



SYNTHESIS, CHARACTERIZATION OF COPPER (II) COMPLEX WITH MIXED LIGANDS OF 1, 10-PHENANTHROLINE, L-PHENYLALANINE AND ETHYLAMINE: STUDIES ON DNA BINDING, NUCLEASE AND BIOLOGICAL ACTIVITIES

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ABSTRACT

A copper (II) complex, (Cu (phen)(L-phe)(Ethylamine))(ClO₄) (phen=1, 10-Phenanthroline, L-Phenylalanine), has been synthesized and characterized by IR, UV-Vis, EPR spectra and elemental analysis. Binding of this copper (II) complex with calf thymus DNA (CT-DNA) was investigated by UV-Visible absorption, fluorescence spectroscopic, cyclic voltametric and viscosity techniques. Also the interaction of pBR322 DNA with the same copper (II) complex was studied using the gel electrophoresis method. The complex was shown to bind DNA by different mode and cleave pBR322 DNA in the presence and absence of ascorbate. The copper (II) complex was tested for its antibacterial and antifungal activity and it was found to have good antibacterial and antifungal activities

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INTRODUCTION

Deoxyribonucleic acid plays an important role in the life process, because it bears heritage information and instructs the biological synthesis of proteins and enzymes through the replication and transcription of genetic information in living cells. DNA is a particularly good target for metal complexes as it offers a wide variety of potential metal binding sites (Pyle *et al.*, 1989). Such sites include the electron rich DNA bases or phosphate groups that are available for direct covalent coordination to the metal center. There are several types of sites in the DNA molecule where binding of metal complexes can occur: (i) between two base pairs (intercalation), (ii) in the minor groove, (iii) in the major groove, and (iv) on the outside of the helix (Pyle *et al.*, 1989).

Transition metal complexes are known to bind to DNA via both covalent and non covalent interactions. In covalent binding the labile ligand of the complexes is replaced by a nitrogen base of DNA such as guanineN7. On the other hand, the non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of cationic metal complexes along outside of DNA helix, along major or minor groove (Rajendran *et al.*, 2009). The interaction of transition metal complexes with DNA has long been a subject of intensive investigation with the perspective of development of newer materials for application in biotechnology and medicine (Cowan, 2001). The effect of size, shape, hydrophobicity, and the charge on the binding of the

complex to DNA has been studied by changing the type of heteroaromatic ligand or metal center (Barton, 1986). In the order to make mixed-ligand coordination compounds intercalate in DNA, the intercalated ligand needs to be flat, with a large surface area and have a special geometry that permits overlapping between the aromatic ring of intercalated ligand and the base pairs in DNA.

Copper complexes of 1, 10-phenanthroline and its derivatives are of great interests since they exhibit numerous biological activities such as antitumor (Ranford *et al.*, 1993), anti-Candida (Majella, *et al.*, 1999), antimycobacterial (Saha *et al.*, 2004), and antimicrobial (Zoroddu *et al.* 1996) activity etc. Moreover, considerable attention has been focused on the use of phenanthroline complexes as intercalating agents of DNA (Erkkila *et al.*, 1999) and as artificial nucleases (Sigman *et al.*, 1993). It is well known that bis(1,10-phenanthroline) - copper (II) shows an efficient DNA cleavage activity in the presence of thiol and hydrogen peroxide (Sigman *et al.*, 1993). Numerous biological experiments have also demonstrated that DNA is the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA, which can cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death (Catherine *et al.*, 2001).

Amino acids are the basic structural units of proteins, and some copper complexes of amino acids were reported to exhibit potent antitumor and artificial nuclease activity (Gracia-Mora *et al.*, 2001). In this context, we focused

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our interests on the development of copper (II) complexes of phenanthroline with amino acids, and investigated their DNA cleavage activity. The selection of L-Phenylalanine as a second ligand in the copper (II) complex may enhance the affinity of the complex towards DNA.

In this paper, we synthesized and characterization of copper (II) complex by IR, EPR spectra and elemental analysis. The binding properties of the title complex to CT-DNA were carried out using UV - Visible absorption, fluorescence spectroscopic, cyclic voltametric and viscosity techniques and gel electrophoresis cleavage of pBR322 DNA in the absence and presence of ascorbic acid. The binding mode of the copper complex to DNA is accessed to be different mode from the experimental results, which implicated that the copper (II) complex can be a candidate for DNA-binding reagents, as well as laying the foundation for the rational design of new useful DNA probes. The gel electrophoresis experiment shown that the copper (II) complex can cleave pBR322 DNA effectively in the presence and absence of ascorbic acid as an effective inorganic nuclease. We have also reported the antimicrobial activities of a sample of copper (II) complex against Gram +ve and Gram -ve bacteria and fungus.

MATERIALS AND METHODS

All the reagents were of analytical grade (Sigma-Aldrich and Merck). Calf thymus DNA obtained from Sigma-Aldrich, Germany, was used as such. The spectroscopic titration was carried out in the buffer (50 mM NaCl-5 mM Tris-HCl, pH 7.1) at room temperature. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance 1.8-1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein (Marmur *et al.*, 1961). Milli-Q water was used to prepare the solutions. Absorption spectra were recorded on a UV-VIS-NIR Cary 5E Spectrophotometer using cuvettes of 1 cm path length, and emission spectra were recorded on a fluorolog. The complex, (Cu (phen) (L-phe) (H₂O)) ClO₄, was prepared as reported earlier in the literature (Subramanian *et al.*, 2001).

Physical measurements

Element analyses were performed by SAIF, Lucknow, India. The conductivity study was taken by using an aqueous solution of complex with an Elico conductivity bridge type CM82 and a dip-type cell and with cell constant 1.0. Absorption spectra were recorded on a UV-VIS-NIR Cary300 Spectrophotometer using cuvettes of 1-cm path length and emission spectra were recorded on a JASCO FP 770 spectrofluorimeter. FT-IR spectra were recorded on a FT-IR Perkin Elmer spectrophotometer with samples prepared as KBr pellets. EPR spectra were recorded on Varian E-112 EPR spectrometer at room temperature and at LNT (Liquid nitrogen temperature, 77 K), the field being calibrated with DPPH = 1, 10-diphenyl-2-picrylhydrazyl (g =2.0037). The antimicrobial screening studies were carried out at micro labs, Arcot, Tamil Nadu, India. The bacteria and fungus species were obtained from National Chemical Laboratory (NCL),

Pune, India. Electrochemical measurements were recorded on a Electrochemical analyzer CH Instrument version 5.01 and model-600C. A three-electrode system comprising of a glassy carbon working electrode, a platinum wire auxiliary electrode and a saturated calomel reference (SCE) electrode were used for voltammetric work. The buffer solution (50 mM NaCl-5 mM Tris-HCl) was used as supporting electrolyte. Agarose gel electrophoresis method was carried out at micro labs, Arcot, Tamil Nadu, India. Water purified using a Milli-Q system was used for all the present studies.

Synthesis of (Cu (Phen) (L-Phe) (EA))ClO₄

About 2.627 g (3 mM) of (Cu (phen) (L-phe)(H₂O)ClO₄) complex dissolved in 50 mL of water. To this 0.2254g (3 mM) of ethylamine in 15 mL of distilled water was added slowly with constant stirring for 30 min. A bluish substance was separated out, which was filtered and dried. Yield, 0.9 g (Found (%): C 49.92, H 4.67, N 10.04, Calc. (%): C 50.0, H 4.56, N 10.14.

DNA binding experiments

The DNA binding experiments were performed at 30.0 ± 0.2° C. The DNA concentration per nucleotide was determined by electronic absorption spectroscopy using the known molar extinction coefficient value of 6600 M⁻¹ cm⁻¹ at 260 nm (Reichmann *et al.*, 1954). Absorption titration experiments of copper (II) complex samples in buffer solution (50 mM NaCl-5 mM Tris-HCl, pH 7.2) were performed by using a fixed complex concentration to which increments of the DNA stock solutions were added. Copper (II) complex-DNA solutions were allowed to incubate for 10 min before the absorption spectra were recorded. For fluorescence quenching experiments DNA was pretreated with ethidium bromide (EB) for 30 min. The copper (II) complexes were then added to this mixture and their effect on the emission intensity was measured. Samples were excited at 450 nm and emission was observed between 500 and 800 nm.

Viscosity experiments were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 30.0±0.1 °C in a thermostatic water bath. Calf thymus DNA samples approximately 200 base pairs in average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility (Chaires *et al.*, 1982). Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio (Cohen *et al.*, 1969), where η is the viscosity of CT DNA in the presence of complex, and η_0 is the viscosity of CT DNA alone.

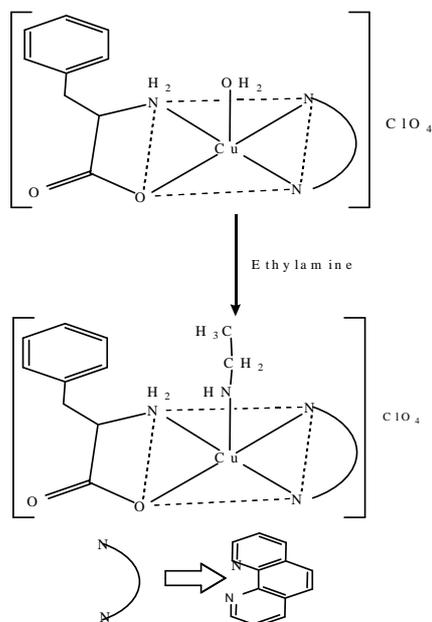
For the gel electrophoresis experiments, supercoiled pBR322 DNA (0.1 µg) was treated with the copper (II) complex in 50 mM Tris-HCl-18 mM NaCl buffer, pH 7.2. The samples were electrophoresed for 3 h at 50 V on a 0.8 % agarose gel in Tris-acetic acid-EDTA buffer. The gel was stained with 0.5 µg/mL of ethidium bromide and photographed under UV light.

Microbial assay

The in vitro antimicrobial screening of the copper (II) complex was tested for its effect on certain human pathogenic bacteria and fungus by disc diffusion method. The complex was stored dry at room temperature and dissolved in DMSO. Both the Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria were grown in nutrient agar medium and incubated at 37° C for 48 h followed by frequent subculture to fresh medium and were used as test bacteria. The fungi *Candida albicans* grown as sabourad dextrose agar medium were incubated at 27° C for 72 h followed by periodic subculturing to fresh medium and were used as test fungus. Then the petriplates were inoculated with a loop full of bacterial and fungal culture and spread throughout the petriplates uniformly with a sterile glass spreader. To each disc the test samples (10 ppm) and reference ciprofloxacin (1 µg/disc) for bacteria) or clotrimazole (10 µg/disc for fungus) was added with a sterile micropipette. The plates were then incubated at 35 ± 2°C for 24–48 h and 27 ± 1°C for bacteria and fungus, respectively. Plates with disc containing respective solvents served as control. Inhibition was recorded by measuring the diameter of the inhibitory zone after the period of incubation. All the experiments were repeated thrice and the average values are presented.

RESULTS AND DISCUSSION

The elemental analyses data were found to be in good agreement, with those of the calculated values. The Λ_M value of the complex in water is 110 Ohm⁻¹ cm² mol⁻¹, which indicated that the complex is 1:1 electrolytes (Geary 1971). The synthetic strategy of the complex is outlined in Scheme 1.



Scheme 1 Synthetic strategy of Cu (II) complex

Spectral and electrochemical characterization

In the IR region, the bands around 1613 cm⁻¹ and 1320 cm⁻¹ can be attributed to the ring stretching frequencies (ν (C=C) and ν (C=N)) of 1,10-phenanthroline (Zhang *et al.*, 2004). The IR values, δ (C-H) 751cm⁻¹ and 718 cm⁻¹ observed for phenanthroline are red shifted to 840 cm⁻¹ and 781 cm⁻¹. These shifts can be explained by the fact that each of the two nitrogen atoms of phenanthroline ligands donates a pair of electrons to the central copper metal forming a coordinate covalent bond (Jin *et al.*, 1996). The bands around 2900 cm⁻¹ and 2850 cm⁻¹ can be assigned to C–C stretching vibration of aliphatic CH₂ of L-phenylalanine and also CH₂ group of ethylamine. The broad band observed around 3425 cm⁻¹ and 3300 cm⁻¹ is assigned to the N–H stretching of L-phenylalanine (Geckeler *et al.*, 1980) and the band around 1086 cm⁻¹ has been assigned to ν (Cl–O) of perchlorate anions (Zhang *et al.*, 2006).

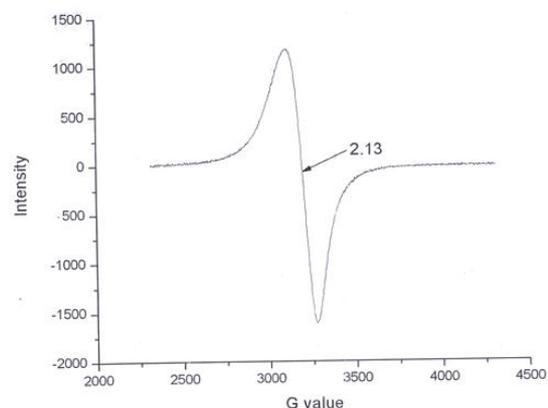


Fig. 1 EPR spectrum of (Cu(Phen)(L-Phe)(EA))ClO₄ in DMSO at liquid nitrogen temperature.

In the UV–Vis region, the intense absorption bands appeared from 240 to 300 nm is attributed to intraligand transitions. Another band which appeared around 270 nm is assigned to ligand field transitions (Liu *et al.*, 1999).

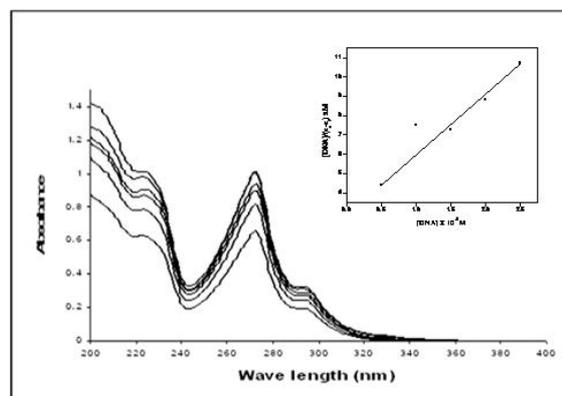


Fig. 2. Absorption spectra of (Cu(Phen)(L-Phe)(EA))ClO₄. In the absence and in the presence of increasing amounts of DNA. (Complex) = 15 µM. (DNA) = (5, 10, 15, 20, 25) µM. Inset: plot of (DNA)/($\epsilon_a - \epsilon_f$) vs. (DNA). Arrow shows the absorbance changes upon increasing DNA concentrations.

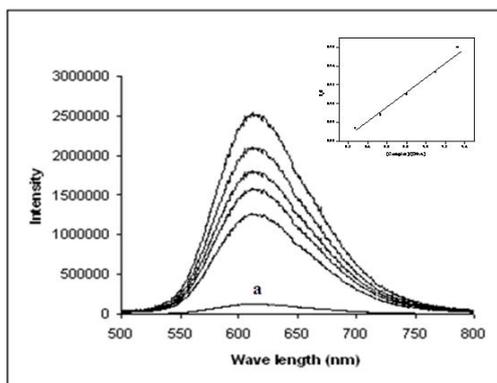


Fig. 3 Emission spectra of EB bound to DNA in the absence (a) and in the presence of (Cu (Phen)(L-Phe)(EA))ClO₄. (Complex) = 8 - 32 x 10⁻⁶ M. (DNA) = 3 x 10⁻⁵ M, (EB) = 3 x 10⁻⁵ M. Arrow shows the intensity changing upon increasing complex. Inset: plot of I₀/I vs. (Complex)/(DNA). Emission spectrum of EB alone (a) concentrations.

The solid state EPR spectra of the copper (II) complex were recorded in X-band frequencies shows Fig.1. At liquid nitrogen temperature the complex exhibits well defined single isotropic feature near g = 2.13. Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines. This intermolecular type of spin exchange is caused by the strong spin coupling which occurs during a coupling of two paramagnetic species.

DNA binding studies

Electronic spectral studies

Electronic absorption spectroscopy was an effective method to examine the binding mode of DNA with metal complexes (Barton *et al.*, 1984). In general, hyperchromism and blue-shift are associated with the binding of the complex to the helix by an intercalative mode involving strong stacking interaction of the aromatic chromophore of the complex between the DNA base pairs. Fig. 2 shows the UV absorption spectral study of copper (II) complex in the absence and presence of DNA. In the ultraviolet region from 240 to 300 nm, the complex had strong absorption peaks at 275 nm, besides a shoulder band around 295 nm. The absorption intensity of the copper (II) complex sample increased (hyperchromism) evidently after the addition of DNA, which indicated the interactions between DNA and the complex. We have observed minor bathochromic shift along with significant hyperchromicity for complex. The intrinsic binding constant, K_b, was determined by using the following equation (Pyle *et al.*, 1989):

$$(DNA) / (\epsilon_a - \epsilon_f) = (DNA) / (\epsilon_o - \epsilon_f) + 1/K_b(\epsilon_o - \epsilon_f)$$

Where (DNA) is the concentration of DNA in base pairs, ε_a, ε_f and ε_o correspond to A_{obsd} / (Cu), the extinction coefficient of the free copper complex and the extinction coefficient of the complex in the fully bound form, respectively, and K_b is the intrinsic binding constant. The ratio of the slope to intercept in the plot of (DNA) / (ε_a-ε_f) versus (DNA) gives the value of K_b and for our copper (II) complex it is 2.784 x 10⁻⁵. The binding propensity of

the phen complex due to the presence of extended planar aromatic ring in phen. Earlier studies on bis-phen copper complex have shown that this complex binds to DNA either by partial intercalation or binding of one phen ligand to the minor groove while the other phen making favourable contacts within the groove (Sigman *et al.*, 1993). The nature of binding of the phen complex is proposed to be similar as observed for the bis-phen species. The K_b values obtained for our copper (II) complex samples are much similar than those for any other known simple mononuclear or binuclear copper (II) complexes including complexes such as (Cu₂phen₂Cl₄) (K_b, 4.75 x 10⁴ M⁻¹) (Zhang, Q., et al), (Cu (phen)₂Cl₂) (K_b, 2.70 x 10³ M⁻¹) (Gupta *et al.*, 2004).

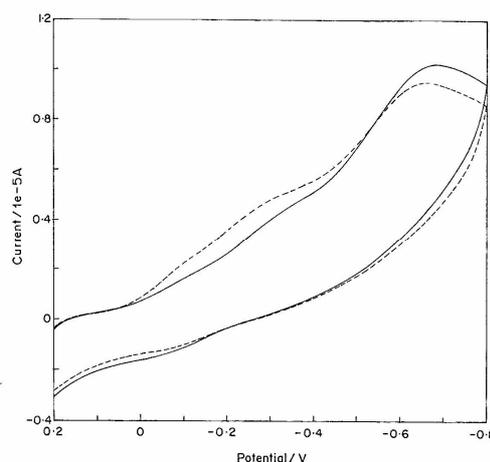


Fig. 4 Cyclic voltammogram of (Cu(Phen)(L-Phe)(EA))ClO₄ (1 mM) complex in the absence (—) and in the presence (---) of CT-DNA (1.5 x 10⁻⁵ M). 5 mM in buffer containing 50 mM NaCl-5 mM Tris-HCl, pH 7.2. Scan

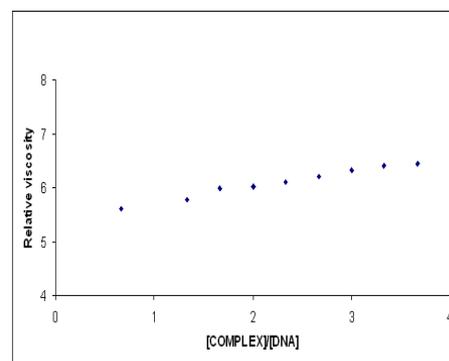


Fig.5 Effect of increasing amount of (Cu(Phen)(L-Phe)(EA))ClO₄(1,15,20,25,30,35,40,45,50 μM) on the relative viscosity of calf thymus DNA (15 μM) in 5mM Tris-HCl/50mM NaCl buffer.

Fluorescence spectral studies

As the copper (II) complexes are non-emissive, competitive binding studies with EthBr were carried out to gain support for the mode of binding of the complexes with DNA. The study involves addition of the complexes to DNA pretreated with EthBr ((DNA)/ (EthBr) = 1) and then measurement of intensity of emission. The observed enhancement in emission intensity of EthBr bound to DNA is due to intercalation of the fluorophore in between

the base pairs of DNA and stabilisation of its excited state (Fig. 3) (LePecq *et al.*, 1967). The addition of the complex to DNA pretreated with EB causes appreciable reduction in the emission intensity. This behaviour can be analysed through the Stern–Volmer equation (Lakowicz *et al.*, 1973), $I_0/I = 1 + K_{sv}r$, where I_0 and I are the fluorescence intensities in the absence and the presence of complex, respectively. K_{sv} is a linear Stern–Volmer quenching constant, r is the ratio of the total concentration of complex to that of DNA. The quenching plot (Fig. 3) illustrates that the quenching of EB bound to DNA by the copper (II) complex is in good agreement with the linear Stern–Volmer equation, which also indicates that the complex binds to DNA. In the plot of I_0/I versus (Complex)/(DNA), K_{sv} is given by the ratio of the slope to intercept. The K_{sv} value for our copper (II) complex thus obtained is 0.593, which is higher than that for ordinary transition metal copper complex (Jiang, C.W., *et al.*). This suggests that our copper (II) complex binds strongly with DNA, which is also consistent with our absorption spectral results.

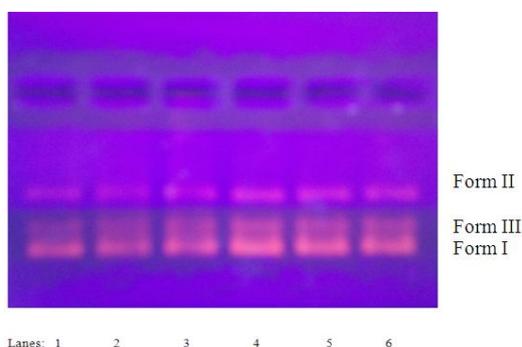


Fig.6 Electrophoretic behaviour of pBR322 DNA by (Cu(Phen)(L-Phe)(EA))ClO₄. Lane 1 pBR322 DNA alone; Lane 2-6: DNA + copper (II) complex in the concentration of 5, 10, 15, 20, 30, 40 μM.

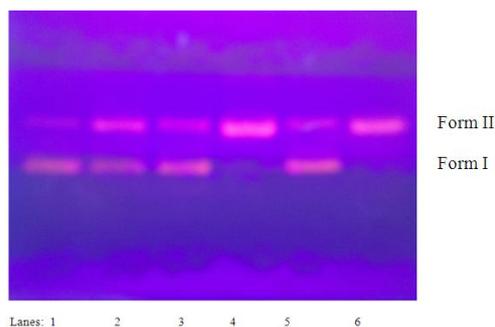


Fig. 7 Electrophoretic separations of pBR322 DNA by (Cu(Phen)(L-Phe)(EA))ClO₄. Lane 1: DNA alone, Lane 2: DNA + Complex (25 μM), Lane 3: DNA + Ascorbic Acid (100 μM), Lane 4: DNA + Ascorbic Acid (100 μM) + Complex (25 μM), Lane 5: DNA + Ascorbic Acid (500 μM), Lane 6: DNA + Ascorbic Acid (500 μM) + Complex (25 μM).

Viscosity studies

To explore further the interaction between the copper (II) complex and DNA, viscosity measurements were carried out on CT DNA by varying the concentration of the complex. Spectroscopic data are necessary, but insufficient to support an intercalative binding mode. Hydrodynamic measurements which are sensitive to

length increases are regarded as the least ambiguous and the most critical tests of binding on solution in the absence of crystallographic structure data (Kelly *et al.*, 1985). A classical intercalation mode causes a significant increase in the viscosity of DNA solution due to the increase in separation of the base pairs at intercalations sites and hence to an increase in overall DNA contours length. A partial and/or nonclassical intercalation of ligand would reduce the DNA viscosity (Yang *et al.*, 1997). The effects of the copper (II) complex on the viscosity of CT DNA solution are given in figure 5.

The plot shows that complex had a reverse effect on the relative viscosity of the CT DNA. With addition of the complex, the relative viscosity of DNA increased. Since the increase is far less than that observed for an intercalator such as EB. This observations leads us to support the above spectral studies which suggest that the complex interact with DNA via partial intercalation between DNA base pairs, which is similar to the interaction of (Cu(phen)₂)²⁺ with DNA (Mahadevan *et al.*, 1998).

Cyclic voltammetric study

Cyclic voltammetric techniques was employed to study the interaction of the present redox active metal complex with DNA with a view to further explore the DNA binding modes assessed from the above spectral and viscometric studies. Typical cyclic voltammetry (CV) behaviors of (Cu(Phen)(L-Phe)(EA))ClO₄ in the absence and presence of CT-DNA are shown in Fig. 4. The cyclic voltammogram of (Cu(Phen)(L-Phe)(EA))ClO₄ in the absence of DNA featured reduction of Cu(II) to the Cu(I) form at a cathodic peak potential (Monica, B., *et al.*), Epc of -0.68 V and anodic peak potential, Epa of -0.4 V. The separation of the anodic and cathodic peak potentials, Ep = -0.28 V. The formal potential E_{1/2}, taken as average of Epc and Epa, is -0.54 V in the absence of DNA. The presence of DNA in the solution at the same concentration of (Cu(Phen)(L-Phe)(EA))ClO₄ causes a considerable decrease in the voltammetric current coupled with a slight shift in the E_{1/2} (E_{1/2} = -0.52 V) to less negative potential. The drop of the voltammetric currents in the presence of CT-DNA can be attributed to diffusion of the metal complex bound to the large, slowly diffusing DNA molecule. Obviously, E_{1/2} undergoes a positive shift (25 mV) after forming aggregation with DNA, suggesting that the copper complex bind to DNA mainly by intercalation binding mode (Carter *et al.*, 1989.), and this result also proves the results obtained from viscosity and absorption spectrum studies again.

Gel electrophoresis studies

The characterization of DNA recognition by transition metal complex has been aided by the DNA cleavage chemistry that is associated with redox-active or photoactivated metal complexes (Sitlani *et al.*, 1992). DNA cleavage is controlled by relaxation of supercoiled circular form of pBR322 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be

observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between.

reported that copper complex show any significant activity against the Gram positive and Gram negative bacteria. Recently Patel *et al.*, 2005, have indicated that the copper (II) complex with L-phenylalanine has exhibited considerable activity against some human pathogens (Patel *et al.*, 2005).

Table 1 Antimicrobial activities of complex

Complex	Diameter of zone inhibition (nm)								
(Cu(Phe)(L-Phen)(EA)ClO ₄)	1	2	3	4	5	6	7	8	9
	26	32	25	21	21	5	15	8	8

1, *Staphylococcus aureus*; 2, *Bacillus cereus*; 3, *Micrococcus luteus*;
4, *Escherichia coli*; 5, *Klebsiella pneumoniae*; 6, *Pseudomonas aeruginosa*;
7, *Aspergillus niger*; 8, *Aspergillus flavus*; 9, *Candida albicans*; Solvent, DMSO

DNA cleavage was analyzed by monitoring the conversion of supercoiled DNA (Form I) to nicked DNA (Form II) and linear DNA (Form III) in aerobic condition. Interestingly, we have found that this copper complex can cleave the supercoiled DNA to nicked and linear DNA at the same time. As shown in Fig. 6, with the increase of the complex concentration, the intensity of the circular supercoiled DNA (Form I) band was found decrease, while that of nicked (Form II) and linear DNA (Form III) bands increase apparently. When the complex concentration was up to 30 μM (lane 5), the circular supercoiled DNA (Form I) band was extremely faint, when it more than 40 μM (lane 6), the circular supercoiled DNA (Form I) band was disappeared completely.

In order to establish the reactive species responsible for the cleavage of the plasmid DNA, we carried out the experiment in the presence of ascorbic acid as reducing agent (Fig. 7). Compared with the control experiments using only the copper (II) complex or ascorbic acid (lane 2, lane 3 and lane 5), the experiment using both copper (II) complex and the same concentration of ascorbic acid (lane 4 and lane 6) showed that the supercoiled DNA (Form I) apparently convert to nicked (Form II) and linear DNA (Form III). Although the ascorbic acid concentration in lane 5 was fivefold of that in lane 3, there is little difference between these two bands. When we add the same concentration of the copper (II) complex to them, an obvious difference occurred. Compared with lane 4, the supercoiled DNA (Form I) completely disappeared and the linear DNA (form III) apparently appeared in lane 6. These results are similar to that observed for some Cu-salen complexes as chemical nuclease (Sigman 1990). It is likely the generation of hydroxyl radical and/or activated oxygen mediated by the copper complex results in DNA cleavage. Further studies are undergoing to clarify the cleavage mechanism.

Antibacterial and Antifungal screening

The copper (II) complex was screened in vitro for its microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method. The complex was found to exhibit considerable activity against Gram positive and Gram negative bacteria and the fungus *C. albicans*. The test solutions were prepared in dimethyl sulfoxide and the results of the antimicrobial activities are summarized in Table 1. Zoroddu *et al.*, 1996, have

In our biological experiments, using copper (II) complex, we have observed antibacterial activity against Gram positive bacteria *Staphylococcus aureus* and *B. subtilis* and Gram negative bacteria *E. coli* and *Pseudomonas aeruginosa*. The copper (II) complex has shown high activity against Gram positive than Gram negative bacteria. The copper (II) complex is also very active against the fungus *C. albicans* than the standard antifungal drug, clotrimazole. It may be concluded that our copper (II) complex inhibits the growth of bacteria and fungi to a greater extent.

CONCLUSION

We described here new copper (II) complexes. Further characterization of the complexes was achieved through physico-chemical and spectroscopic methods. The effectiveness of binding of complex is being confirmed by means of hyperchromism in the electronic spectral studies and decrease in intensity of emission in the case of emission spectral studies. Besides that, the effectiveness of binding is also confirmed by the viscometric and cyclic voltametric studies. This shows that the complex is partial intercalative and also groove mode with DNA base pairs effectively. The super coiled DNA is being cleaved in the electrophoresis by the complex which confirms that the complex is having the ability to act as potent DNA cleaving agent. The complex (Cu (Phen)(L-Phen)(EA)ClO₄) exhibit good antimicrobial activity.

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