Copper complexes as chemical nucleases

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Abstract. Redox active mononuclear and binuclear copper(II) complexes have been prepared and structurally characterized. The complexes have planar N-donor heterocyclic bases like 1,10-phenanthroline (phen), dipyridoquinoxaline (dpq) and dipyridophenazine (dppz) ligands that are suitable for intercalation to B-DNA. Complexes studied for nuclease activity have the formulations $[Cu(dpq)_2(H_2O)]$ $(ClO_4)_2.H_2O$ (**1**), $[\{CuL(H_2O)\}_2(m\text{-}ox)](ClO_4)_2$ (L = bpy, **2**; phen, **3**; dpq, **4**; and dppz, **5**) and $[Cu(L)(salgly)]$ (L = bpy, **6**; phen, **7**; dpq, **8**; and dppz, **9**), where salgly is a tridentate Schiff base obtained from the condensation of glycine and salicylaldehyde. The dpq complexes are efficient DNA binding and cleavage active species. The dppz complexes show good binding ability but poor nuclease activity. The cleavage activity of the *bis*-dpq complex is significantly higher than the *bis*-phen complex of copper(II). The nuclease activity is found to be dependent on the intercalating nature of the complex and on the redox potential of the copper(II)/copper(I) couple. The ancillary ligand plays a significant role in binding and cleavage activity.

Keywords. Copper(II) complexes; nuclease activity; catalytic properties; DNA binding.

1. Introduction

Chemical nucleases are redox active coordination complexes that cleave DNA by an oxidative pathway. Direct scission of DNA by oxidizing or hydrolytic agents is the subject matter for several current review articles[1–10]. Redox-active transition metal complexes in the presence of oxidants have been extensively used for DNA cleavage reactions. In a typical reaction, an oxidising agent like O_2, H_2O_2 or a peracid is added in addition to the transition metal complex. There are also examples where chemically or electrochemically generated metal in high oxidation state can act as an oxidant in the presence of a reductant. Generally, metal complexes capable of abstracting a ribosyl hydrogen are as well expected to oxidize guanine or other nucleobases. Current efforts are on to design transition metal complexes as chemical nucleases suitable for plasmid nicking or in a direct strand scission.

Among the transition metal based cleaving reagents, the chemistry of copper, particularly copper phenanthroline complexes, is primarily sugar directed. Copper complexes usually do not mediate nucleobase oxidation, but are responsible for direct strand scission by hydrogen atom abstraction from the deoxyribose moiety. Sigman and coworkers have shown that *bis*-(1,10-phenanthroline)copper(I) complex in presence of

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H_2O_2 efficiently cleaves DNA [1,2]. The importance in DNA binding and cleavage by redox and photoactive metal complexes is primarily for examining the sequence specificities of DNA binding using a variety of intercalating ligands by 'footprinting' methods [1,2,11–15]. Although the true binding mode of the *bis*-phen complex to DNA is presently unknown, it has been suggested that the nuclease activity of $[Cu(phen)_2]^+$ is related to the partial intercalation or binding of one phen ligand to the minor groove of DNA while the other phen ligand makes favourable contact within the groove.

The key intermediate in nucleic acid oxidation is the formation of an active Cu(III)-OH species leading to the generation of freely diffusible HO• which abstracts proton from the deoxyribose C-4′ or C-1′ position. The formation of Cu(I)OOH or Cu(III)-OH has earlier been proposed for the nucleobase oxidation involving $CuCl_2$ in presence of H_2O_2 or O_2 with a reductant like ascorbate, reduced glutathione or NADH in DNA strand breaking giving the order $T > G > C > A$ [16,17]. The 1:1 complex formed by $CuCl_2$ with the anticancer drug famotidine has been shown as a better catalyst than $CuCl_2$ for sulfite autooxidation leading to DNA damage [18,19]. Similarly Cu^{2+} ion and H_2O_2 in the presence of L-dopa or a catechol, greatly enhance DNA oxidation [20].

The nuclease activity of $[Cu(phen)_2]^+$ was discovered by Sigman and coworkers in 1979 while they were investigating the inhibition of the poly $[d(A–T)]$ directed polymerization catalysed by *E. coli* DNA polymerase I in presence of phen ligand [21]. They have observed that DNA strand scission requires the mole ratio of 2:1 for the 1,10 phenanthroline and copper in this complex. The role of phen ligand is to modulate the redox potential of the Cu(II)/Cu(I) couple and to bind DNA leading to the occurence of the oxidative chemistry of the cuprous complex at the surface of the nucleic acid [1,2]. The novel nuclease activity of the *bis*(phen)copper complex has prompted us to synthesize redox active copper complexes having planar N-donor heterocyclic ligands suitable for nuclease activity studies. Herein we report the binding and nuclease activities of three types of copper(II) complexes, viz. $[Cu(dpq)_2(H_2O)](ClO_4)_2.H_2O$ (**1**), $[\{Cu(L)(H_2O)\}_2$ (***m*ox)]$(ClO_4)_2$ (L = bpy, **2**; phen, **3**; dpq, **4**; dppz, **5**) and $[Cu(salgly)(L)]$ (salgly, a tridentate Schiff-base derived from salicylaldehyde and glycine; L = bpy, **6**; phen, **7**; dpq, **8**; dppz, **9**), where dpq and dppz are dipyridoquinoxaline and dipyridophenazine, respectively.

2. Results and discussion

2.1 *DNA binding and nuclease activity of $[Cu(dpq)_2(H_2O)](ClO_4)_2.H_2O$ (**1**)*

Dipyridoquinoxaline (dpq) ligand with an extended planar quinoxaline moiety is expected to be a better binder to DNA compared to 1,10-phen and other substituted phen ligands. The dpq complex is likely to show enhanced nuclease activity than its phen analogue. This part of the work stems from our interest to explore the DNA binding and nuclease activity of the *bis*-dpq complex of copper(II) [22].

Complex **1** was prepared from a reaction of copper(II) perchlorate with dipyridoquinoxaline in methanol. The complex was structurally characterized. A perspective view of the molecule is shown in figure 1. The complex has a CuN_4O coordination geometry with a distorted trigonal bipyramidal structure. It exhibits a visible spectral *d–d* band at 718 nm in MeCN. The complex is redox active and displays a Cu(II)/Cu(I) couple at 0·09 V with a ΔE_p value of 229 mV at a scan rate of 50 mV s^{-1}. The complex undergoes facile reduction with sodium ascorbate in an aqueous-DMF
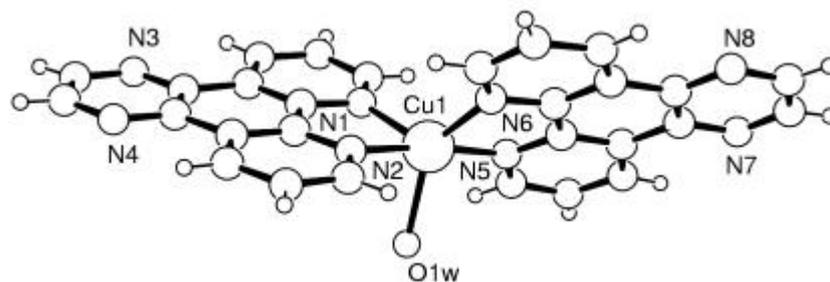
**Figure 1.** A perspective view of the cationic complex $[Cu(dpq)_2(H_2O)]^{2+}$.
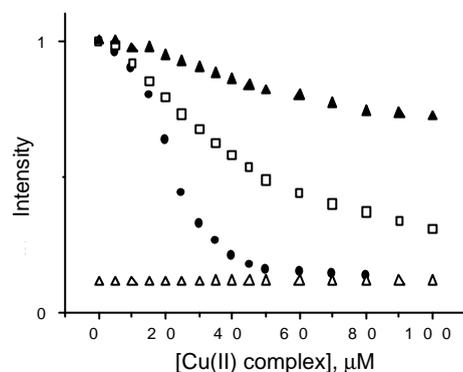
Figure 2. Effect of addition of *bis*-chelate copper(II) complexes (dpq, •; phen, □; bpy, ▲) to the emission intensity of the CT DNA-bound ethidium bromide at different complex concentrations. The emission intensity of EB in absence of DNA is also shown (Δ).

medium (4:1 *v/v*) to form an unstable brown copper(I) species which readily converts to **1** on exposure to air.

The binding of complex **1** with calf thymus DNA has been studied by fluorescence spectral method. Competitive binding studies have been made using ethidium bromide (EB) intercalator which showed enhanced emission intensity compared to free EB [23,24]. Intercalation of the complex to the DNA displaces bound EB and the emission intensity reduces due to fluorescence quenching of the free EB molecules by water [25,26]. Figure 2 shows the decrease of the emission intensity of the EB on addition of complex **1**. A comparison of the data can be made with the binding studies done using analogous bpy and phen complexes. The bpy species shows no apparent intercalation. Complex **1** with a greater slope has significantly higher binding affinity than its phen analogue.

DNA cleavage studies have been made by gel electrophoresis using supercoiled pUC19 DNA in 50 mM Tris-HCl/50 mM NaCl buffer (*p*H 7·2). Complex **1** on reaction with the DNA in presence of ascorbic acid (H$_2$A) shows nuclease activity which is better than its phen analogue (figure 3). The bpy complex, $[Cu(bpy)_2]^{2+}$ does not show any apparent cleavage. At a concentration of 15 μM, the dpq complex is able to convert 75%
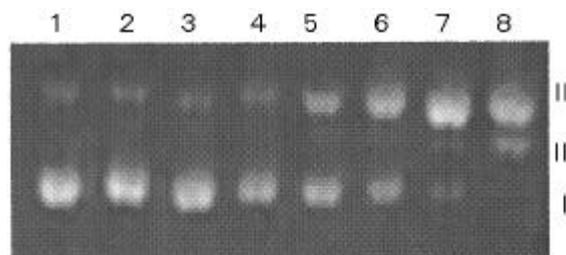
Figure 3. Cleavage of SC pUC19 DNA (0·5 μg) by copper complexes in presence of ascorbic acid (H$_2$A, 100 μM) in a 50 mM Tris-HCl + 50 mM NaCl buffer at 37°C. Lane 1, DNA control; lane 2, DNA + complex **1**; lane 3, DNA + H$_2$A; lane 4, DNA + *bis*-bpy Cu(II) complex + H$_2$A; lanes 5 and 6, DNA + *bis*-phen Cu(II) complex + H$_2$A; lanes 7 and 8, DNA + **1** + H$_2$A [complex concentrations: 15 μM for lanes 5 and 7; 30 μM for lanes 2, 4, 6 and 8].

of the initial supercoiled (SC) to nicked circular (NC) form whereas the phen analogue can convert only 17% of the SC. At a higher concentration of 30 μM, the dpq complex shows complete cleavage while the phen analogue displays only 40% conversion.

2.2 *DNA binding and nuclease activity of [{Cu(L)(H$_2$O)}$_2$($\mathbf{\mu}$ox)](ClO$_4$)$_2$ (2–5)*

Rill and coworkers have earlier shown that a mole ratio of 2:1 for the phen and copper is needed for observing efficient nuclease activity, regardless of the reducing agent used in the cleavage reaction[27]. This part of the work stems from our interest to study the DNA binding and cleavage properties of dicopper(II) complexes by covalently linking two mono-phen copper and related units by a planar oxalate bridge to form a dinuclear species having a ligand to copper mole ratio as 1:1[26]. We have prepared four such complexes of general formulation [{Cu(L)(H$_2$O)}$_2$($\mathbf{\mu}$ox)](ClO$_4$)$_2$ (L = bpy, **2**; phen, **3**; dpq, **4**; dppz, **5**) from a reaction of Cu(ClO$_4$)$_2$.6H$_2$O with the bidentate N-donor heterocyclic base (L) and sodium oxalate in aquous ethanol. The complexes display characteristic infrared bands in the ranges 1651–1662 and 1077–1098 cm^{-1} for the oxalate and perchlorate, respectively. The complexes show a *d–d* band in the range 650–720 nm in aqueous DMF solution. Complexes **1** and **3** have been structurally characterised. A perspective view of complex **3** is shown in figure 4. The structure consists of a centrosymmetric complex in which the Cu(dpq)(H$_2$O) units are bridged by an oxalate. The separation between two copper centres is 5·138(2) Å. In the square-pyramidal geometry, the deviation of copper from the basal plane is 0·062 Å. The angle formed between two basal planes is 2·6°.

The complexes are redox active and show two cyclic voltammetric responses in the potential range 0·07 to –0·31 V vs SCE in a dmf-50 mM Tris-HCl/0·1 M KCl buffer (1:1 *v/v*, *p*H 7·2). They are assignable to the Cu$_2$(II)/Cu(II)Cu(I) and Cu(II)Cu(I)/Cu$_2$(I) couples. A ΔE_p value in the range 100–250 mV at 50 mV s^{-1} with the ratio of the cathodic to anodic peak currents as unity suggests the quasireversible nature of the electron transfer process. The $E_{1/2}$ values for the Cu$_2$(II)/Cu(II)Cu(I) couple are –0·05, –0·04, 0·02 and 0·07 V for the complexes **2–5** respectively. The $E_{1/2}$ values for the Cu(II)Cu(I)/Cu$_2$(I) couple are –0·31, –0·27, –0·22 and –0·20 V, respectively, for **2–5**. The complexes
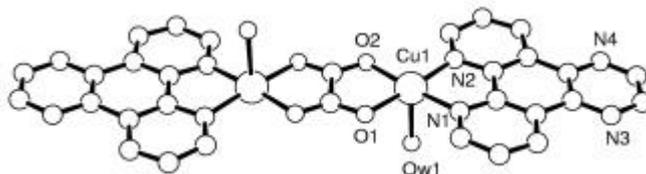
Figure 4. A perspective view of the cationic complex in $[Cu_2(ox)(dpq)_2 (H_2O)_2](ClO_4)_2 \cdot 2H_2O$.
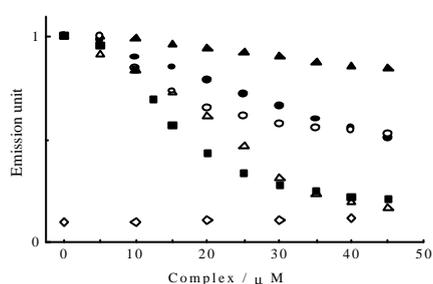

Figure 5. The emission intensity of CT DNA-bound ethidium bromide (12·5 μM) at different complex concentrations for the complexes $[\{LCu(H_2O)\}_2(\mu\text{-ox})](ClO_4)_2$ (L = bpy, ▲; phen, ; dpq, ■; dppz, Δ) and the *bis*-phen copper(II) species (●). The emission intensity of EB in absence of DNA in 5 mM Tris-HCl + 50 mM NaCl buffer (*p*H 7·2) containing 3% dmf at 25°C is also shown (◊).

undergo facile reduction with ascorbic acid to form an unstable brown Cu(I) species which converts to the copper(II) complex on exposure to dioxygen. The catalytic process is effective with a mole ratio of the ascorbic acid to the complex as ~100 for complexes **2** and **3** and ~60 for complexes **4** and **5**.

The binding ability of the complexes **2–5** to the calf thymus DNA has been studied by fluorescence spectral method. The decrease in the emission intensity of the EB on addition of the dimeric complexes and the mononuclear *bis*-phen copper(II) complex is shown in figure 5. The bpy complex showing a near zero slope is essentially unable to dislocate the DNA-bound EB molecules due to lack of any intercalation ability of the bpy ligand. The dpq and dppz complexes showing higher slopes than the *bis*-phen copper(II) species indicate significant binding ability which follows the order: **4** > **5** > **3** > Cu(phen)$_2^{2+}$ **2**.

The complexes are nuclease active. The cleavage activity of the complexes has been determined from gel electrophoresis based on their ability to convert supercoiled (SC) pUC19 DNA to nicked circular (NC) DNA form (figure 6, table 1). There are several interesting findings in this study. The complexes **2–5** have a Cu: heterocyclic base ratio as 1:1. The bpy complex has been found to be nuclease inactive and a poor binder to DNA. This clearly suggests that the DNA binding in the form of intercalation could be an important factor for the cleavage activity. The luminescence experiments monitoring the emission of DNA bound EB strongly suggest the intercalative mode of binding.
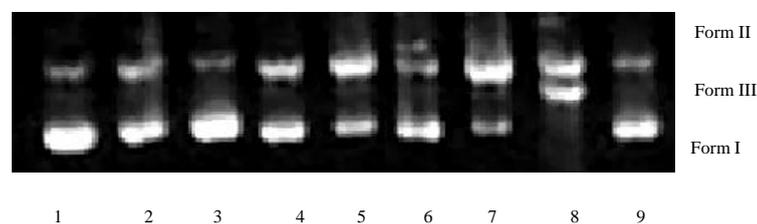
Figure 6. Cleavage of SC pUC19 DNA (0·5 μg) by the binuclear complexes (30 μM) in presence of ascorbic acid (H_2A). Lane 1, DNA control; lane 2, DNA + H_2A; lane 3, DNA + complex **4**; lane 4, DNA + H_2A + mono-phen Cu(II) species; lane 5, DNA + H_2A + *bis*-phen Cu(II) species; lanes 6–9, DNA + H_2A + complexes **2–5**, respectively.

Table 1. DNA (pUC19) cleavage data of the complexes **2–5** along with the mono and *bis*-phen complexes of copper(II).

Sl. no. [a]	Reaction condition [b]	Form (%)		
		SC	NC	Linear
1	DNA control	90	10	
2	DNA + ascorbic acid (H_2A)	83	17	
3	DNA + $[Cu_2(dpq)_2(H_2O)_2(ox)](ClO_4)_2$ (**4**)	90	10	
4	DNA + H_2A + $[Cu(phen)(H_2O)_2](ClO_4)_2$	70	30	
5	DNA + H_2A + $[Cu(phen)_2(H_2O)](ClO_4)_2$	40	60	
6	DNA + H_2A + $[Cu_2(bpy)_2(H_2O)_2(ox)](ClO_4)_2$ (**2**)	80	20	
7	DNA + H_2A + $[Cu_2(phen)_2(H_2O)_2(ox)](ClO_4)_2$ (**3**)	28	72	
8	DNA + H_2A + $[Cu_2(dpq)_2(H_2O)_2(ox)](ClO_4)_2$ (**4**)	0	55	45
9	DNA + H_2A + $[Cu_2(dppz)_2(H_2O)_2(ox)](ClO_4)_2$ (**5**)	78	22	

[a] The serial numbers correspond to the lanes shown in figure 6. [b] Concentrations of different species: H_2A 100 μM; copper complexes 30 μM; DNA ≈ 500 ng. Reaction medium: DMF-50 mM tris-HCl/50 mM NaCl buffer (1:1 *v/v* *p*H 7·2). SC (supercoiled), NC (nicked circular) and linear are different forms of DNA

Complexes **3–5** are avid binders to DNA. The significant binding observed for the dpq and dppz complexes could be related to the presence of extended aromatic ring making intercalation more favourable between the *p*-system of the ligand and the DNA base pairs. The dpq and dppz complexes, however, drastically differ in their cleavage activities in presence of a reducing agent like ascorbic acid. The anomalous activity of **5** could be related to the enhancement of the stability of the cuprous complex in presence of hydrophobic interactions with the DNA. A stable copper(I) complex is unlikely to react with dioxygen to form the reactive copper-oxo species. The other possibility is the difference in the groove selectivity of the dpq and dppz ligands. Control experiments done by the research group of Collins and Barton have shown that while phen and dpq ligands prefer to intercalate through the minor groove[28–30], complexes containing dppz bind to DNA through the major groove[31–33]. The mechanistic pathways for DNA cleavage involving the minor and major grooves are known to yield different scission products[1–8]. It is likely that the dpq complex intercalates through the minor groove, while the dppz complex **5** may prefer the major groove for binding.

Catalytic studies done on the complexes **2–5** in a buffer medium in presence of ascorbic acid show that a cleavage of the dimeric unit to two monomeric species takes place as $[\{Cu^{II}L(H_2O)\}_2(\boldsymbol{m}ox)](ClO_4)_2 + H_2A \rightarrow 2[LCu^I(H_2O)(ClO_4)] + A + H_2C_2O_4$. Such a monomeric complex containing the dppz ligand is likely to be stable in the cuprous form due to hydrophobic interactions of the extended aromatic rings of dppz with the DNA helix. This could be a possibility for the nuclease inactivity of the complex.

2.3 DNA binding and nuclease activity of [Cu(salgly)(L)].4H₂O (6–9)

In order to understand the influence of ancillary ligand on the DNA binding and cleavage activity, we have prepared a series of mixed ligand copper(II) complexes containing a tridentate ONO-donor Schiff base ligand (salgly), obtained from the condensation of salicylaldehyde with glycine. The complexes of formulation [Cu(salgly)(L)] (**6–9**) where L = bpy (**6**), phen (**7**), dpq (**8**) and dppz (**9**) have been prepared by reacting [Cu(salgly)(H₂O)] with L in aqueous methanol. The complexes were characterized from elemental analytical and spectral data. The complexes display a *d–d* band in the range 650–700 nm in methanol. Complex **6** with four waters of crystallization has been structurally characterized and a perspective view of the complex is shown in figure 7. The complexes have a distorted square-pyramidal (4+1) structure with a N₂O₂ basal plane. The heterocyclic ligand occupies the axial-equatorial sites of the square-pyramidal structure. The water molecules show chemically significant intra and intermolecular hydrogen bonding interactions.

The DNA binding studies of the complexes **6–9** have been made by fluorescence spectral method by monitoring the decrease in the emission intensity of the ethidium bromide molecules bound to DNA on addition of the metal complex. Figure 8 shows the variation of the binding ability of different complexes. While [Cu(salgly)(dpq)] (**8**) shows similar binding ability as $[Cu(phen)_2]^{2+}$, the bpy complex **6** is a poor binder to CT DNA. The complexes [Cu(salgly)(phen)] (**7**) and [Cu(salgly)(dppz)] (**9**) show similar binding ability. It is interesting that dppz ligand with an extended *p*-aromatic ring is less
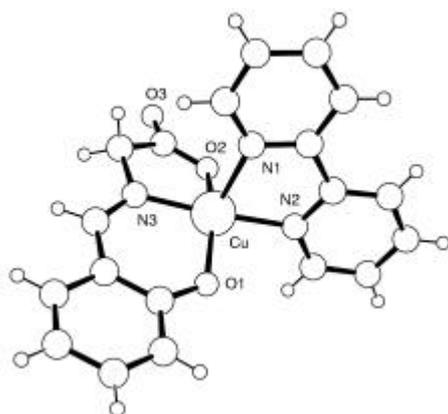
Figure 7. A perspective view of [Cu(salgly)(bpy)] (**6**).
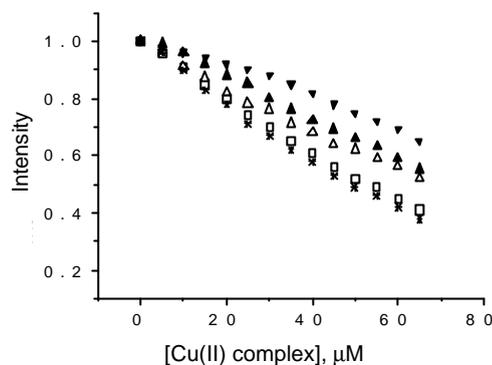
Figure 8. Effect of addition of copper(II) complexes of formulation [Cu(salgly)(L)] (L = bpy, ▼; phen, Δ; dpq,   and dppz,   ▲) along with [Cu(phen)$_2$]$^{2+}$ (∗) to the emission intensity of CT DNA-bound ethidium bromide (12·5 μM) in 5 mM Tris-HCl + 50 mM NaCl buffer (*p*H 7·2) containing 1% DMF at 25°C.
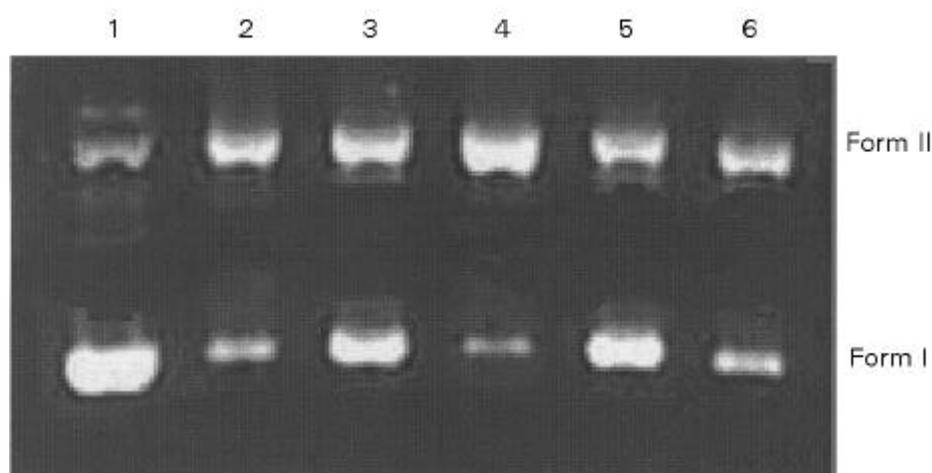
Figure 9.   Cleavage of SC pUC19 DNA (0·5 μg) by the Schiff base complexes (30 μM) in the presence of ascorbic acid (H$_2$A, 100 μM) in a 50 mM Tris-HCl + 50 mM NaCl buffer at 37°C. Lane 1, DNA control; lane 2, DNA + H$_2$A + *bis*-phen copper(II) complex; lane 3, DNA + H$_2$A + complex **7** (L = phen); lane 4, DNA + H$_2$A + complex **8** (L = dpq); lane 5, DNA + H$_2$A + complex **6** (L = bpy); lane 6, DNA + H$_2$A + complex **9** (L = dppz).

efficient binder than phen and dpq. The reason could be the specificity of groove choice for these planar ligands. While phen and dpq may prefer minor groove, dppz may bind through the major groove.

The cleavage of pUC19 DNA by the complexes **6–9** in presence of ascorbic acid (H$_2$A) is studied. The agarose gel electrophoresis diagram is shown in figure 9. It is apparent from the cleavage pattern that the cleavage efficiency follows the order: Cu(phen)$_2$$^{2+}$ ≈

8 > **7** > **9** > **6**. While the bpy and dppz complexes are found to be poor cleavers of DNA, the dpq complex has similar activity as the *bis*-phen copper complex.

3. Experimental

All reagents and chemicals were purchased from commercial sources. Solvents were purified by standard procedures. Dipyridoquinoxaline and dipyridophenazine were prepared by literature methods [28,34,35]. The glycinatosalicylaldimine Schiff base containing precursor complex $[Cu(salgly)(H_2O)].0.5H_2O$ was prepared by a known procedure [36]. Calf thymus (CT) DNA and supercoiled (SC) pUC19 DNA were purchased from Bangalore Genie (India). Agarose (molecular biology grade) and ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide) were from Sigma (USA). Tris-(hydroxymethyl) aminomethane. hydrochloride (Tris-HCl) buffer solutions were prepared using deionised, sonicated triple distilled water.

The CHN analyses were carried out on a Perkin-Elmer 2400 instrument. All the complexes gave satisfactory elemental analysis data. The infrared, electronic and fluorescence spectra were recorded on a Bruker Equinox 55, Hitachi U-3400 and Perkin-Elmer LS 50B spectrophotometers, respectively. Electrochemical measurments were made at 25°C on a EG&G PAR Model 253 Versa Stat Potentiostat/Galvanostat using a three-electrode setup comprising of a glassy carbon working, platinum wire auxiliary and a saturated calomel reference electrode (SCE). The experimental data were uncorrected for junction potentials.

Complex $[Cu(dpq)_2(H_2O)](ClO_4)_2.H_2O$ (**1**) was prepared in high yield by reacting 1·0 mmol of $Cu(ClO_4)_2.6H_2O$ with 2·0 mmol of dpq in 15 ml MeOH under stirring condition for 0·5 h at 25°C. The solid thus formed was isolated, washed with cold methanol and dried in vaccuo over P_4O_{10}. The oxalato bridged dicopper(II) complexes $[\{CuL(H_2O)\}_2(\mathbf{\mu}ox)](ClO_4)_2$ (**2–5**) were prepared by following a general procedure in which 1·0 mmol of $Cu(ClO_4)_2.6H_2O$ was reacted with 1·0 mmol of L and 0·5 mmol of sodium oxalate in 70 ml aqueous ethanol. The complexes were isolated in 70% yield as a blue solid after washing the crude solid with ethanol and water followed by drying over P_4O_{10}. Complexes $[Cu(salgly)(L)].4H_2O$ (**6–9**) were prepared by reacting an equimolar mixture of $[Cu(salgly)(H_2O)].0.5H_2O$ and L in 30 ml aqueous methanol (1:5 *v/v*) under stirring for 1 h at 25°C. The complexes were isolated in ~80% yield as a dark green solid on evaporation of the solvent and finally crystallized from an aqueous methanolic solution of the complex by evaporation at 5°C. Complexes $[Cu(dpq)_2(H_2O)](ClO_4)_2.H_2O$ (**1**), $[\{CuL(H_2O)\}_2(\mathbf{\mu}ox)](ClO_4)_2$ (L = bpy, **2**; dpq, **4**) and $[Cu(salgly)(bpy)].4H_2O$ (**6**) were characterized by single crystal X-ray crystallography. A detailed report on the crystal structures will be published elsewhere.

In the DNA binding studies, the concentration of the CT DNA was determined by recording the absorption intensity at 260 nm with a molar extinction coefficient value $6600 \, dm^3 \, mol^{-1} \, cm^{-1}$ [37]. The experiments were done using 0·1 mM complex concentration in the absence and presence of CT DNA varying from 0 to 250 μM. In order to compare the relative binding of the complexes to DNA with respect to the *bis*-phen complex of copper(II), ethidium bromide bound CT DNA solution in Tris-HCl/NaCl buffer (*p*H 7·2) was treated with an increasing amount of the complex solution. The fluorescence intensities at 610 nm (510 nm excitation) of the ethidium bromide in the bound form were plotted against the complex concentration.

The cleavage of DNA was monitored using agarose gel electrophoresis. In a typical experiment, supercoiled pUC19 DNA (6 μL, ~0·5 μg) in Tris-HCl buffer (50 mM) with 50 mM NaCl (*p*H 7·2) was treated with the metal complex (30 μM) and ascorbic acid (H_2A, 100 μM) followed by dilution with the Tris-HCl buffer to a total volume of 20 μL. The samples were incubated for 1 h at 37°C. Loading buffer containing 25% bromophenol blue, 0·25% xylenecyanol and 30% glycerol (3 μl) was added and the sample was subjected to electrophoresis using 0·8% agarose gel containing 1·0 μg/ml ethidium bromide at 40 V for 3 h in Tris-borate-EDTA (TBE) buffer. Bands were visualised by UV light and photographed. The cleavage activity was estimated by determining the extent of conversion of SC DNA to NC DNA using the BIORAD gel documentation system. The data were corrected for the low level of the NC present in the original sample and for the low affinity of ethidium bromide binding to SC compared to NC and linear forms of DNA [38].

4. Conclusions

Redox active copper(II) complexes having planar N-donor heterocyclic ligands are found to be nuclease active in presence of ascorbic acid. The complexes having 2,2′-bpy ligand are poor binder and cleaver to DNA. The copper(II) complexes having dipyrido-quinoxaline ligand are good binders to DNA and in presence of ascorbic acid, they show nuclease activity. The cleavage activity of the copper-dpq complexes are comparable to that of the well explored nucleolytic reagent *bis*-phen complex of copper(I). The copper(II) complexes having dipyridophenazine (dppz) ligand are found to be poor cleaver to DNA in presence of H_2A. They, however, show different binding ability to DNA in the copper(II) form. The results are of importance in the development of the chemistry of nuclease active redox based copper complexes.

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