Dipyridophenazine Complexes of Cobalt(III) and Nickel(II): DNA-Binding and Photocleavage Studies

S. Arounaguiri and Bhaskar G. Maiya*

School of Chemistry, University of Hyderabad, Hyderabad 500 046, India

Received July 13, 1995

Introduction

Metal complexes of the type $[M(LL)_3]^{n+}$, where LL is either 1,10-phenanthroline (phen) or a modified phen ligand, are particularly attractive species for developing new diagnostic and therapeutic agents that can recognize and cleave DNA.[1,2] The ligands or the metal in these complexes can be varied in an easily controlled manner to facilitate an individual application, thus providing an easy access for the understanding of details involved in DNA-binding and cleavage.[1,3] Currently, a great deal of attention is being paid to DNA interactions of mixed-ligand ruthenium(II) complexes that contain both phen (or bpy = 2,2′-bipyridyl) and modified phen (or modified bpy) ligands, the latter of which was so designed to augment the intercalative interaction by the complexes.[4−8] Among various such complexes studied so far, $[Ru(phen)_2DPPZ]^{2+}$ and $[Ru(bpy)_2DPPZ]^{2+}$ (DPPZ = dipyrido[3,2-*a*:2′,3′-*c*]phenazine, a modified phenanthroline ligand) have been reported to be avid binders of DNA and more importantly, to be remarkable luminescent reporters of DNA structure.[5] In addition, it has been recently shown, by Barton and co-workers, that the application of mixed-ligand ruthenium(II) complexes of the type $[Ru(phen)_2(LL')]^{2+}$ (LL′ = a modified phenanthroline ligand belonging to the DPPZ family) permits variations in geometry, size, hydrophobicity, and hydrogen-bonding ability of the complexes and allows a variation in the strength of their DNA-binding and "light-switching" ability.[6]

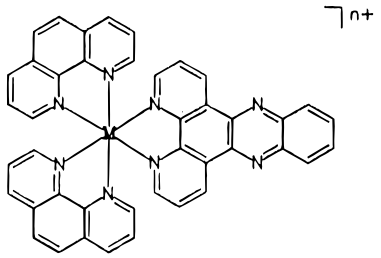

Figure 1. Structures of the investigated complexes.

Notwithstanding this well-documented importance of DPPZ in DNA interactions of the complexes containing it, binding studies using such complexes having a metal ion other than ruthenium have attracted much less attention; exceptions being recent reports on $[Os(phen)_2DPPZ]^{2+}$ and $[Re(py)_2(CO)_3DPPZ]^{+}$ (py = pyridine).[9] Moreover, apart from a study which reports on the electrochemically initiated cleavage of DNA by $[Ru(O)(tpy)DPPZ]^{2+}$ (tpy = terpyridine),[8] to our knowledge, nuclease activity of no other DPPZ complex has ever been tested. Clearly, further studies using various $[M(phen)_2DPPZ)]^{n+}$ complexes are needed to evaluate the influence of metal-ion-induced geometry, charge, spin-state, redox potential, etc. changes on the DNA binding and cleavage mechanisms in this important class of complexes as was the case with the previously reported metallodervatives (M = Ru, Rh, Co, Cr, etc.) of phen or modified phen ligands.[1,3,10] Such studies are also needed to serve as complementary studies to those involving $[Ru(phen)_2(LL')]^{2+}$ type complexes mentioned above. This manuscript reports on the synthesis, characterization, DNA-binding, and photochemical DNA cleavage characteristics of $[M(phen)_2DPPZ)]^{n+}$ where M = Co(III) or Ni(II) and n = 3 or 2, respectively. Structures of these investigated complexes are given in Figure 1.

Experimental Section

Materials. All common chemicals, solvents as well as cobalt(II) and nickel(II) salts, 1,10-phenanthroline monohydrate and 1,2-diaminobenzene were purchased either from BDH (Bombay, India) or Merck (Bombay, India). CT DNA, tetrabutylammonium chloride ((TBA)Cl) and tetrabutylammonium hexafluorophosphate ((TBA)PF_6) were obtained from Sigma Chemicals. The supercoiled pBR 322 DNA (Bangalore Genie, Bangalore, India) was used as received. Agarose (molecular biology grade) and ethidium bromide were purchased from Bio-Rad. All the solvents were purified before use as per the standard procedures.[11] Deionized, triply distilled water was used for preparing various buffers.

(1) See for example (and also references therein): (a) Sigman, D. S. *Acc. Chem. Res*. **1986**, *19*, 180. (b) Barton, J. K. *Science* **1986**, *233*, 727. (c) *Metal-DNA Chemistry*; Tullis, T. D., Ed.; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989. (d) Pyle, A. M.; Barton, J. K. *Prog. Inorg. Chem*. **1990**, *38*, 413. (e) Turro, N. J.; Barton, J. K.; Tamalia, D. A. *Acc. Chem. Res*. **1991**, *24*, 332. (f) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev*. **1993**, 93, 2295. (g) Murphy, C. J.; Barton, J. K. *Methods Enzymol*. **1993**, *226*, 576.

(2) For recent examples, see: (a) Tysoe, S. A.; Morgan, R. J; Baker, A. D.; Strekas, T. C. *J. Phys. Chem*. **1993**, *97*, 1707. (b) Carlson, D. L.; Huchital, D. H.; Mantilla, E. J.; Sheardy, R. D.; Murphy Jr., W. R. *J. Am. Chem. Soc*. **1993**, *115*, 6424. (c) Liu, F. L., Meadows, K. A.; McMillin, D. R. *J. Am. Chem. Soc*. **1993**, *115*, 6699. (d) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *32*, 2573. (e) Lecomte, J. P.; Mesmaeker, A. K. D.; Orellana, G. *J. Phys. Chem*. **1994**, *98*, 5382. (f) Naing, K.; Takashani, M.; Taniguchi, M.; Yamagishi, A. *Inorg. Chem*. **1995**, *34*, 350.

(3) (a) Barton, J. K.; Raphael, A. L. *J. Am. Chem. Soc*. **1984**, *106*, 2466. (b) Kelly, J. M.; Tossi, A. B.; McConnell, D. J.; OhUigin, C. *Nucleic Acid. Res*. **1985**, *13*, 6017. (c) Barton, J. K.; Raphael, A. L. *Proc. Natl. Acad. Sci. U.S.A*. **1985**, *82*, 6460. (d) Krischenbaum, M. R.; Tribolet, R.; Barton, J. K. *Nucleic Acid Res*. **1988**, *16*, 7943. (e) Mei, H. -Y; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A*. **1988**, *85*, 1339. (f) Sammes, P. G.; Yahioglu, G. *Chem. Soc. Rev*. **1994**, 327.

(4) (a) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc*. **1989**, *111*, 3051. (b) Tossi, A. B.; Kelly, J. M. *Photochem. Photobiol*. **1989**, *49*, 545 (c) Morgan, R. J.; Chatterjee, S.; Baker, A. D.; Strekas, T. C. *Inorg. Chem*. **1991**, *30*, 2687. (d) Sitlani, A. S.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc*. **1992**, *114*, 2303.

(5) (a) Friedman, A. E.; Chambron, J. C.; Sauvage, J. P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc*. **1990**, *112*, 4960. (b) Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acid Res*. **1991**, *19*, 2595. (c) Chambron, J.-C.; Sauvage, J.-P. *Chem. Phys. Lett*. **1991**, *182*, 603. (d) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809. (e) Turro, C.; Bossman, S. H.; Jenkins, Y.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc*. **1995**, *117*, 9026.

(6) Hartshorn, R. M.; Barton, J. K. *J. Am. Chem. Soc*. **1992**, *114*, 5919.

(7) Hiort, C. H.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc*. **1993**, *115*, 3448.

(8) Gupta, N.; Grover, N.; Neyhart, G. A.; Liang, W.; Singh, P.; Thorp, H. H. *Angew. Chem., Int. Ed. Engl*. **1992**, *31*, 1048.

(9) (a) Holmlin, R. E.; Barton, J. K. *Inorg. Chem*. **1995**, *34*, 7. (b) Yam, V. W.-W.; Lo, K. K.-W.; Cheung, K.-K.; Kong, R. Y.-C. *J. Chem. Soc., Chem. Commun*. **1995**, 1191.

(10) (a) Barton, J. K.; Paranawithana, S. R. *Biochemistry* **1986**, *25*, 2205. (b) Subramanian, R.; Meares, C. F. *J. Am. Chem. Soc*. **1986**, *108*, 6427. (c) Basile, L. A.; Barton, J. K. *J. Am. Chem. Soc*. **1987**, *109*, 7548. (d) Basile, L. A.; Raphael, A. L.; Barton, J. K. *J. Am. Chem. Soc*. **1987**, *109*, 7550.

(11) Perrin, D. D.; Armango, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon: Oxford, England, 1986.

S0020-1669(95)00868-8 CCC: $12.00

Synthesis. **$[Co(phen)_2DPPZ](PF_6)_3\cdot 5H_2O$.** To a 50 mL ethanolic solution of $[Co(phen)_2Cl_2]Cl\cdot 3H_2O$[12] (578 mg, 1 mM) was added a 423 mg (1.5 mM) sample of DPPZ.[13] The resulting solution was refluxed for 1 h and further stirred for 4−5 h under nitrogen. It was filtered, and the complex was precipitated upon addition of a saturated ethanolic solution of ammonium hexafluorophosphate. The complex was filtered and further dried under vacuum before being recrystallized (acetone−ether).

Anal. Calcd for $C_{42}H_{34}N_8O_5P_3F_{18}Co$: C, 41.74; H, 2.84; N, 9.27. Found: C, 41.13; H, 2.95; N, 9.13. FABMS (*m/z*): $[M - PF_6]^+$, 991; $[M - 2PF_6]^{2+}$, 846. IR (KBr): 3400, 1608, 1523, 1506, 1433, 858, and 715 cm^{-1}. UV−visible (CH_3CN), λ_{max}, nm (log ϵ): 220 (5.22), 282 (5.11), 330 (4.31), 348 (4.23), 359 (4.29), and 377 (4.23). ^1H NMR (DMSO-d_6, 200 MHz), ppm (TMS): 9.95 (dd, 2H), 9.21 (d, 2H), 9.18 (d, 2H), 8.59 (m, 8H), 8.32 (d, 4H), 8.00 (m, 4H), 7.68 (d, 4H).

$[Ni(phen)_2DPPZ](PF_6)_2\cdot 4H_2O$. This complex was prepared, starting from $[Ni(phen)_2Cl_2]$[14] (490 mg, 1 mM) and DPPZ (423 mg, 1.5 mM), in a manner analogous to that described above for the cobalt complex.

Anal. Calcd for $C_{42}H_{34}N_8O_4P_2F_{12}Ni$: C, 47.44; H, 3.22; N, 10.54. Found: C, 47.36; H, 3.01; N, 10.45. FABMS (*m/z*): $[M - PF_6]^+$, 845; $[M - phen - 2PF_6]^{2+}$, 520. IR (KBr): 3408, 1624, 1516, 1494, 1423, 848, and 727 cm^{-1}. UV−visible (CH_3CN), λ_{max}, nm (log ϵ): 226 (4.92), 273 (5.03), 325 (4.14), 348 (4.12), 358 (4.13), and 376 (4.12). μ_{eff} (solid, 293 ± 2 K): 3.16 μ_B.

Each hexafluorophosphate salt was dissolved in minimum amount of acetone, and a saturated solution of (TBA)Cl in acetone was added dropwise until the precipitation was complete. The water-soluble chloride salts thus obtained were filtered, washed thoroughly with acetone, and vacuum dried. Recovery was about 90% of the theoretical yield in each case.

Methods. UV−visible and IR spectra were recorded with Shimadzu Model UV-160 A (coupled with a temperature controller Model TCC-240 A) and JASCO Model FT-IR spectrophotometers, respectively. The ^1H NMR spectra were recorded with a Bruker NR-FT 200 spectrometer, and magnetic susceptibility measurements were carried out using a CAHN (Model 6612) magnetic susceptibility system. Cyclic and differential-pulse voltammetric experiments were performed on a Princeton Applied Research electrochemical work station as described previously.[15] FABMS spectra were recorded with a JEOL SX 102/DA-600 mass spectrometer.

DNA-Binding and Cleavage Experiments. The concentration (base-pairs) of CT DNA was measured by using its known extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}).[16] Buffer A (5 mM tris, pH 7.1, 50 mM NaCl) was used for absorption titration experiments and buffer B (1 mM phosphate, pH 7.0, 2 mM NaCl) was used for thermal denaturation and differential-pulse voltammetric experiments. The chloride salts of the complexes were used in studies with DNA. Absorption titration experiments were performed by maintaining the metal complex concentration constant (10−15 μM) and varying the nucleic acid concentration (0−100 μM). The absorption data were analyzed for an evaluation of the intrinsic binding constant, K_b, using a reported procedure[17] which had been previously employed for obtaining the K_b values of several mixed-ligand ruthenium (II) complexes.[4a] 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 each investigated complex. The melting temperature (T_m) and the curve width σ_T (=temperature range between which 10% and 90% of the absorption increase occurred) were calculated as described.[3b] Differential-pulse voltammetric experiments (highly polished glassy carbon electrode) were performed for 0.1 mM of the complexes in the absence and in the presence of increasing amounts (0−3 mM) of CT DNA.

For the gel electrophoresis experiments, supercoiled pBR 322 DNA (100 μM) in tris-HCl buffer (pH = 8.0) was treated with an 100 μM of the metal complex, and the mixture was 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 of ethidium bromide for 0.5 h after which it was analyzed using the UVP gel documentation system GDS 2000 and was also directly photographed and developed as described previously.[18] Irradiation experiments were carried out by keeping the pre-incubated (dark, 1 h) samples inside the sample chamber of a JASCO Model FP-777 spectrofluorimeter ($\lambda = 350 \pm 5$ nm; slit width = 5 nm)

Results and Discussion

Synthesis and Characterization. $[Co(phen)_2DPPZ]^{3+}$ and $[Ni(phen)_2DPPZ]^{2+}$ were synthesized by the reaction of the corresponding dichloro complexes with DPPZ. Each new complex showed satisfactory elemental analysis, FABMS and IR data (see Experimental Section). While the d^6, diamagnetic cobalt complex was further characterized by its ^1H NMR spectrum, the nickel complex, upon incorporating the diamagnetic correction, gave a μ_{eff} of 3.16 μ_B consistent with the presence of a d^8, paramagnetic nickel(II) ion in it.[14] The UV−visible spectra (200−400 nm) of both the complexes were seen to be dominated by bands due to the transitions involving phen and DPPZ ligands.[19] On the basis of the spectral data of DPPZ, $[Ru(phen)_2DPPZ]^{2+}$,[5d] and $[Ru(bpy)_2DPPZ]^{2+}$,[13b] the bands seen at 348, 360, and 376 nm in both $[Co(phen)_2DPPZ]^{3+}$ and $[Ni(phen)_2DPPZ]^{2+}$ have been assigned to $\pi-\pi^*$ transitions of the metal-bound DPPZ.

The cyclic voltammogram of $[Co(phen)_2DPPZ]^{3+}$ (CH_3CN, 0.1 M (TBA)PF_6; Pt-working electrode) showed peaks at +0.40, −0.95, −1.17, −1.70, and −1.83 V (*vs* SCE). Wave analysis suggested that while the peaks observed at +0.40, −0.95, and −1.17 V represent diffusion-controlled, reversible/quasi-reversible, one-electron transfer reactions,[20] those observed at −1.70 and −1.83 V are totally irreversible under identical experimental conditions. On the basis of the reported electrochemical data of $[Co(phen)_3]^{2+}$ [21] (+0.39, −0.96 V under our experimental conditions), the peak observed in the positive scan region for $[Co(phen)_2DPPZ]^{3+}$ can be ascribed to the Co(III)/Co(II) redox couple and that observed at −0.95 V to the Co(II)/Co(I) couple. The peaks at −1.17, −1.70, and −1.83 V have been assigned to electron additions onto the Co(III)-bound DPPZ and phen ligands, respectively.[19] The ligands on $[Ni(phen)_2DPPZ]^{2+}$ could be reduced at −1.29 and −1.72 V (DPPZ) and at −1.90 V (phen).

DNA-Binding Studies. Addition of increasing amounts of CT DNA resulted in hypochromism and bathochromic shifts of the peak maxima in the UV−visible spectra of both $[Co(phen)_2DPPZ]^{3+}$ and $[Ni(phen)_2DPPZ]^{2+}$. As seen in Figure 2a, the lowest energy band of $[Ni(phen)_2DPPZ]^{2+}$ shows a bathochromic shift of 7 nm in the presence of CT DNA. The

(12) Vlcek, A. A. *Inorg. Chem.* **1967**, *6*, 1425.
(13) (a) Dickenson, J. E.; Summers, L. A. *Aust. J. Chem.* **1970**, *23*, 1023. (b) Amouyal, E.; Homsi, A.; Chambron, J.-C; Sauvage, J.-P. *J. Chem. Soc., Dalton Trans* **1990**, 1841.
(14) Harris, C. M.; Mckenzie, E. D. *J. Inorg. Nucl. Chem.* **1967**, *29*, 1047.
(15) (a) Sirish, M.; Maiya, B. G. *Photochem. Photobiol. A: Chem.* **1994**, *85*, 127. (b) Rao, T. A.; Maiya, B. G. *Polyhedron* **1994**, *13*, 1863.
(16) (a) Reichmann, M. E.; Rice, S. A.; Thomas, C. A.; Doty, P. *J. Am. Chem. Soc.* **1954**, *76*, 3047.
(17) Wolfe, A.; Shimer, G. H.; Meehan, T. *Biochemistry* **1987**, *26*, 6392.
(18) (a) Mehta, G.; Sambaiah, T.; Maiya, B. G.; Sirish, M.; Chatterjea, D. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2667. (b) Mehta, G.; Sambaiah, T.; Maiya, B. G.; Sirish, M.; Dattagupta, A.; *Tetrahedron. Lett.* **1994**, *35*, 4201. (c) Mehta, G.; Sambaiah, T.; Maiya, B. G.; Sirish, M.; Dattagupta, A. *J. Chem. Soc. Perkin Trans.* 1. **1995**, 295.
(19) (a) Ackermann, M. N.; Interrante, L. V. *Inorg. Chem.* **1984**, *23*, 3904. (b) Fees, J.; Kaim, W.; Moscherosch, M.; Matheis, W.; Klima, J.; Krejcik, M.; Zalis, S. *Inorg. Chem.* **1993**, *32*, 166.
(20) Here 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 potentials, respectively and $\Delta E_p = 60-80$ mV where E_p is the peak potential for these reductions (see: Nicholson, R. S.; Shain, I. *Anal. Chem.* **1964**, *36*, 706).
(21) Maki, N.; Tanaka, N. In *Encyclopedia of Electrochemistry of The Elements*; Bard, A. J., Ed.; Marcel Dekker: New York, 1975; Vol. 3, pp 43−210 and references cited therein.

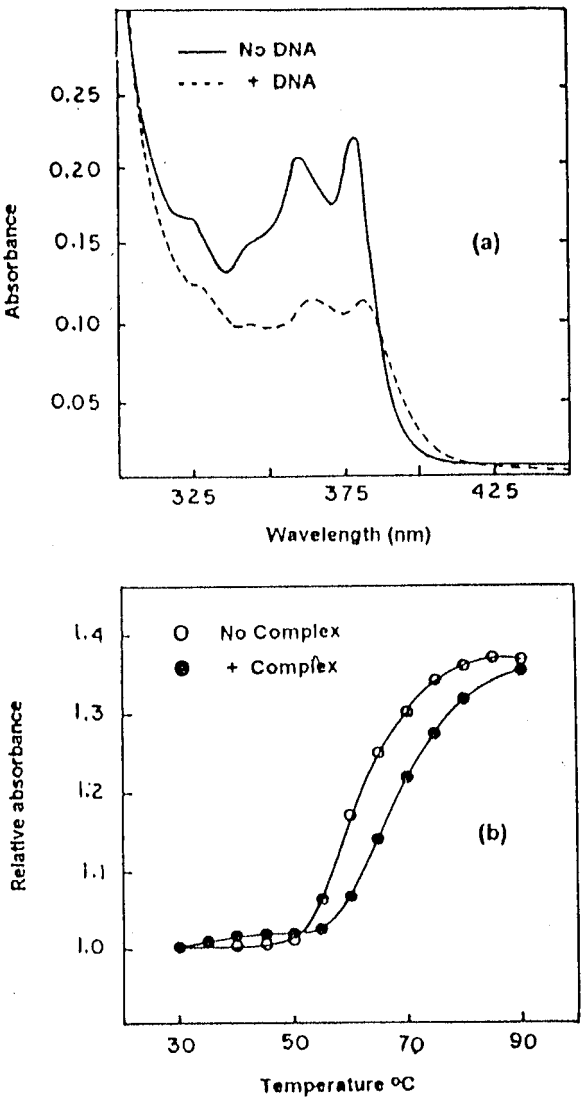

Figure 2. (a) UV−visible spectra (buffer A) of $[Ni(phen)_2DPPZ]^{2+}$ (15 μM) in the absence and in the presence of CT DNA (40 μM) (b) Melting curves (buffer B) of CT DNA (160 μM) in the absence and in the presence of $[Co(phen)_2DPPZ]^{3+}$ (8 μM).

corresponding band in the cobalt complex, under similar experimental conditions, was seen to be red-shifted by 6 nm. Absorption titration curves with the absorbance being measured between 300 and 430 nm revealed that isosbestic points are present at 388 and 416 nm and at 385 and 414 nm for the nickel (Figure 2a) and cobalt complexes, respectively. These spectral changes are reminiscent of those reported for DNA interactions of several tris-chelated, mixed-ligand complexes containing phen and/or DPPZ including $[Ru(phen)_2DPPZ]^{2+}$.[1,4−7] The intrinsic binding constant, K_b, which was obtained by monitoring the change in absorbance at 376 nm with increasing concentration of DNA is as high as $(9 \pm 2) \times 10^5$ M^{-1} for both the complexes investigated in this study.[22] $[Ru(bpy)_2DPPZ]^{2+}$, $[Ru(phen)_2DPPZ]^{2+}$, $[Os(phen)_2DPPZ]^{2+}$, and $[Ru(H_2O)DPPZ(tpy)]^{2+}$ have been reported to strongly bind to DNA ($K_b \cong 10^6-10^7$ M^{-1}) by an intercalative mode.[5−9] Results of various spectroscopic and biochemical studies have suggested that it is the DPPZ ligand that intercalates between the base-pairs of DNA in these mixed-ligand complexes. On the basis of the similarities in structures, absorption titration characteristics, and apparent binding constants between the previously studied DPPZ complexes mentioned above and $[Co(phen)_2DPPZ]^{3+}$ and $[Ni(phen)_2DPPZ]^{2+}$, it can be suggested that DNA-binding by the latter complexes also involves an intercalation of DPPZ. This suggestion is further supported by thermal denaturation and differential-pulse voltammetric experiments, the results of which are summarized below.

Thermal denaturation experiments carried out on CT DNA in the absence of any added complex revealed that the T_m and σ_T values for the duplex are 60 ± 2 and 22 ± 1 °C, respectively, under our experimental conditions. Addition of $[Co(phen)_2DPPZ]^{3+}$ or $[Ni(phen)_2DPPZ]^{2+}$ ([DNA]/[complex] = 20) increased T_m by 8 ± 1 °C for the former (see Figure 2b) and by 6 ± 1 °C for the latter complex.[23] By contrast, addition of either $[Co(phen)_3]^{3+}$ or $[Ni(phen)_3]^{2+}$ to DNA increased the T_m value by <5 °C. The σ_T values are 24 ± 1 and 26 ± 1 °C for $[Co(phen)_2DPPZ]^{3+}$ and $[Ni(phen)_2DPPZ]^{2+}$, respectively.

Differential-pulse voltammetric experiments carried out for $[Co(phen)_3]^{3+}$ and $[Co(phen)_2DPPZ]^{3+}$ in both the presence and the absence of CT DNA have revealed that decrease in the peak-current due to Co^{III}/Co^{II} redox couple ($E_{1/2}$ ($Co^{III/II}$) = −0.02 V vs SCE for both the complexes in buffer B) in the presence of a 30-fold molar excess of DNA (in comparison with the peak-current in buffer B) is more pronounced for the DPPZ-containing complex (≅75%) compared to that for the tris-phen complex (≅50%). Bard et al.[24] have previously shown that addition of DNA reduces the cyclic and differential-pulse voltammetric peak-currents of the Co^{III}/Co^{II} couple for $[Co(phen)_3]^{3+}$ and have also estimated K_b to be $(1.6 \pm 0.2) \times 10^4$ M^{-1} under experimental conditions similar to those employed in this study.[24b] Our results are consistent with this data and the pronounced reduction of the peak-current observed for $[Co(phen)_2DPPZ]^{3+}$ can be explained if the affinity toward DNA is higher for this DPPZ-containing complex than that for $[Co(phen)_3]^{3+}$.[25]

Photocleavage of DNA. Only $[Co(phen)_2DPPZ]^{3+}$ was found to effect the photocleavage of the supercoiled pBR 322 DNA. Control experiments suggested that untreated DNA does not show any cleavage in the dark and even upon irradiation by a 350 nm light (compare lanes 1 and 2, Figure 3). Similarly, DNA nicking was not observed for pBR 322 treated with $[Co(phen)_2DPPZ]^{3+}$ in the dark experiments (lane 3, Figure 3). On the other hand, irradiation of DNA in the presence of the complex for 20 min caused the generation of relaxed circular DNA as shown in lane 4 of Figure 3. Increasing the irradiation time to 60 min. resulted in further relaxation of the duplex only in the presence of the complex unaffecting the untreated sample (compare lanes 2 and 5 in Figure 3).[26] Control

(22) These K_b values can be taken only as the lower limit of the effective binding constants for these complexes because they bind too strongly even at micromolar concentrations of DNA as was the case with the previously studied DPPZ complexes.[5−9] Thus, although the two complexes investigated in the present study provide a good opportunity to compare directly the binding of isosteric intercalating species of +2 and +3 charge to DNA, such a comparison could not be made.

(23) T_m and σ_T values increased with increasing addition of the metal complex as expected.[3b]

(24) (a) Carter, M. T.; Bard, A. J. *J. Am. Chem. Soc.* **1987**, *109*, 7528. (b) Carter, M. T.; Rodriguez, M.; Bard, A. J. *J. Am. Chem. Soc.* **1989**, *111*, 8901.

(25) Indeed, a rough estimate of K_b (using a method adopted from ref 24) for the binding of $[Co(phen)_2DPPZ]^{3+}$ with DNA by the differential-pulse voltammetric method is $\cong 10^6$ M^{-1}, a value that is close to the one obtained by the absorption titration method.

(26) The mobility change of pBR 322 that is evident in the presence of $[Co(phen)_2DPPZ]^{3+}$ as shown in Figure 3 was also noticed when the nickel(II) analogue was employed, but not when $[Co(phen)_3]^{3+}$ and $[Ni(phen)_3]^{2+}$ were employed in the gel electrophoresis experiments. However, no change in the mobility has been observed when the DNA samples containing $[Co(phen)_2DPPZ]^{3+}$ (irradiated or not) were ethanol precipitated prior to loading onto the gel suggesting that DNA binding by this complex involves noncovalent interactions.

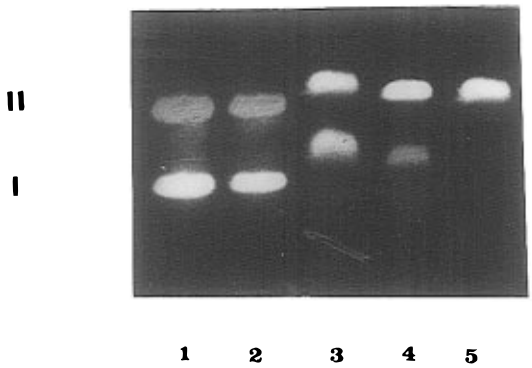

Figure 3. Light-induced nuclease activity of $[Co(phen)_2DPPZ]^{3+}$ (numbers given in brackets refer to % of the open circular form of DNA, form II, in each case) Dark and light experiments: lanes 1 and 2, untreated pBR 322 (100 μM) in the dark [55] and upon irradiation (60 min) [52]; lanes 3, 4, and 5, pBR 322 + $[Co(phen)_2DPPZ]^{3+}$ (100 μM) in the dark [46] and upon irradiation for 20 min [66] and 60 min [100], respectively.

experiments have suggested that phen and DPPZ (free ligands, dissolved in 10% DMF) are not detectably active under our irradiation conditions. In addition, no perceptible DNA cleavage was observed when samples of pBR 322 containing $[Co(phen)_3]^{3+}$ or $[Co(phen)_2(phen\text{-}dione)]^{3+}$ (phen-dione = 1,10-phenanthroline-5,6-dione, a precursor used in the synthesis of DPPZ) were irradiated at 350 nm, as both these complexes do not appreciably absorb at this wavelength.[27] Finally, the d^8 $[Ni(phen)_2DPPZ]^{2+}$ system did not show any light-induced nuclease activity, probably because of the paramagnetic nature of the complex that, in principle, would render the excited state of the molecule ineffective.

In summary, the results described in this study while underscoring the importance of DPPZ in the DNA-binding also demonstrate that substitution by different metal ions can bring about subtle modulation in the properties and, consequently, in the DNA interaction of this new class of mixed-ligand complexes containing the versatile dipyridophenazine ligand.

Acknowledgment. We are grateful to the Indo-French Centre for Promotion of Advanced Research (Centre Franco-Indien Pour la Promotion de la Recherche Avancee), New Delhi, for their financial support of this research. In addition, we thank Dr. Aparna Dattagupta for her kind help in carrying out the electrophoresis experiments and CDRL, Lucknow, India, for recording the mass spectra. We also thank the referees for their helpful and constructive suggestions. S.A. acknowledges a research fellowship from the UGC.

IC9508684

(27) However, $[Co(phen)_2DPPZ]^{3+}$, $[Co(phen)_3]^{3+}$, and $[Co(phen)_2(phen\text{-}dione)]^{3+}$ all effect DNA nicking when irradiated at 313 nm where they show more equal absorbance (log ϵ values at 313 nm are 4.43, 4.16, and 4.10 for the three complexes in that order) than at 350 nm. The relative efficiencies of the photocleavage reactions roughly follow a trend: $[Co(phen)_2DPPZ]^{3+}$, 1 > $[Co(phen)_3]^{3+}$, 0.9 > $[Co(phen)_2(phen\text{-}dione)]^{3+}$, 0.7. Further studies that inquire into the mechanism of DNA cleavage by these complexes using various "inhibitors" are currently in progress.