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Copper(II) Terpyridine Complexes: Effect of Substituent on DNA Binding and Nuclease Activity

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Mononuclear copper(II) terpyridine complexes, [Cu(ttpy)Cl]Cl (**1**) and [Cu(itpy)Cl]Cl (**2**) (ttpy = tolylterpyridine and itpy = imidazolylterpyridine) were synthesized and characterized. The interaction of the complexes with DNA was studied by electronic and CD spectroscopy, viscosity and gel electrophoresis. Absorption titrations, viscosity and CD experiments reveal an intercalative mode of DNA binding for these complexes. The binding constant values for **1** and **2** are $(5.6 \pm 0.2) \times 10^4$ and $(1.4 \pm 0.2) \times 10^4$ M^{-1}, respectively, and suggest moderate binding of these complexes to DNA. From computational studies, it was found that the aromatic π cloud is more uniformly distributed in the case of tolylterpyridine (complex **1**), which possibly leads to better stacking interactions with the DNA bases and hence a higher binding constant value for complex **1**. From the gel electrophoresis experiments, it is inferred that both complex **1** and **2** cleave plasmid DNA in the presence of ascorbic acid and the cleavage efficiency of complex **1** is greater than that of complex **2**.

Introduction

Small molecules that bind and cleave double-stranded DNA under physiological conditions have found wide applications in the field of medicine, molecular biology and biotechnology.[1–5] Transition-metal complexes characterized by high stability, structural versatility and unique spectroscopic and redox properties are exploited largely in many of these efforts. These metal complexes are capable of binding to DNA by a multitude of interactions and cleave DNA by virtue of their intrinsic chemical, electrochemical and photochemical reactivities.[6–10] In the majority of the complexes studied, the metal ion usually serves as the redox centre and the ligand is responsible for DNA recognition. The modes of recognition are primarily based upon intercalation, groove-binding, hydrogen bonding and electrostatic interactions.

Since the success of platinum complexes as anticancer drugs, studies on the interaction of transition-metal complexes with nucleic acids has been a topic of interest in biochemical and coordination chemical research.[11] Ruthenium(II) polypyridyl complexes have attracted comprehensive attention due to their significance in the development of new therapeutic agents and novel nucleic acid structural probes.[12–14] A number of dinuclear iron complexes are known to exhibit hydrolytic cleavage of duplex DNA.[15–16] Nickel(II) and cobalt(II) macrocyclic complexes have been investigated as potential site-specific DNA modification agents.[17–18] The other metal that has been most widely used in these studies is copper; complexes of copper with different types of ligands have been tested as potential chemical nucleases.[19–22] Among them, 1,10-phenanthroline and its derivatives have attracted great attention due to their high nucleolytic efficiency.[23–26] Studies by Sigman and coworkers have shown that bis(1,10-phenanthroline)copper(I) complex efficiently cleaves DNA through the formation of a sugar hydrogen abstracting active oxygen species.[27–28] They have also been broadly used as foot printing agents of both proteins and DNA,[29] probes of the dimensions of the minor groove of duplex structures,[30] and identifiers of transcription starting sites.[31]

Our laboratory has been focusing on DNA interaction studies of bis-chelated copper(II) complexes with various tridendate terpyridine ligand systems.[32,33] The present work stems from our interest to explore the DNA binding properties and nuclease activity of 1:1 copper(II) complex with these ligand systems and to examine whether they exhibit a significant change in the binding properties as compared to the bis-chelated complexes. In this paper, we report the synthesis and DNA interaction and cleavage studies of copper(II) complexes with two terpyridine derivatives, namely tolyl terpyridine (ttpy), and imidazolyl terpyridine (itpy) ligands, the structures of which are shown in Figure 1. The DNA interaction has been investigated using a host of physical methods like electronic absorption spectroscopy, viscosity measurements, electrochemical methods and circular dichroic studies and the cleavage reactions have been assayed using agarose gel electrophoresis.

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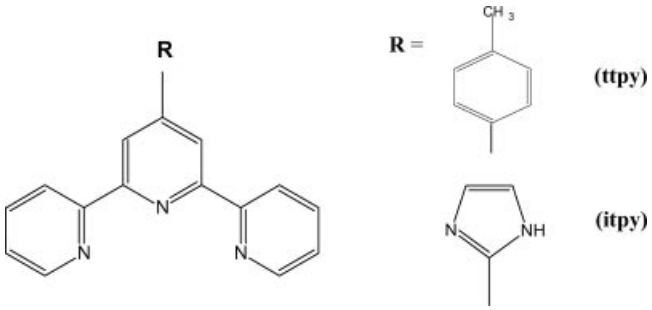

Figure 1. The structures of the ttpy and itpy ligands.

Results and Discussion

Synthesis and Spectral Properties

Complexes **1** and **2** could be obtained in good yields and sufficiently pure forms by mixing hot methanolic solutions of copper chloride and the respective terpyridine ligands in equimolar quantities. The authenticity of the complexes was ascertained from mass spectral analysis. The ESI mass spectra of the complexes show base peaks assignable to the parent ion $[Cu{\cdot}L{\cdot}Cl]^+$, where L corresponds to ttpy and itpy for complexes **1** and **2**, respectively.

The electronic spectra of the complexes exhibit intense intraligand bands in the region 280–350 nm and a broad ligand field band in the region 550–800 nm (Table 1). The absorptions in the UV region show a shift to higher wavelengths relative to the unsubstituted terpy complex (228 and 324 nm). This shift is due to the lowering of the LUMO (π*) energy in the ttpy ligand owing to a more extended conjugation relative to the terpy ligand. In the case of copper(II) complexes, three d–d transitions are possible: $d_{xz},d_{yz}\rightarrow d_{x^2-y^2}$, $d_{z^2}\rightarrow d_{x^2-y^2}$, $d_{xy}\rightarrow d_{x^2-y^2}$. However, only a single broad band is observed for both the copper(II) complexes, which indicates the total sum of all the above transitions. The broadness associated with the d–d bands is generally taken as an indication of the geometrical distortion of the complex from perfect planar symmetry.

Table 1. Electronic and EPR (taken at 77 K) spectral parameters for complexes **1** and **2**.

Complex	Electronic spectra[a] λ [nm] (ε, $M^{-1}cm^{-1}$)	g values	A values
Complex **1**	287 (18,050) 318 sh (11,800) 690 br (160)	$g_\parallel = 2.36$ $g_\perp = 2.06$	$A_\parallel = 161$ G
Complex **2**	288 (17,765) 338 (22,995 350 sh (23,595) 710 br (153)	$g_\parallel = 2.39$ $g_\perp = 2.08$	$A_\parallel = 165$ G

[a] br = broad, sh = shoulder.

EPR spectra of complexes **1** and **2** (frozen solutions at 77 K) in DMSO reveal an axial nature with four well-resolved peaks of low intensity in the low-field region and one intense peak in the high-field region. No band corresponding to $M_s = \pm 2$ was observed in the spectra, ruling out any copper–copper interaction. Figure 2 shows the EPR spectrum of complex **1** and the EPR parameters are provided in Table 1. From the observed values for both complex **1** and **2**, it is clear that $g_\parallel > g_\perp$, which indicates that the unpaired electron predominantly lies in the $d_{x^2-y^2}$ orbital.[34] The ratio of $g_\parallel/A_\parallel$ may be considered as a diagnostic parameter of the stereochemistry of the complex, and the range reported for square-planar complexes is 105–135 G.[35] In the present case, this value is higher than 135 G, indicating a considerable tetrahedral distortion around the copper site that could be due to the rigidity of the terpy moiety and the geometrical constraint it imposes on the central metal ion.

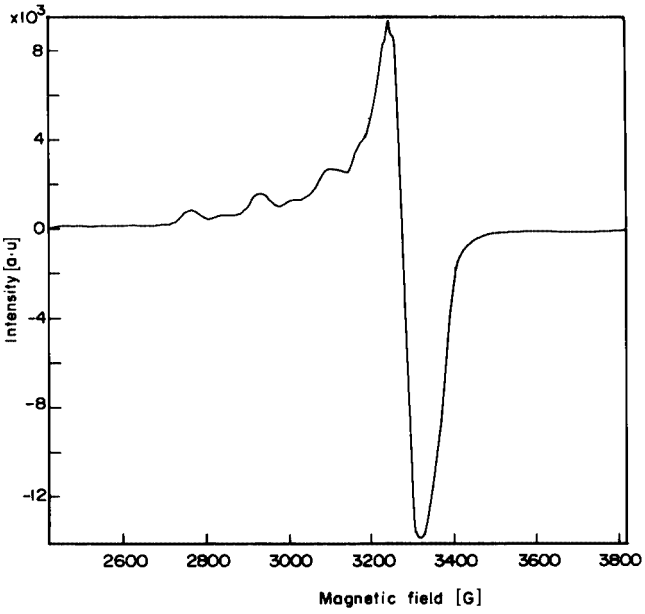

Figure 2. EPR spectrum of a frozen solution of complex **1** at 77 K in DMSO (microwave frequency = 9.5 GHz, power = 3 mW, modulation frequency = 100 kHz, modulation amplitude = 3 G).

Cyclic voltammetric studies to establish the redox behaviour of the copper(II) complexes reveal a non-Nernstian, almost irreversible redox process involving the Cu^{II}/Cu^{I} couple near –0.2 V for both of the complexes (values provided in Table 2). The anodic to cathodic peak current ratio of around 0.2 for both the complexes suggests poor reversibility of the electron transfer process. Figure 3 depicts the cyclic voltammograms of complexes **1** and **2**. The Cu^{II}/Cu^{I} redox potential of complex **1** is slightly more negative relative to **2**, which indicates that the +1 oxidation state is more stabilized in complex **2** than it is in complex **1**.

Table 2. Electrochemical behaviour of complexes **1** and **2**.[a]

Complexes	$E_{1/2}$ [V] CV	$E_{1/2}$ [V] DPV	ΔE_p [mV]	i_{pa}/i_{pc}
1	–0.257	–0.248	101	0.25
2	–0.223	–0.224	109	0.20

[a] Reference electrode: SCE; supporting electrolyte: TBAP.

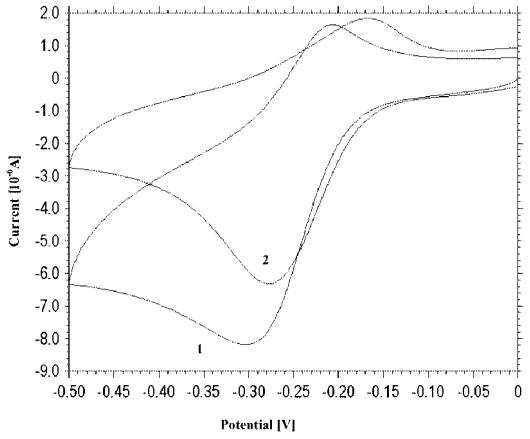

Figure 3. Cyclic voltammograms of complexes **1** and **2** (0.2 mM); scan rate 50 mV s^{-1}; supporting electrolyte, TBAP (100 mM), reference electrode SCE.

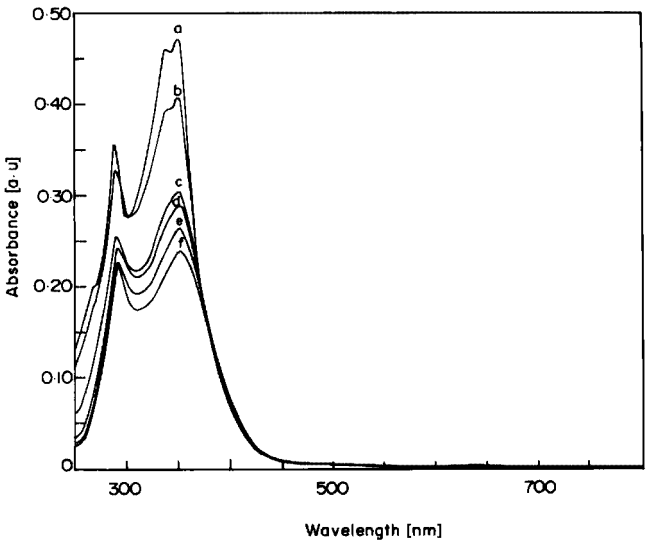

Figure 4. Absorption spectra of complex **2** (20 μM) in the absence (a) and presence (b–f) of increasing concentrations of CT DNA (40–120 μM).

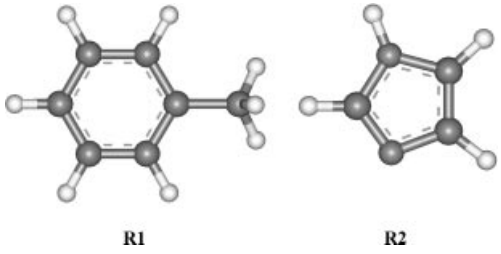

Figure 5. Optimized molecular structures of **R1** (toluene) and **R2** (imidazole) obtained at the B3LYP/6-31G* level of calculation (grey spheres represent carbon atoms; black spheres represent nitrogen atoms).

Absorption Titration Experiments

The interaction of the metal complex with DNA causes significant changes in the ligand-centred spectral transitions of the complex and these changes are monitored to calculate the binding constant. In the presence of increasing concentrations of DNA, both **1** and **2** were found to exhibit hypochromism with an almost negligible shift in the absorption maxima. This decrease in the intensity of the spectral band is typical of an intercalative mode of binding wherein the interacting molecule is sandwiched between the DNA base pairs.[36,37] The planarity and extended aromaticity of the terpyridine ligand systems bring about the stacking of the molecule between the DNA bases. The spectral changes on interaction of complex **2** with DNA are shown in Figure 4. The binding constant K_b was estimated to be $(5.6 \pm 0.2) \times 10^4$ and $(1.4 \pm 0.2) \times 10^4$ M^{-1} for complexes **1** and **2**, respectively. The binding constant values for these complexes are of the same order of magnitude as those observed for the corresponding bis-chelate systems reported in our earlier publications.[32] Also, the mode of binding remains intercalative in both cases. This suggests that the major factor influencing the mode and extent of binding could be the large size of the ligand system. The planar terpyridine ring appears to dictate the binding characteristics irrespective of the geometry of the complex (four or six coordinate).

To further explore the influence of substituents on the binding constant values, computational studies were carried out for complexes **1** and **2**. As seen from Figure 1, complexes **1** and **2** differ only in their substituents (R group). Therefore, calculations were carried out by considering only the tolyl and imidazolyl groups (**R1** and **R2**, respectively) as representative of **1** and **2**. Hydrogen atoms were added in the R groups to satisfy the valency (optimized molecular structures shown in Figure 5).

The structures were optimized at the B3LYP/6-31G* level of theory by using the Gaussian 98W suite of programs.[38] Density functional theory (DFT) based descriptors such as chemical hardness (η) and electrophilicity (ω) were calculated for **R1** and **R2**[39] and the results are presented in Table 3. Nucleus independent chemical shift (NICS) criteria proposed by Schleyer[40] is also calculated at the centre of the rings. All the three parameters, namely, η, ω and NICS are measures of reactivity and stability. From the table, it is observed that **R1** is less hard and more reactive than **R2**. This is also implied in the values of ω; the ω value of **R1** is higher than that of **R2**, indicating the higher reactivity of **R1**. NICS values indicate the degree of aromaticity: the more negative the NICS value, the more aromatic the system and higher the stability. From Table 3, we observe that the NICS value of **R2** is greater than that of **R1**, indicating the higher reactivity and lesser stability of **R1**. Thus, the tolyl-substituted molecule, complex **1**, binds to DNA more strongly [$(5.6 \pm 0.2) \times 10^4$ M^{-1}]

Table 3. Chemical hardness (η), electrophilicity (ω) and nucleus independent chemical shift [NICS(0)] values of R1 and R2 calculated by using the B3LYP/6-31G* level of theory.

Species	η [eV]	ω [eV]	NICS(0) [ppm]
Toluene	3.27	1.49	–9.6
Imidazole	3.51	0.97	–14.4

relative to the imidazole substituted molecule, complex **2** [$(1.4 \pm 0.2) \times 10^4$ M^{-1}].

In addition to this, the molecular electrostatic potential map of **R1** and **R2** was calculated at the B3LYP/6-31G* level of theory and is presented in Figure 6. The Gaussview package was used for viewing the map.[41] In the case of **R2**, the π cloud is substantially influenced by the electronegative nitrogen atom of the imidazole ring, but in **R1**, the aromatic π cloud is uniformly distributed above and below the molecular plane, which could bring about favourable stacking interactions with the DNA bases during intercalation (Figure 6). Hence, intercalation in this case would be comparatively more favourable than it is in **R2**. Thus, computational studies clearly show that the tolyl substituent makes complex **1** a better intercalator relative to imidazolyl-substituted complex **2**.

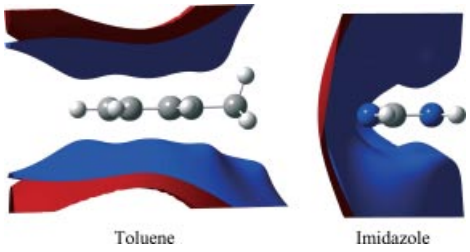

Figure 6. Total electron density map of **R1** and **R2** obtained at 0.003 au surface.

Viscosity Experiments

Measurement of DNA viscosity is regarded as the least ambiguous and the most critical test of the binding mode of DNA in solution and affords stronger arguments for an intercalative DNA binding mode. The viscosity of DNA is enhanced in a complete or partial intercalative mode of binding where an increase in the length of the polynucleotide occurs as a result of the destacking of the base pairs.[42] A partial nonclassical mode of binding could bend or kink the DNA helix, reduce its effective length and thereby its viscosity. The effect of increasing concentrations of complexes **1** and **2** on the specific relative viscosity of DNA is shown in Figure 7. The relative viscosity of DNA increases steadily with an increase in the concentration of the complex and this result parallels the pronounced absorbance hypochromism that was observed for the complexes and further confirms their intercalative mode of binding. However, the increase in viscosity is less than that observed for proven intercalators.[43] Among the two, the increase in the relative viscosity is more pronounced for complex **1** than for **2**, which parallels the trend in DNA binding studies.

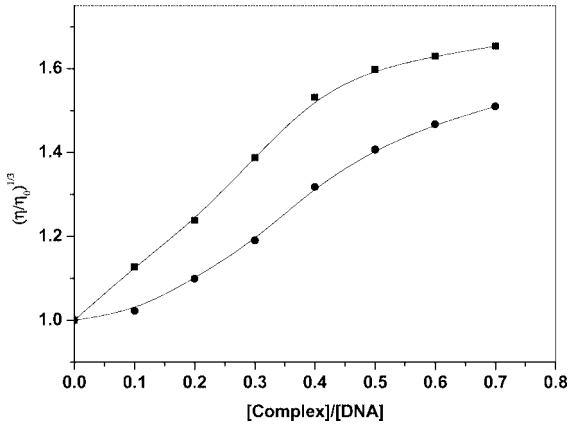

Figure 7. Effect of increasing concentration of complexes **1** (square) and **2** (circle) on the relative viscosity of CT DNA (200 μM).

Circular Dichroism

Circular dichroic spectral techniques may give us useful information on how the conformation of the DNA chain is influenced by the bound complex. The CD spectrum of CT DNA consists of a positive band at 275 nm that can be due to base stacking and a negative band at 245 nm that can be due to helicity and it is also characteristic of DNA in a right-handed B form.[44] Thus, simple groove binding and electrostatic interaction of small molecules with DNA show less or no perturbations on the base stacking and helicity bands, whereas intercalation enhances the intensities of both the bands stabilizing the right-handed B conformation of CT DNA as observed for classical intercalators.[45] The CD spectrum of CT DNA was monitored in the presence of increasing amounts of complexes **1** and **2**; the changes observed in the case of complex **2** are shown in Figure 8. The positive band showed an enhancement in the molar ellipticity with a slight redshift of the band maxima whereas the negative band showed a decrease in the intensity when the complex concentration was progressively increased. Similar changes were observed in the CD spectrum of DNA in the case of complex **2**. These observations are supportive of the intercalative mode of binding of these complexes, where in the stacking of the complex molecules between the base pairs of DNA leads to an enhancement in the positive band and the partial unwinding of the helix is reflected in the decreased intensity of the negative band. It is also evi-

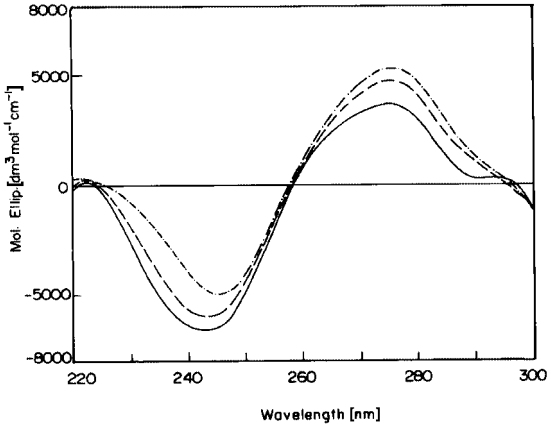

Figure 8. CD spectra of CT DNA (200 μM) in the absence (– – –) and presence of 20 (·-·-·-·-·-) and 40 μM (- - - - - - - -) of complex **1**.

dent from the CD spectra that binding of the complex does not lead to any significant change in the conformation of CT DNA.

Gel Electrophoresis Studies

To determine the ability of the present copper complexes to serve as metallonucleases, DNA cleavage studies were performed on plasmid pBR322 DNA and the results were monitored by gel electrophoresis. The naturally occurring super coiled form (Form I), when nicked, gives rise to an open circular relaxed form (Form II). This upon further cleavage results in the linear form (Form III). When subjected to gel electrophoresis, Form I shows a relatively faster migration than Forms II and III. Form II migrates very slowly owing to its relaxed structure whereas the linear form shows a migration in between Form I and Form II. Figure 9 shows the electrophoretic pattern of plasmid DNA treated with complexes **1** and **2**. Control experiments suggest that untreated DNA and DNA incubated with either complex or ascorbic acid alone does not show any significant DNA cleavage (Figure 9, lanes 1–3) but in the presence of ascorbic acid, the complexes were found to exhibit nuclease activity. In the presence of 25 μM of complex **1** and 75 μM of ascorbic acid, slight cleavage of plasmid DNA occurs as is evident from lane 4 (Figure 9) whereas there is hardly any cleavage in lane 5 (Figure 9), which represents the same concentration of the complex and reductant for **2**. On increasing the concentration of the complex to 50 μM while maintaining the concentration of the reducing agent at 75 μM, the cleavage was found to be much more efficient, as is evident from the increase in Form II (Figure 9, lanes 6 and 7). A further increase in the concentration of the complex to 75 μM and ascorbic acid to 150 μM leads to complete conversion of the supercoiled form to Form II and III (Figure 9, lanes 8 and 9). From Table 4 it is clear that complex **1** exhibits better nuclease activity than **2**.

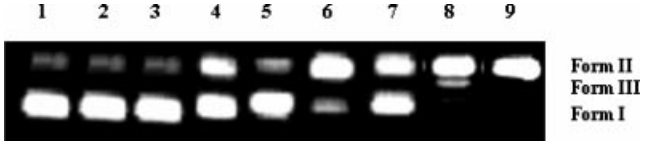

Figure 9. Cleavage of pBR322 DNA by complexes **1** and **2** in the presence of ascorbic acid. DNA (250 ng) was incubated with **1** and **2** for 60 min. in Tris buffer (pH 7.5). Lane 1, DNA control; lane 2, DNA + **1** (75 μM) alone; lane 3, DNA + ascorbic acid (150 μM); lane 4, DNA + **1** (25 μM) + ascorbic acid (75 μM); lane 5, DNA + **2** (25 μM) + ascorbic acid (75 μM); lane 6, DNA + **1** (50 μM) + ascorbic acid (75 μM); lane 7, DNA + **2** (50 μM) + ascorbic acid (75 μM); lane 8, DNA + **1** (75 μM) + ascorbic acid (150 μM); lane 9, DNA + **2** (75 μM) + ascorbic acid (150 μM).

To quantify the nuclease activity of the complexes, rate constants were determined for both complex **1** and **2** by further studying the extent of DNA cleavage in a time dependent manner. The complex concentration was maintained at 75 μM for both **1** and **2** as maximum DNA damage occurs at this particular concentration. The results of gel

Table 4. Extent of DNA cleavage by complexes **1** and **2**.

Complexes	Concentration of Complex [μM]	%SC	%NC	%L[a]
1	25	65	35	
	50	14	86	
	75		85	15
2	25	89	11	
	50	53	47	
	75	2	98	

[a] SC = supercoiled; NC = nicked circular; L = linear.

electrophoresis were subjected to densitometric quantification and the kinetic parameters were analyzed by assuming a simple pseudofirst-order process for conversion of Form I to Form II. The rate constants determined from the plot of ln(%SC DNA) versus time (shown in Figure 10) were found to be 4.32×10^{-4} s^{-1} for complex **1** and 3.14×10^{-4} s^{-1} for complex **2**. The higher k value for **1** is clearly indicative of a better nuclease activity of **1** relative to **2**.

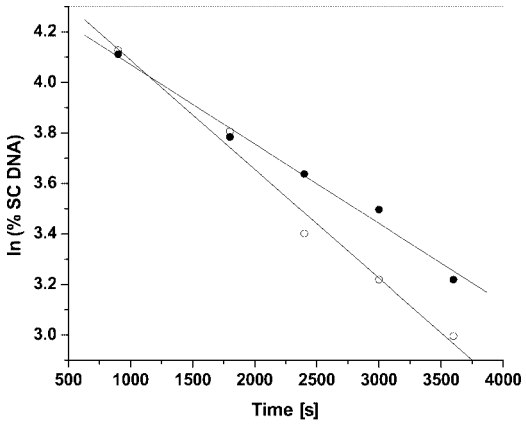

Figure 10. Plot of ln(%SC DNA) versus time for a complex concentration of 75 μM; complex **1** (○), Complex **2** (●).

To explain the cleavage efficiency of **1** being greater than **2** it is essential to look at the probable mechanistic pathway for this process. Because the complexes exhibit nuclease activity only in the presence of ascorbic acid, it is clear that the copper(II) complex in both cases is first reduced to a copper(I) complex, which then reacts with molecular oxygen to produce reactive oxygen species (ROS). A species with a more negative redox potential is always a better reducing agent and would reduce molecular oxygen much more readily thereby causing efficient DNA cleavage. In the present case, because complex **1** has a more negative redox potential than **2**, the former is a better DNA cleaving agent than the latter. Similar observations were also reported by other research groups.[46,47] Moreover, the higher binding affinity of complex **1** towards DNA relative to **2**, could also possibly be another reason for the higher cleavage efficiency of complex **1**. Further studies are under progress to investigate the DNA cleavage in detail.

Conclusions

The present communication reports the synthesis and characterization of two four-coordinate copper(II) complexes with tridentate terpyridine ligands. On the basis of electronic and EPR spectral studies, the complexes are suggested to have a four-coordinate geometry with considerable tetrahedral distortion in the square plane. The complexes are found to adopt an intercalative mode of binding with moderate binding strengths. The influence of the substituents of the terpyridine ligand was discussed on the basis of computational studies. The tolyl substituent in complex **1** is less hard and more reactive relative to the imidazolyl substituent of complex **2**. Also, the aromatic π cloud almost uniformly lies above and below the molecular plane in complex **1**, which leads to its better intercalative binding with DNA. They exhibit efficient nuclease activity in the presence of ascorbic acid and the differences in their cleavage efficiencies were explained on the basis of spectral and electrochemical results.

Experimental Section

General: 2-Acetyl pyridine, tolualdehyde, *p*-bromobenzaldehyde and imidazole 2-carboxaldehyde were obtained from Sigma Aldrich, USA and used as received. Tris(hydroxymethyl) methylamine (Tris) was obtained from SRL Chemicals. Calf Thymus DNA (CT DNA) was obtained from Fluka Chemicals. All chemicals and reagents used were of analytical grade received from Ranbaxy, Mumbai. Stock solution of DNA was prepared by stirring a sample dissolved in 10 mM (Tris) buffer, (pH 7.2) at 4 °C. The solution was dialyzed exhaustively against the Tris buffer for 48 h and filtered through a membrane filter obtained from Sartorius (45 μM). The filtered DNA solution in the buffer gave a UV absorbance ratio (A_{260}/A_{280}) of about 1.8, indicating DNA was sufficiently free from protein.[48] The concentration of DNA was determined by using an extinction coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm.[49]

[Cu(ttpy)Cl]Cl (1): The complex was prepared in good yield from the reaction of $CuCl_2{\cdot}2H_2O$ in methanol with tolyl terpyridine ligand, which was prepared by a procedure reported in the literature.[50] The ligand (0.323 g, 1 mmol) and $CuCl_2{\cdot}2H_2O$ (0.170 g, 1 mmol) were dissolved in methanol individually and the solutions were warmed. The hot solution of the ligand was added slowly with constant stirring to copper chloride when the colour changed to intense green. The solution was cooled to room temperature and the green precipitate of the copper–ttpy complex separated out and was filtered and dried. IR [KBr disc]: $\tilde{\nu}$ = 3069, 1619, 1451, 1098 cm^{-1}. MS (ESI): *m*/*z* = 421 $[M - L{\cdot}Cl]^+$. $C_{22}H_{17}Cl_2CuN_3$ (456.5): calcd. C 57.83, H 3.72, N 9.20, Cu 13.91; found: C 57.78, H 3.69, N 9.25, Cu 13.93. The complex is soluble in DMSO and partially soluble in methanol.

[Cu(itpy)Cl]Cl (2): The complex was prepared by a similar procedure to that of complex **1**, by taking $CuCl_2{\cdot}2H_2O$ (0.170 g) and itpy (0.299 g) in the mole ratio 1:1; the ligand was prepared by a similar procedure as that of ttpy. IR (KBr disc): $\tilde{\nu}$ = 3068, 1616, 1450, 794 cm^{-1}. MS (ESI): *m*/*z* = 397 $[M - L{\cdot}Cl]^+$. $C_{18}H_{13}Cl_2CuN_5$ (432.5): calcd. C 49.94, H 3.00, N 16.18, Cu 14.68; found C 49.87, H 2.94, N 16.09, Cu 14.60. The solubility of the complex is similar to that of **1**.

Physical Measurements: ESI mass spectra were recorded with a Finnigan LCQ Advantage mass spectrometer equipped with an electron spray source. Infrared spectra were recorded for the complexes and the ligand by using a Perkin–Elmer RX-1 FTIR spectrometer and the samples were prepared by KBr mull sampling technique. Electronic spectra were recorded with a Perkin–Elmer Lamda35 double beam spectrophotometer. Circular dichroic spectra were recorded with a J-715 spectropolarimeter (Jasco) at 25 °C with a 0.1-cm pathlength cuvette. Cyclic voltammetry was performed on CH-Instruments, electrochemical analyzer, CH-620 B. X-band electron paramagnetic resonance (EPR) spectra at room temperature and liquid nitrogen temperature were recorded with a Bruker EMX 6/1 spectrometer and the field calibration was done by using diphenylpicrylhydrazyl (DPPH).

DNA Binding and Cleavage Studies – Absorption Titration Experiments: The electronic spectra of complexes **1** and **2** were monitored in the absence and presence of DNA in the UV region. Absorption titration experiments were conducted by maintaining the metal ion concentration constant (20 μM) and varying the concentration of DNA (0–150 μM). In the reference cell, a DNA blank was placed so as to cancel any absorbance due to DNA at the measured wavelength. From the absorption titration data the binding constant (K_b) was determined by using the following equation.

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

where ε_a, ε_f and ε_b corresponds to A_{obs}/[Cu], the extinction coefficient for the free copper complex and the extinction coefficient for the complex in the bound form, respectively. A plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus [DNA] gives K_b as the ratio of the slope to the intercept. Density functional theory calculations were performed to explore the reactivity, stability and electron density parameters of the terpyridine substituents. From the theoretical investigation, the influence of substituent on the magnitude of binding constant is explained.

Viscometric experiments were carried out by using an Ostwald-type viscometer of 2 mL capacity, thermostatted in a water bath maintained at 25 °C. The flow rates for the buffer (10 mM), DNA (200 μM) and DNA in the presence of copper(II) complex at various concentrations (20–140 μM) were measured with a manually operated timer at least three times to agree within 0.2 s. The relative viscosity was calculated according to the relation, $\eta = (t - t_0)/t_0$ where t_0 is the flow time of the buffer and t is the observed flow time for DNA, in the presence and absence of the complex. A plot of $(\eta/\eta_0)^{1/3}$ versus $1/R$ (R = [DNA]/[Complex]) was constructed from viscosity measurements.[51]

Voltammetric experiments were carried out by using a standard three-electrode system comprising glassy carbon working electrode with 3 mm diameter, platinum auxillary electrode and saturated calomel reference electrode (SCE). The samples were prepared in DMSO, and the solutions were deoxygenated by purging nitrogen gas for 10 min. prior to the measurements. The experiments were carried out with a complex concentration of 0.2×10^{-3} M by using tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte.

Circular dichroic spectrum of DNA in the absence and presence of complexes was recorded with a J-715 spectropolarimeter (Jasco) at 25 °C with a 0.1-cm pathlength cuvette. The spectra were recorded for 200 μM of DNA in the absence and presence of 5–40 μM of the copper(II) complex in the region 220–300 nm.

Cleavage of plasmid DNA was monitored by using agarose gel electrophoresis. Supercoiled pBR322 DNA (180 ng) in tris(hydroxymethyl)methylamine (Tris) buffer was treated with copper(II) complex (25–75 μM) and ascorbic acid (75–150 μM). The samples were incubated for 60 min at 35 °C. A second set of experiments was performed by maintaining the complex concentration at 75 μM and

ascorbic acid at 150 μM and varying the incubation time from 0–60 min. The agarose gel (0.8%) containing 0.5 μg of ethidium bromide was prepared and the electrophoresis of the DNA cleavage products was performed on it. The gels were run at 100 V for 1 h in Tris(boric acid) ethylenediaminetetraacetic acid (TBE) buffer and the bands were identified by placing the stained gel under an illuminated UV lamp. The fragments identified were photographed by using a gel documentation system.

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