free BSA, 1 mM $MgCl_2$), 2 or 3 units of topoisomerase, and $[Ru(phen)_2(qdppz)]^{2+}$ (or $[Ru(phen)_2(hqdppz)]^{2+}$) (1−9 μM) were incubated for 30 min at 37 °C. The reactions were stopped by the addition of 250 mM EDTA and 10% SDS. The samples were then electrophoresed in a 1% agarose gel as described above. The extent of helix unwinding was determined by adopting a method described in the literature,[34,35] eq 1, where σ is the

$$\sigma = r_c\Phi/18 \quad (1)$$

superhelical density of the plasmid, r_c equals the amount of the ruthenium complex ions bound per nucleotide when all of the superhelices are removed, and Φ is the unwinding angle. Two independent control experiments were carried out under the same set of experimental conditions. In the first control, no drug was used and, here, no form I could be detected upon electrophoresis of the samples treated only with topoisomerase. In the other control, where EtBr was used as the intercaltor, the value of Φ calculated (26 ± 4°) was found to be nearly equal to that reported in the literature (24 ± 2°).[35]

Care was taken to avoid the entry of direct, ambient light into the samples during all experiments, and unless otherwise specified, all experiments were carried out at 293 ± 3K.

Results and Discussion

The "atypical" electronic structure of dppz, which has been proposed[36,37] to endow it with features of both an α-diimine chelate and a 1,4-diazine moiety, and the consequent structural and electronic features of the complexes derived from this versatile ligand seem to have made them attractive candidates for use in various applications.[1−15] Derivatization of dppz with quinone—a ubiquitous electron-deficient group known for its reversible redox chemistry and DNA-binding ability[38]—was

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expected to provide an opportunity to examine the redox tuning of the DNA interactions by the ensuing complexes. Thus, we attempted to assess the effects due to the additional, electroactive quinone/hydroquinone component on the DNA-binding and -photocleavage efficacies of $[Ru(phen)_2(qdppz)]^{2+}$ and $[Ru(phen)_2(hqdppz)]^{2+}$ in this study.[39] Before we take up these issues, it is instructive to examine the details concerning the syntheses and characterizations of these new complexes in light of those of the other structurally and functionally similar systems reported previously.

A. Design, Synthesis, and Characterization. Each synthetic step presented here is straightforward and provides a good yield of the desired product in pure form. qdppz was characterized earlier by UV−visible and infrared methods,[23] and our data agree with the reported data. Additional characterization of this ligand has been carried out in the present study by elemental analysis, FAB-MS, ^{1}H NMR, and electrochemical methods (see the Experimental Section). These data are also consistent with the structure and integrity of the compound.

The PF_6 salt of $[Ru(phen)_2(qdppz)]^{2+}$ gave a satisfactory elemental analysis, and it showed the expected pattern in its FAB-MS spectrum. In addition, the peak positions and their intensities in the ^{1}H and ^{13}C NMR spectra of the complex gave sufficient evidence for its structure. The complex also showed the characteristic MLCT band at 440 nm and bands due to the intraligand transitions at 388 (qdppz) and 263 (phen) nm in the UV−visible spectrum. The spectra of phen, qdppz, and $[Ru(phen)_2(qdppz)](PF_6)_2$ given in Figure 2 serve to illustrate this point. The infrared spectra of qdppz and $[Ru(phen)_2(qdppz)]^{2+}$ each showed a peak at 1670 cm^{-1} ascribable to quinone carbonyl stretching, thus confirming that chelation of qdppz to ruthenium has not affected the quinone part of this ligand. The well-defined[40] cyclic voltammetric responses seen at +1.36 and −0.37 V for the complex can be assigned to Ru^{III}/Ru^{II} and $qdppz/qdppz^{\bullet -}$ couples, respectively. The spectral and redox characteristics of $[Ru(phen)_2(qdppz)]Cl_2$ were found to be essentially similar to those described above for the PF_6 salt.

$[Ru(phen)_2(hqdppz)]^{2+}$ could be obtained by the reduction of $[Ru(phen)_2(qdppz)]^{2+}$ with $Na_2S_2O_4$, and the process could be reversed by oxidation with $Ce(NH_4)_2(NO_3)_6$ to reproduce the quinone form. Electrochemical and fluorescence methods have provided further support for the reversible nature of this quinone/hydroquinone redox reaction. The reduced complex obtained via chemical means could be characterized by UV−visible, NMR, and electrochemical methods (see the Experimental Section). In particular, the complex shows only the MLCT band (442 nm) at $\lambda > 350$ nm, with the quinone $\pi-\pi^*$ transition (388 nm) of its precursor $[Ru(phen)_2(qdppz)]^{2+}$ having clearly disappeared from the UV−visible spectrum, Figure 3.[41] Equally important is the appearance of a pair of ^{13}C NMR signals at 156.5 and 157.6 ppm ascribable to the phenolic carbon atoms of the complex instead of those due to the quinone carbon atoms on $[Ru(phen)_2(qdppz)]^{2+}$ (182.8 and 183.5 ppm).

B. DNA Binding. Initially, the interaction of $[Ru(phen)_2(qdppz)]Cl_2$ with DNA was monitored by absorption titration (MLCT band) and thermal denaturation (monitoring A_{260} of DNA) methods. In the presence of increasing amounts of calf-thymus (CT) DNA, the complex showed bathochromic shifts (4 ± 1 nm) and hypochromism (50 ± 6% at $[DNA]_{phosphate}/[Ru] = 4$) in the UV−visible spectrum and increased values (at $[DNA]_{phosphate}/[Ru] = 25$) of both the DNA melting temperature ($\Delta T_m = 6 \pm 1$ °C) and the curve width ($\Delta\sigma_T = 4 \pm 1$ °C) in the thermal denaturation experiments.[42] These observations are reminiscent of those reported earlier for various metallointercalators[43] and suggest that $[Ru(phen)_2(qdppz)]Cl_2$ binds strongly to DNA by an intercalative mode. Indeed, the apparent binding constant K_b for the interaction of $[Ru(phen)_2$-

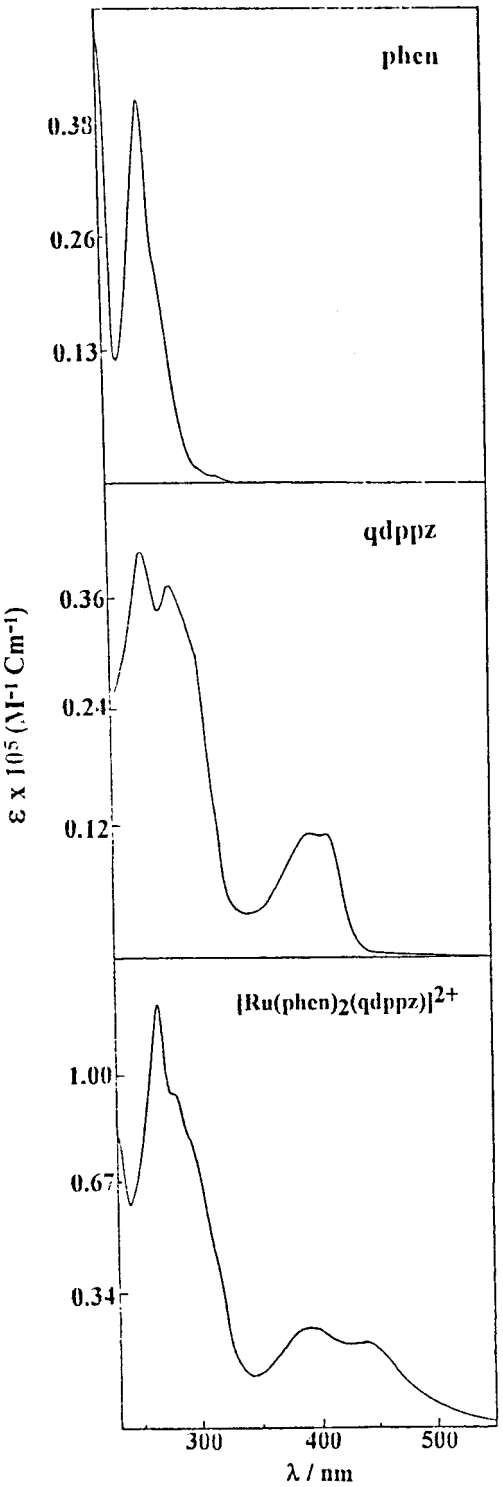

Figure 2. UV−visible spectra of phen, qdppz, and $[Ru(phen)_2(qdppz)]^{2+}$ in CH_3CN.

(39) Cation-binding properties of a series of ruthenium(II) complexes containing crown-ether-appended dppz have been reported: Yam, V. W.-W.; Lee, V. W.-M.; Ke, F.; Siu, K.-M. *Inorg. Chem.* **1997**, *36*, 2124.

(40) i_p vs $v^{1/2}$ = constant, where i_p is the peak current and v is the scan rate, $i_{p_a}/i_{p_c} = 0.9-1.0$, where i_{p_a} and i_{p_c} refer to anodic and cathodic peak currents, respectively, and $\Delta E_p = 60-80$ mV, where E_p is the peak potential for these electron-transfer processes. See: Nicholson, R. S.; Shain, I. *Anal. Chem.* **1964**, *36*, 706.

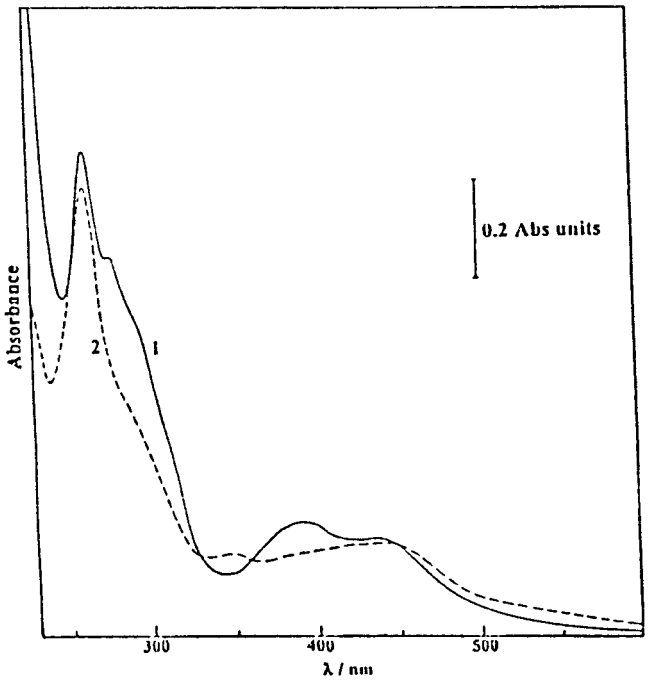

Figure 3. UV−visible spectra of (1) $[Ru(phen)_2(qdppz)]^{2+}$ and (2) $[Ru(phen)_2(hqdppz)]^{2+}$ in CH_3CN/H_2O (10:1, v/v). The reduced complex was obtained by dithionite reduction of 1×10^{-5} M $[Ru(phen)_2(qdppz)]^{2+}$.

$(qdppz)]^{2+}$ with DNA is $>10^6$ M^{-1} as estimated from the absorption titration data.[44]

On the other hand, the value of K_b is only $(1 \pm 0.2) \times 10^5$ M^{-1} for $[Ru(phen)_2(hqdppz)]^{2+}$ (buffer A, $Na_2S_2O_4$) as compared to the strong binding exhibited by $[Ru(phen)_2(qdppz)]^{2+}$.[45] This reduced complex also showed bathochromic shifts (maximum: 4 ± 1 nm) and hypochromism (maximum: $25 \pm 3\%$) during UV−visible titration with DNA at 293 ± 3 K. However, thermal denaturation experiments could not be satisfactorily carried out with $[Ru(phen)_2(hqdppz)]^{2+}$ due to the appearance of turbidity (probably due to the presence of dithionite or $(TBA)PF_6/(TBA)Cl$) for DNA solutions containing this complex at higher temperatures.

It is of interest to know which ligand of the available two in $[Ru(phen)_2(qdppz)]^{2+}$, i.e., phen or qdppz, intercalates with DNA. In this regard, it may be noted that the strength of DNA binding by $[Ru(phen)_2(qdppz)]^{2+}$ reported here is higher than that of DNA binding by $[Ru(phen)_3]^{2+}$ but it is in the same range as that for the Os(II), Ru(II), Ni(II), and Co(III) complexes containing dppz (or modified dppz).[3−10,16] Although this observation argues in favor of an interaction of the bound qdppz with DNA, we note that it is only indirect evidence. This is mostly because the UV−visible method employed here for the estimation of the binding constant does not monitor exclusive properties of the individual ligands in $[Ru(phen)_2(qdppz)]^{2+}$. We adduce direct evidence for the intercalation of metal-bound qdppz with DNA by the application of viscometric, topoisomerase assay, and electrochemical methods.

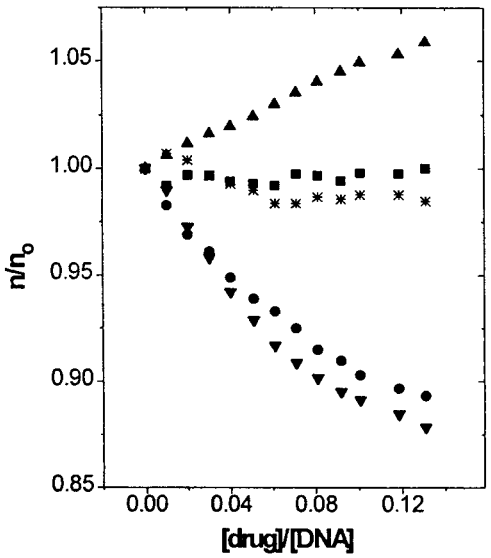

Figure 4. Results of viscometric titrations carried out for CT DNA (300 μM in base pairs, buffer C) in the presence of (▲) $[Ru(phen)_2(qdppz)]^{2+}$ (3−40 μM), (■) $[Ru(phen)_3]^{2+}$ (3−40 μM), (●) $Na_2S_2O_4$ (9−120 μM), and (▼) $Na_2S_2O_4$ (9−120 μM) + $[Ru(phen)_2(qdppz)]^{2+}$ (3−40 μM). The plot marked with asterisks was obtained by subtracting the data for $Na_2S_2O_4$ alone from those for $Na_2S_2O_4$ + $[Ru(phen)_2(qdppz)]^{2+}$.

Plots of η/η_0 vs [drug]/DNA are presented in Figure 4. As seen, while $[Ru(phen)_3]^{2+}$ does not affect the DNA viscosity as reported previously,[30] there is a positive change of viscosity with increasing addition of the complex for $[Ru(phen)_2(qdppz)]^{2+}$, suggesting intercalation.[29,30] On the other hand, a significant negative change in the viscosity of DNA can be noticed for $[Ru(phen)_2(hqdppz)]^{2+}$, which is generated in situ in the viscometric cell by dithionite reduction of $[Ru(phen)_2(qdppz)]^{2+}$ ($[Ru(phen)_2(qdppz)]^{2+}/Na_2S_2O_4 = 1/3$, mol/mol). However, it should be noted that, as such, addition of dithionite alone to DNA solutions results in negative changes in the viscosity.[46] Thus, the "net" viscosity change observed for $[Ru(phen)_2(hqdppz)]^{2+}$ is similar to that exhibited by $[Ru(phen)_3]^{2+}$, as seen in Figure 4.

The extent of DNA helix unwinding by a noncovalently bound species may be quantitated by examining the change in the superhelical density in a plasmid after relaxation of the

(41) Interestingly, $[Ru(phen)_2(DPPN)]^{2+}$, where DPPN is benzo[*i*]dipyrido-[3,2-*a*:2′,3′-*c*]phenazine, has been reported to show a transition at 391 nm that is ascribable to the $\pi-\pi^*$ transition of the "benzene-fused phenazine" moiety.[3d,7b] However, such a transition is not apparent in the spectrum of $[Ru(phen)_2(hqdppz)]^{2+}$, incorporating the "naphthalene-fused phenazine" ligand. It is possible that the $\pi-\pi^*$ transition is broad and red-shifted and that there is an overlap of this band with the MLCT band of this ruthenium complex. In fact, $[Ni(phen)_2(hqdppz)]^{2+}$ (obtained by dithionite reduction of the corresponding quinone complex) shows broad absorption between 300 and 400 nm: Eswaramoorthy, K.; Maiya, B. G. Unpublished results.

(42) T_m and σ_T values increased with increasing addition of the metal complex, as expected.

(43) Long, E. C.; Barton, J. K. *Acc. Chem. Res.* **1990**, *23*, 271.

(44) In a plot of percent hypochromism versus the ratio of nucleotide phosphate to ruthenium ($R_{NP:Ru}$), absorption saturation was evident at $R_{NP:Ru} \sim 3$, suggesting essentially stoichiometric binding, even at the lowest possible complex concentration ([Ru] $\sim$ 2 μM) under our experimental conditions. A simple fit of this plot indicates K_b with a lower limit of $\sim 10^6$ M^{-1}. These experiments actually require concentrations that are several orders of magnitude higher than K_b. Therefore, data are not sufficient to establish the K_b value with acceptable accuracy for $[Ru(phen)_2(qdppz)]^{2+}$. *This observation is entirely consistent with that reported for the majority of previously studied dppz-based complexes.*[3−11]

(45) K_b is estimated by following a method described by: Wolfe, A.; Shimer, G. H.; Meehan, T. *Biochemistry* **1987**, *26*, 6392.

(46) A mixture of $Na_2S_2O_4$ and Na_2SO_4 also effected negative changes in the DNA viscosity in a fashion similar to that noted for $Na_2S_2O_4$ alone in Figure 4. Addition of salts is known to influence the viscosity of DNA; see, for example, refs 47−49.

(47) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18.

(48) Cavalieri, L. F.; Rosoff, M.; Rosenberg, B. H. *J. Am. Chem. Soc.* **1956**, *78*, 5239.

(49) Cory, M.; McKee, D. D.; Kagan, J.; Henty, D. W.; Miller, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 2528.

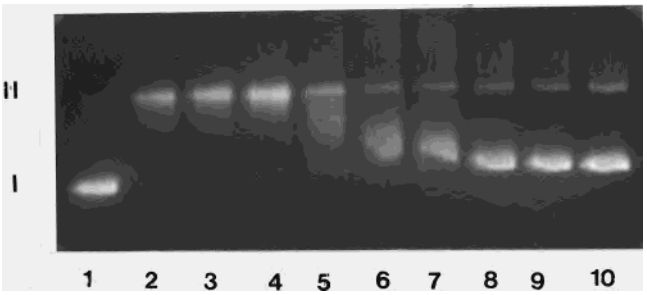

Figure 5. Agarose gel showing the unwinding of pBR 322 by $[Ru(phen)_2(qdppz)]^{2+}$ after incubation with topoisomerase I in the presence of increasing amounts of ruthenium complex. While lane 1 is the pBR 322 control (without incubation), lane 2 shows the DNA after incubation with topoisomerase in the absence of the complex. Lanes 3−10 show the topoisomers after incubation of DNA, topoisomerase, and 1.1, 2.2, 3.3, 4.4, 5.5, 6.6, 8, and 9 μM $[Ru(phen)_2(qdppz)]^{2+}$, respectively.

plasmid in the presence of a bound complex with topoisomerase I.[34,35] Figure 5 shows the unwinding of pBR 322 DNA by $[Ru(phen)_2(qdppz)]^{2+}$ following incubation with topoisomerase I in the presence of increasing amounts of the ruthenium complex. From these titrations, an unwinding angle of 34 ± 10° per ruthenium bound is obtained for $[Ru(phen)_2(qdppz)]^{2+}$. This value is consistent with those observed for other strongly intercalating ruthenium complexes such as $[Ru(bpy)_2(phi)]^{2+}$ (phi = 9,10-phenanthrenequinonediimine)[50] and $[Ru(bpy)_2(dppz)]^{2+}$.[51] On the other hand, a topoisomerase assay carried out in the presence of the reduced complex $[Ru(phen)_2(hqdppz)]^{2+}$ gave a lower value for the unwinding angle (16 ± 7°) per bound ruthenium, as expected.

Electrochemical methods, although easily adapted to monitor DNA interactions with small molecules, have rarely been employed for this purpose in the case of metallopolypyridyls.[16,52−55] The differential-pulse voltammetric method was employed in the present study to monitor DNA binding by $[Ru(phen)_2(qdppz)]^{2+}$. In buffer B, ruthenium-bound qdppz could be reduced at −0.16 V, a potential that is well within the solvent discharge limit and far away from the peaks due to the Ru^{III}/Ru^{II}, Ru^{II}/Ru^{I}, and phen/phen$^{\bullet-}$ redox couples. Coulometric studies revealed that this electrode process involves a $2e^-$ ($2H^+$) transfer and generates $[Ru(phen)_2(hqdppz)]^{2+}$. Successive additions of CT DNA to a solution of $[Ru(phen)_2(qdppz)]^{2+}$ resulted in diminution of the peak current (maximum: 40 ± 5%) and cathodic shifts in the peak potential (maximum: 33 ± 2 mV) in the differential-pulse voltammograms as shown in Figure 6. While the decrease in peak current is in conformity with the proposal that ruthenium-bound qdppz intercalates with DNA,[16,52−55] the cathodic shift of the peak potential observed here merits further discussion.

It was previously shown that binding of a metal complex to DNA can bring about a shift in the redox potential if one redox state is more strongly bound than the other.[52−54] The change in the binding constant can be determined according to eq 2, where

$$E^{b}{}_0' - E^{f}{}_0' = (RT/nF)\log(K_{red}/K_{ox}) \qquad (2)$$

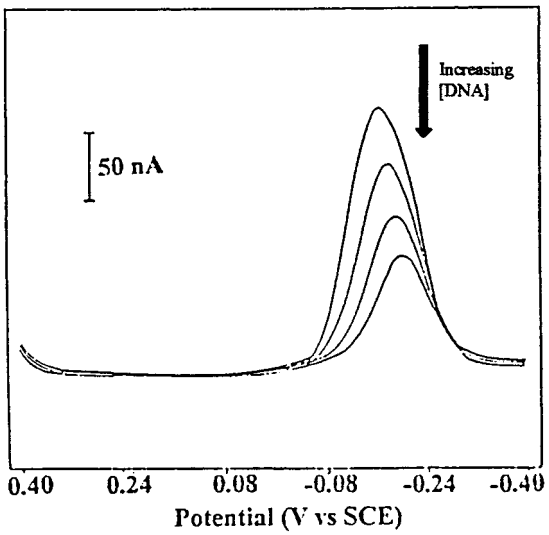

Figure 6. Differential-pulse voltammograms (scan rate = 10 mV/s; modulation amplitude = 10 mV pp) of $[Ru(phen)_2(qdppz)]^{2+}$ (0.1 mM, buffer B) in the absence (top) and in the presence of increasing amounts of CT DNA (0.3, 0.9, and 3.0 (bottom) mM).

$E^{b}{}_0'$ and $E^{f}{}_0'$ are the thermodynamic redox potentials for the bound and free complexes, respectively, n is the number of electrons transferred, K_{red}/K_{ox} is the ratio of binding constants for the reduced and oxidized species, and other parameters have their usual meanings. By substituting appropriate values to suit the electrochemistry of $[Ru(phen)_2(qdppz)]^{2+}$ and from a limiting shift of 33 mV, we calculate that $K([Ru(phen)_2(qdppz)]^{2+})/K([Ru(phen)_2(hqdppz)]^{2+})$ is ≈12.6.[56] Thus, the reduced species binds to DNA less strongly than $[Ru(phen)_2(qdppz)]^{2+}$, and this result is in agreement with the known intercalative ability of the quinone moiety.[38] Results of absorption titration experiments carried out with the reduced complex also suggest the same; vide supra.[57]

C. Luminescence Studies. Electrochemical on/off switching of luminescence exhibited by the $2e^-/2H^+$ couple $[Ru(phen)_2(qdppz)]^{2+}/[Ru(phen)_2(hqdppz)]^{2+}$ was already communicated by us. While the oxidized form of the complex was found to be totally nonluminescent, the electrochemically reduced form was found to emit light of 601 nm in aqueous (5−10%) CH_3CN solutions. This process was found to be reversible. During the present study, we noticed that both $[Ru(phen)_2(qdppz)]^{2+}$ and $[Ru(phen)_2(hqdppz)]^{2+}$ remain essentially nonluminescent in the presence of DNA. To rationalize this finding, we carried out a series of luminescence experiments with these redox-related complexes in aqueous, aqueous micellar, and nonaqueous media. The results are summarized below.

$[Ru(phen)_2(qdppz)]^{2+}$ was found either to be weakly luminescent or to be nonluminescent ($\phi < 10^{-4}$) in both rigorously dried nonaqueous solvents and in aqueous CH_3CN (10% H_2O),

(50) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809.
(51) Pyle, A. M.; Rehmann, J. P.; Meshoyeer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051.
(52) Carter, M. T.; Bard, A. J. *J. Am. Chem. Soc.* **1987**, *109*, 7528.
(53) Carter, M. T.; Rodriguez, M.; Bard, A. J. *J. Am. Chem. Soc.* **1989**, *111*, 8901.
(54) Carter, M. T.; Bard, A. J. *Bioconjugate Chem.* **1990**, *1*, 257.
(55) Grover, N.; Gupta, N.; Singh, P.; Thorp, H. H. *Inorg. Chem.* **1992**, *31*, 2014.
(56) Note here that $n = 2$ for the reduction of $[Ru(phen)_2(qdppz)]^{2+}$ in aqueous solutions and that the electrode process (*and not the overall electrochemical reaction*) was found to be not strictly reversible and diffusion controlled during the cyclic voltammetric experiments in buffer B ($E_{p_c} - E_{p_a} > 50$ mV, and $i_{p_c}/\nu^{1/2}$ is not a constant with respect to scan rates (ν) ranging between 50 and 500 mV/s). However, a tendency toward reversibility was noticed in the presence of DNA. In any case, we note that these electrochemical results do not have any significant effect on the value of the ratio of binding constants.
(57) K_b obtained by the absorption titration method is close to that predicted on the basis of electrochemical redox potential data using eq 2 (i.e., $10^6/12.6 = 8 \times 10^4\ M^{-1} \sim (1 \pm 0.2) \times 10^5\ M^{-1}$).

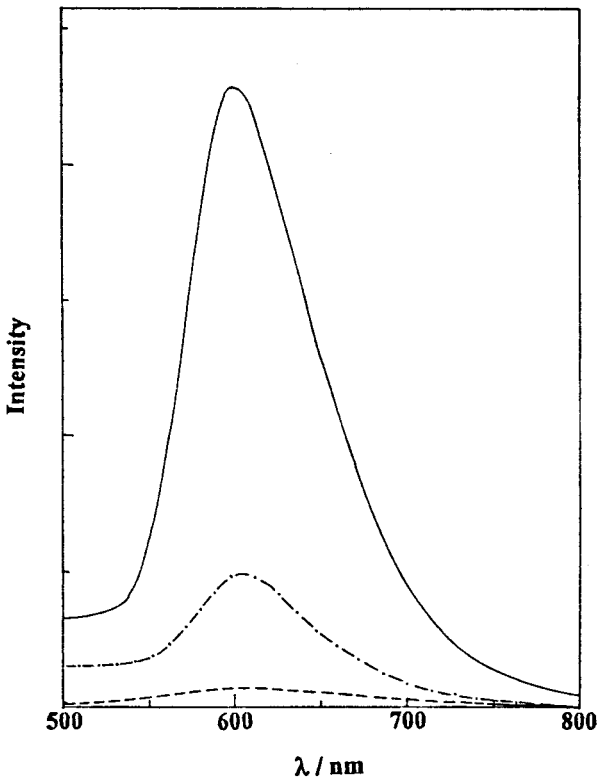

Figure 7. Luminescence spectra ($\lambda_{exc} = 440$ nm) of 10 μM $[Ru(phen)_2(hqdppz)]^{2+}$ (obtained by dithionite reduction of $[Ru(phen)_2(qdppz)]^{2+}$) in water (---), 0.1 M SDS (−•−), and CH_3CN/H_2O (10:1, v/v) (—).

buffer A, and micellar solutions.[17] $[Ru(phen)_2(hqdppz)]^{2+}$, as obtained by in situ dithionite reduction, was also found to be essentially nonluminescent in aqueous solutions with or without buffer A, as was the case with its oxidized form. However, this reduced complex showed MLCT[47] luminescence ($\lambda_{em(max)} = 601$ nm) in micellar and aqueous CH_3CN solutions with quantum yields of approximately 0.002 and 0.01, respectively (Figure 7).

The weak luminescence observed for $[Ru(phen)_2(qdppz)]^{2+}$ in nonaqueous solvents can be rationalized, primarily, in terms of an intramolecular photoinduced electron transfer (PET) quenching of its MLCT state by the appended quinone fragment. Metal complexes bound to quinone-substituted ligands, such as $Re(qdppz)(CO)_3Cl$[23] and $[Ru(bpy)_2(bpy\text{-}BQ)]^{2+}$ (where bpy-BQ is a quinone appended bipyridine ligand),[57] were reported earlier. In both the cases, a PET from the MLCT state to the quinone acceptor was proposed to be responsible for the apparent lack of emission from these complexes. In the case of $[Ru(phen)_2(qdppz)]^{2+}$, an additional process, involving the sensitivity of the excited state to quenching by water and the subsequent increase in the nonradiative decay rate in an aqueous environment, can also be invoked. Indeed, in aqueous solutions, the excited state of $[Ru(phen)_2(dppz)]^{2+}$ was reported to be highly quenched due to proton transfer from the solvent to the dipyridophenazine ligand. [58−62]

As far as $[Ru(phen)_2(hqdppz)]^{2+}$ is concerned, the PET reaction does not operate in this hydroquinone-containing complex. Thus, the lack of luminescence observed in water and in aqueous buffered solutions can be explained solely on the basis of a proton-transfer quenching of the excited state of the complex. As expected, "partial recovery" occurs in SDS and CH_3CN solutions, where the complex can, in principle, reside in an increasingly more hydrophobic micellar environment and the dipyridophenazine ligand is protected from water. These observations were quite helpful in our attempt to distinguish between the DNA-binding modes of these complexes as discussed below.

While the lack of luminescence for the DNA-intercalated oxidized form can be due, in most part, to an intramolecular electron-transfer quenching, that for the reduced complex is quite curious and, moreover, is in stark contrast to the luminescence characteristics of $[Ru(phen)_2(dppz)]^{2+}$ in the presence of DNA. The strong binding of $[Ru(phen)_2(dppz)]^{2+}$ to DNA was reported to give rise to the so-called "molecular light switch effect", where the nearly undetectable emission from the MLCT excited state in water becomes strongly enhanced upon binding, assigned to intercalation of the planar dppz ligand between the base pairs of DNA.[58−61] The apparent lack of emission from $[Ru(phen)_2(hqdppz)]^{2+}$ in the presence of DNA thus clearly indicates that the dipyridophenazine part of hqdppz is residing in a hydrophilic environment.[62] This is possible if either this hydroquinone ligand is not a good intercalator or the complex is intercalating through its phen ligand. Both of these suppositions are consistent with the results of DNA binding of $[Ru(phen)_2(hqdppz)]^{2+}$ as probed by absorption titration, viscometry, differential-pulse voltammetry, and the topoisomerase assay (vide supra).

D. DNA Photocleavage. Control runs in the agarose gel electrophoresis experiments suggested that untreated plasmid pBR 322 DNA does not show any perceptible cleavage in the dark and even upon irradiation by 440 ± 5 nm light for 2 h (lanes 1 and 2, Figure 8). Similarly, DNA nicking was not observed for the plasmid treated with $[Ru(phen)_2(qdppz)]Cl_2$ in the dark run (lane 3). However, increased streaking and retardation in the DNA mobility are seen due to intercalation by this complex.[43] Irradiation of DNA in the presence of the complex for 25 min caused complete conversion of the supercoiled form (form I), generating relaxed circular DNA (form II) under similar experimental conditions (lane 4). The reduced complex, obtained from dithionite reduction of $[Ru(phen)_2(qdppz)]Cl_2$, was also seen to cleave DNA, albeit with less efficiency (see lanes 4 and 5). In summary, these results demonstrate that the DNA-photocleavage efficiencies of the two new complexes follow a trend that is consistent not only with their DNA-binding abilities but also with the known capabilities of Co(III) and Ru(II) complexes containing the parent ligand dppz.[17]

(58) Goulle, V.; Harriman, A.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun*. **1993**, 1034.
(59) Turro, C.; Bossman, S. H.; Jenkins, Y.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc*. **1995**, *117*, 9026.
(60) Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc*. **1994**, *116*, 10286.
(61) Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acids Res*. **1991**, *19*, 2595.

(62) A reviewer has suggested that intercalation of $[Ru(phen)_2(hqdppz)]^{2+}$ might thus involve the "hydroquinone" part of hqdppz, as is the case with several anthracycline antibiotics, but that it is sterically reasonable for the "dipyridophenazine" part of both hqdppz and qdppz to intercalate. We believe that the former mode of binding is more likely because binding by the "hydroquinone" part would leave the "dipyridophenazine" part of the ligand to the aqueous phase, thus explaining the nonluminescent nature of the complex in DNA solutions. In the latter case, the "quinone" and "hydroquinone" parts of these ligands might cause steric clashes with the DNA at the intercalation site. Such a steric clash is expected to render $[Ru(phen)_2(qdppz)]^{2+}$ a weakly binding complex, and this, certainly, is not the case. As far as $[Ru(phen)_2(hqdppz)]^{2+}$ is concerned, intercalation by the "dipyridophenazine" part of hqdppz and subsequent steric clash of the "hydroquinone" part with the DNA would probably rationalize the lower K_b value observed for this complex, but they are inconsistent with its nonluminescent nature in DNA solutions.

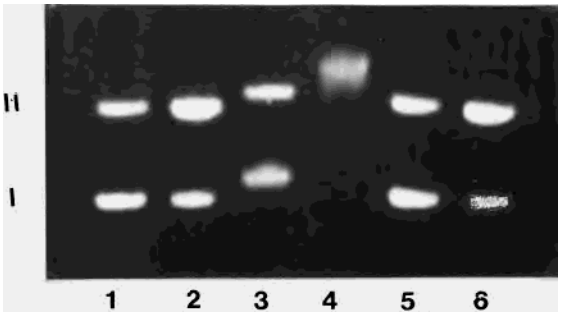

Figure 8. Light-induced nuclease activities of $[Ru(phen)_2(qdppz)]^{2+}$ and $[Ru(phen)_2(hqdppz)]^{2+}$. Dark and light experiments: lanes 1 and 2, untreated pBR 322 (100 μM) in the dark and upon irradiation; lanes 3 and 4, pBR 322 + $[Ru(phen)_2(qdppz)]^{2+}$ in the dark and upon irradiation; lanes 5 and 6, pBR 322 + $[Ru(phen)_2(hqdppz)]^{2+}$ in the dark and upon irradiation. In each case, [DNA nucleotide phosphate]/[drug] = 10.

A comparison could made of the DNA-photocleavage abilities of $[Ru(phen)_2(qdppz)]^{2+}$ and $[Ru(phen)_2(hqdppz)]^{2+}$ with those of the related complexes $[Ru(phen)_3]Cl_2$ and $[Ru(phen)_2(dppz)]Cl_2$ by the agarose gel electrophoresis method. Under comparable experimental conditions, DNA-nicking efficiencies of these complexes were seen to roughly follow the trend $[Ru(phen)_3]Cl_2 \ll [Ru(phen)_2(hqdppz)]Cl_2 \leq [Ru(phen)_2(dppz)]Cl_2 < [Ru(phen)_2(qdppz)]Cl_2$.[63] While DNA photocleavage by $[Ru(phen)_3]Cl_2$ has been reported[64,65] to involve an $^1O_2$-based mechanism and, to a large extent, that by $[Ru(phen)_2(dppz)]Cl_2$ is also expected to involve oxygen-centered reactive species including $^1O_2$,[66] the natures of the reactive intermediates as well as the mechanisms of their actions involved in the efficient DNA photocleavages by the two new complexes observed in this study have not yet been explored in detail. However, it is interesting to note that whereas excitation of $[Ru(phen)_2(hqdppz)]^{2+}$ at 440 nm can activate only the MLCT state, that of $[Ru(phen)_2(qdppz)]^{2+}$ can, in principle, activate both its MLCT and localized quinone ($\pi-\pi^*$) states owing to a partial overlap of the corresponding absorption bands (see Figure 3). While irradiation into the MLCT band of $[Ru(phen)_2(qdppz)]^{2+}$ can generate a species containing oxidized ruthenium and reduced qdppz ($1e^-$ transfer), direct excitation of the bound qdppz is expected to provide the triplet quinone. Both these quinone-based, transient species are known to be potent DNA-cleaving agents capable of reacting with the duplex via various mechanisms,[38,67] thus explaining the superior DNA-nicking ability of $[Ru(phen)_2(qdppz)]^{2+}$ compared to the other complexes investigated in this study.

Conclusions

In summary, the new ruthenium(II) complex $[Ru(phen)_2(qdppz)]^{2+}$, endowed with a novel, quinone-fused dipyridophenazine ligand, is not only an avid binder of DNA but also an efficient photocleaver of the plasmid. The corresponding reduced species, $[Ru(phen)_2(hqdppz)]^{2+}$, also binds and photocleaves DNA, albeit with less efficiency. These results, together with our earlier finding that the redox couple $[Ru(phen)_2(qdppz)]^{2+}/[Ru(phen)_2(hqdppz)]^{2+}$ represents an "electro−photo switch",[17] testify to the importance of quinone/hydroquinone moieties present in these complexes and further suggest that they may be useful in the design of photonucleases and molecule-based electronic devices.

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(64) The observed differences in the DNA-photocleavage efficiencies of these complexes do not seem to arise from differences in their light absorption abilities, since their molar extinction coefficients at 440 nm are found to be close to each other ($\log \epsilon = 4.3 \pm 0.2$).
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(67) The excited state of this complex was seen to generate $^1O_2$ in DMF, as evidenced by the decrease of absorbance due to 1,3-diphenylisobenzofuran present in solution during the irradiation.