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DNA Binding and Biological Activities of Ternary Copper(II) Complexes Containing L-valine and Urea

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ABSTRACT

Ternary copper complexes [Cu(phen)(L-val)U)] 1 and [Cu(bpy)(L-val)U)] 2 (phen - 1,10-phenanthroline bpy - 2,2'-bipyridyl, L-val - L-valine and U - Urea), have been synthesized and characterized by CHN analysis, electronic absorption, infrared, and electron paramagnetic resonance spectral studies. They have been tested for their in vitro DNA binding activities by the spectroscopic methods such as ultraviolet-visible spectral studies, emission spectral studies, viscosity, and cyclic voltammetry measurement. The antibacterial and antifungal studies suggested that complexes possess potential biological activities.

Key words: *L-valine, Heterocyclic bases, Cu(II) complex, DNA binding.*

1. INTRODUCTION

During the past few decades, important research directions in bioinorganic and medicinal chemistry are the development of new anticancer drugs [1,2]. Today cisplatin is the one of the most successful anticancer drug; however, its applications are restricted due to its side effects [3]. The majority of these drugs are not active against cancer cell lines and metastasis (secondary) cancers [4]. To overcome these challenges, significant attempts have been made to replace the cisplatin with appropriate metal-based anticancer drugs. Bioinorganic and medicinal chemists have paid much attention on the design of new metal-based anticancer agents with enhance antimicrobial activity, good selectivity, and low toxicities [5,6]. Particularly, copper(II) complexes have been widely studied because of strong interactions with nucleus base pairs through different modes [7,8]. The phen/bpy complexes have shown good binding activity with DNA, also polypyridyl complexes have a vital role in antimicrobial and anticancer studies [9-13]. The copper(II) complexes containing bpy or phen have concerned much their much more active in the presence of the heterocyclic ligands with DNA. Our group recently focused on phen/bpy containing Cu(II) complexes with various aminoacid are effectively binding with good DNA binding affinity and anticancer activity [14-18]. All of the above facts have induced our interest in the present work. Ternary copper complexes [Cu(phen)(L-val)U)] (1) and [Cu(bpy)(L-val)U)] (2) have been synthesized and characterized also the DNA binding/cleavage and cytotoxic study of our complexes carried out.

2. EXPERIMENTAL

2.1. Materials and Methods

All the chemicals and reagents were obtained from Sigma Aldrich and used without further purification. Calf thymus DNA (CT-DNA) and ethidium bromide (EB) also were obtained from Sigma Aldrich. Ultraviolet-visible (UV-vis) spectrometer was employed to check the solution of CT-DNA purity (A260: A280>1.80) and the concentration (ε=6600 M^{-1} cm^{-1} at 260 nm) in the buffer. The elemental analyses were performed in the Euro-E 3000. The UV-vis absorption spectra were recorded using a Shimadzu UV-2450 and fluorescence emission spectra were recorded using a Jobvin Yvon flurolog-3-11 spectrofluorimeter. The infrared (IR) spectra (KBr pellet) were recorded using an Perkin Elmer system one Fourier-transform IR spectrometer in the range of 4000-450 cm^{-1}. The electron paramagnetic resonance (EPR) spectrum was recorded on the JEOL Model JES FA 200 spectrometer. Viscosity experiments were carried on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained at 28.0 ± 0.5°C. Cyclic voltammetry experiments were recorded on CHI 602D (CH Instruments Co., USA) electrochemical analyzer under oxygen-free conditions using a three-

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electrode cell in water solution with KCl (0.1 M) as the supporting electrolyte.

2.2. Synthesis of [Cu(phen)(L-valine)U]NO_3 (1)

The complex [Cu(phen)(L-val)(H_2O)](NO_3) was synthesized according to a published method [19]. To the aqueous solution of [Cu(phen)(L-val)(H_2O)] (NO_3) (1 mmol) was added urea (1 mmol) the color of the solution change from blue-to-bluish green. The resulting solution was stirred for 4 h and then solution complex was filtered. The filtrate was kept for slow evaporation, after 2 weeks bluish-green-colored complex was separated out. Yield: 72%; analytically (%) calculated for $C_{18}H_{22}CuN_6O_6$: C, 44.86; H, 4.60; N, 17.44. Found: C, 45.21; H, 5.72; N, 16.64. IR (KBr pellet): 3396, 3213, 3134, 2949, 1639, 1585, 1381, 1311, 1124, 852, 719 cm^{-1}. UV-Vis (λ, nm): 272, 294 and 610.

2.3. Synthesis of [Cu(bpy)(L-val)U]NO_3 (2)

Preparation of complex 2 is same as described above. Yield: 76%; analytically (%) calculated for $C_{16}H_{22}CuN_6O_6$: C, 41.97; H, 4.84; N, 18.35. Found: C, 42.67; H, 4.13; N, 18.91. IR (KBr pellet): 3483, 3142, 3062, 2873, 1737, 1608, 1587, 1431, 1369, 1350, 1315, 1259, 1224, 1070, 833 cm^{-1}. UV-vis (λ, nm): 300, 310 and 614.

The detailed experimental setup of DNA binding, 3-(4,5-dimethythiazol-.2-yl)-2,5-diphenyl tetrazolium bromide assay and antimicrobial activity described in Baskaran *et al*. [20], Santhakumar and Arumugham [21], Gopinathan *et al*. [22].

3. RESULTS AND DISCUSSION

3.1. General Aspects

The ternary L-valine copper(II) complexes containing heterocyclic bases are prepared in high yield from a general reaction in which a copper(II) salt is reacted with N,N-donor heterocyclic base, namely, phen and bpy, L-valine and urea (Scheme 1). The complex formulated as [Cu(L-val)(HB)(U)]NO_3 where HB is the heterocyclic bases (phen and bipy). A d-d band near 610 nm in water due to d-d transition and band near 390 nm is assignable due to intraligand transitions (Figure 1). In the IR region (Figure 2), for complex 1 the band around 3396 cm^{-1} (1) and 3483 cm^{-1} (2) can be assigned to γ (N-H) stretching frequency of amino acid. The IR values, γ(C-H) at 2945 cm^{-1} and 2873 cm^{-1} observed for heterocyclic bases for

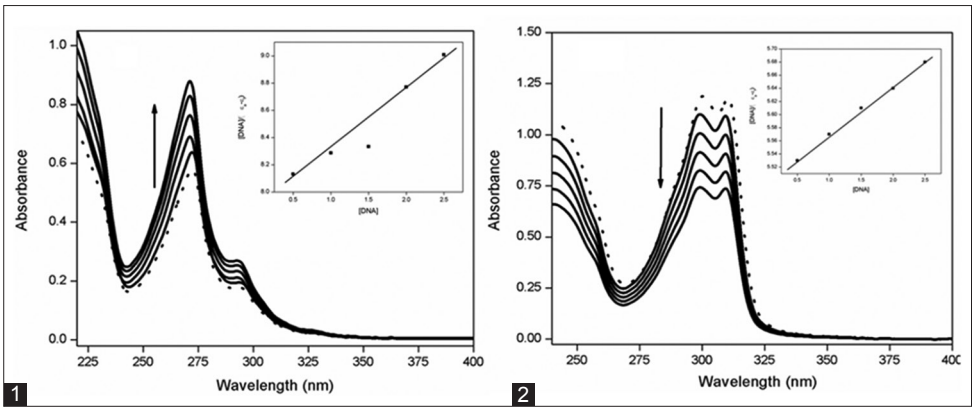


Figure 1: Absorption spectral traces on addition of calf thymus (CT)-DNA to complexes 1 and 2 (shown by arrow). Inset plot of [DNA]/($\varepsilon_a-\varepsilon_f$) versus [DNA] for absorption titration of CT-DNA with complexes.

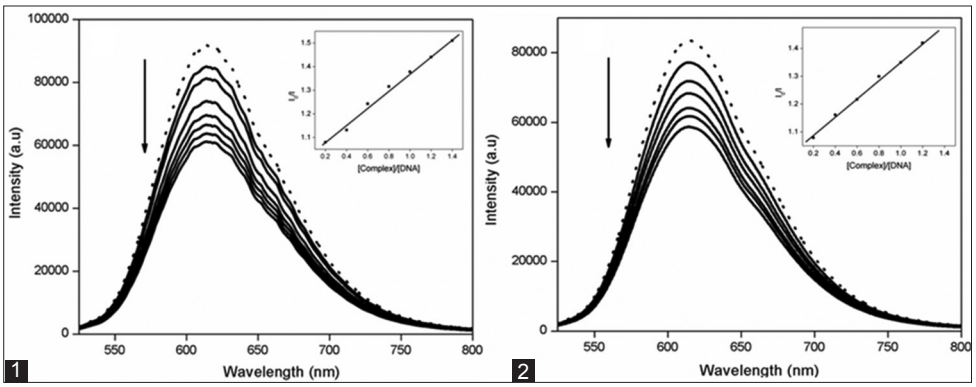


Figure 2: Emission spectra of ethidium bromide bound to DNA in the absence (dotted line) and the presence (dashed line) of complexes 1 and 2. Arrow (↓) shows the intensity changes upon increasing the concentration of the complex. Inset: Stern–Volmer quenching curves.

complex 1 and 2, respectively. The coordination of nitrogen atoms of heterocyclic base with copper metal ion can be examined by δ(C-H) for phenanthroline 853 cm^{-1} and 737 cm^{-1} is shifted to 833 cm^{-1} and 727 cm^{-1} and the band around 1311 cm^{-1} (1) and 1315 cm^{-1} (2) has been assigned for γ(N-O) of nitrate ion. EPR spectra (Figure 3) of mononuclear complexes copper(II) species with S=1/2, those with two signals ($g_\perp$ and $g_\parallel$), on comparing these two signals $g_\perp(x,y)>g_\parallel(z)$ ($B_\perp(x,y)<B_\parallel(z)$) representing elongated axial symmetry of the spin tensor.

3.2. Spectral Studies on DNA Interaction

3.2.1. Electronic spectral studies

The binding complexes (1 and 2) to CT-DNA have been studied by electronic spectral techniques. The absorption spectral traces of complex (1) with increasing concentration of CT DNA are shown in Figure 1. We have observed a minor hypsochromic shift of 2 nm along with significant hyperchromicity for the phen and hypochromicity for bpy complex. The intrinsic DNA binding constants (Kb) of the complexes to CT-DNA are obtained by monitoring the change of the absorption intensity of the spectral bands with increasing concentration of CT DNA, keeping the complex concentration constant. The bpy complex shows weak binding to the DNA due to less extended planarity compared to phen, which is consistent with the observed trend in hypochromism [23]. The intrinsic binding constant (Kb), was determined from the following equation:

$$\frac{[DNA]}{(\varepsilon_a-\varepsilon_f)}=\frac{[DNA]}{(\varepsilon_b-\varepsilon_f)}+\frac{1}{Kb(\varepsilon_b-\varepsilon_f)} \quad (1)$$

The extinction coefficient (ε_a) was obtained by calculating Aobsd/[Cu]. The terms ε_f and ε_b correspond to the extinction coefficients of free (unbound) and fully bound complexes, respectively. A plot of [DNA]/(ε_a–ε_f) versus [DNA] will give a slope 1/(ε_a–ε_f) and an intercept 1/Kb (ε_a–ε_f). Kb is the

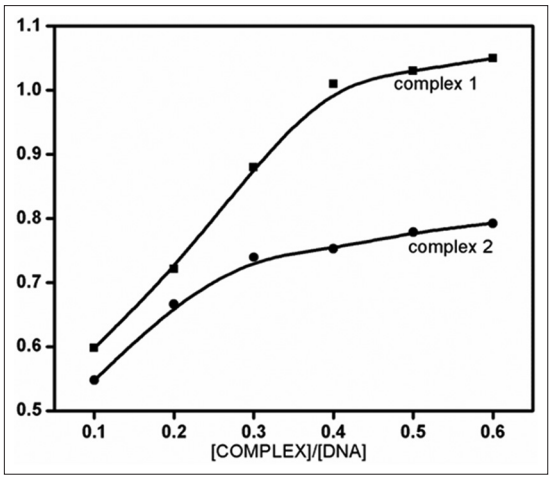
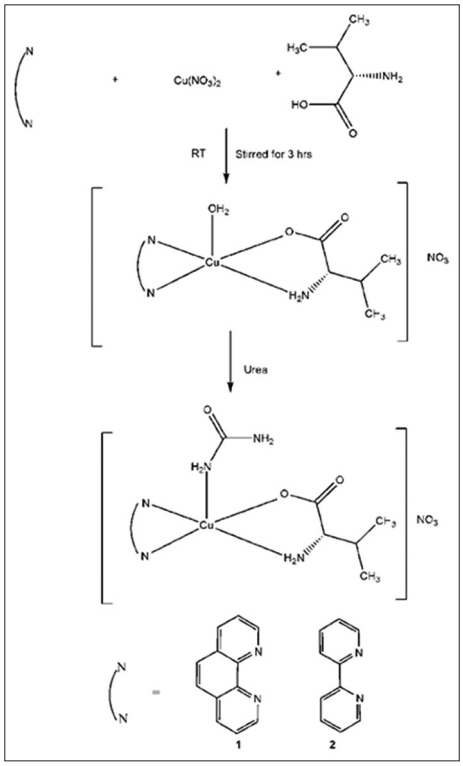


Figure 3: Viscosity of complexes 1 and 2 with calf thymus DNA.



Scheme 1: Synthesis of complexes 1 and 2.

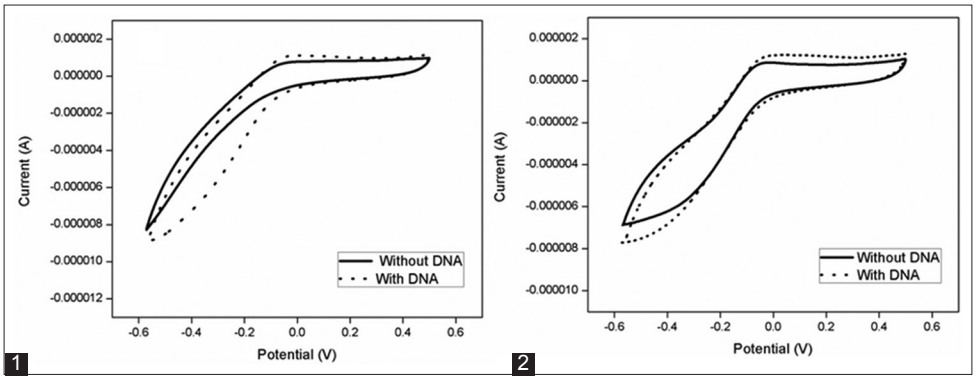


Figure 4: Cyclic voltammogram of complexes (1 and 2) in the absence and presence of calf thymus DNA.

ratio of the slope and the intercept (Equation 1). The Kb values for complexes 1 and 2 are 4.48×10^5 M^{-1} and 7.46×10^4 M^{-1}, respectively. The higher binding propensity of the phen complex in comparison to their bpy could be due to the presence of extended planar aromatic rings facilitating non-covalent interactions with the DNA molecule. Earlier studies on bis-phen copper complexes have shown that this complex binds to DNA either by partial intercalation or by binding of one phen ligand to the minor groove while the other phen making favorable contacts within the groove [24]. The nature of binding of the phen complex is proposed to be similar as observed for the bis-phen species.

3.2.2. Fluorescent spectral studies

To further investigate the interaction between the complex and CT-DNA, the EB fluorescence displacement experiments were also employed. The fluorescence intensity of DNA is negligible; meanwhile, the EB in buffer solution is not significant due to quenching by the solvent molecules. However, if the complex can intercalate into DNA, the binding sites of the DNA available for EB will decrease, and hence the intensity of EB will be quenched. Therefore, EB can be used to explore the interaction of complex with DNA. It should be pointed out that the inner-filter effect (IFE), which refers to the absorbance of light at the excitation or emission wavelength by other compounds present in the solution, can also lead to the decrease in fluorescence intensity. The decrease in the fluorescence intensity of solution induced by the occurrence of IFE could lead to a non-linear relationship between the observed fluorescence intensity and the concentration of the fluorophore, as well as result in the introduction of large errors in the data interpretation. Therefore, it is necessary to consider the IFE and eliminate its interference with the results (Equation 2). In our study, IFE was corrected with the following equation:

$$F_{corr} = F_{obs} \times 10^{\frac{A_{ex} \times d_{ex}}{2} + \frac{A_{em} \times d_{em}}{2}} \quad (2)$$

Where, F_{obs} and F_{corr} are the measured fluorescence and the correct fluorescence intensity, respectively, d_{ex} and d_{em} are the cuvette pathlength in the excitation and emission direction (in cm), and A_{ex} and A_{em} stand for the measured change in absorbance value at the excitation and emission wavelength, respectively. After the removal of IFE, the fluorescence intensities of EB bound to CT-DNA at 614 nm, as illustrated in Figure 2 show remarkable decreasing trends with the increasing concentration of the complexes, indicating that some EB molecules were released into solution after the exchange with the two complexes, resulting in the fluorescence quenching of EB. These observations may be interpreted as the intercalation of the complexes between the base pairs of CT-DNA. The quenching of EB bound to the DNA by the complexes is in agreement with the linear Stern–Volmer (Equation 3) equation [25]:

$$\frac{I_0}{I} = 1 + K_{sv}[Q] \quad (3)$$

Where, I_0 and I represent the fluorescence intensities in the absence and presence of complex, respectively. K_{sv} is a linear Stern–Volmer quenching constant, Q is the concentration of complex. In the quenching plot (insets in Figure 4) of I_0/I versus [complex], the K_{sv} values for complexes 1 and 2 are 4.72 and 3.69, respectively. Thus, the evidences observed in the EB fluorescence displacement experiments are in agreement with the conclusion derived by the electronic absorption spectra measurements.

3.3. Viscometry Studies

The values of relative specific viscosity $(\eta/\eta_0)^{1/3}$, where η and η_0 are the specific viscosities of DNA in the presence and absence of the complexes, were determined and plotted against values of [complex]/[DNA] and specific viscosity (Figure 3). A small to large increase in viscosity of DNA is observed for almost both complexes, the increase in viscosity of DNA by 1 is more than 2. However, lower than that for the potential intercalator, namely, EB suggesting insertion of planar phen ring into the base pairs of DNA viscosity enhancement for the phen complex 1, which is higher than bpy complex 2, is traced to the presence of the central planar aromatic ring in the former. Thus, all these observations suggest that it is the central aromatic rings of phen, which are involved in intercalative mode of DNA binding [26,27].

3.4. Cyclic Voltammetric (CV) Studies

The CV response for complexes 1 and 2 in Tris–HCl buffer (pH 7.28) in the presence and absence of CT DNA is shown in Figure 4. In the forward scan, a single cathodic peak was observed hardly, in the reverse scan, anodic peak was observed, which indicates that the process is reversible. When CT-DNA is added to a solution of complexes, marked decrease in the peak current and potential values were observed. In complex 1, before addition of DNA the anodic peak was not clear, after the addition of DNA, the anodic peak observed at −0.426 V. In complex 2, when addition of DNA the anodic peak shifted from −0.483 V to −0.499V, the slight decrease in peak current of complexes after the addition of DNA due to the binding of complex to the DNA [28].

3.5. Antibacterial and Antifungal Activity

The copper(II) complexes were screened *in vitro* for its microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method. The complexes were found to exhibit considerable activity

Table 1: Antibacterial and antifungal activity of copper (II) complexes.

Micro organisms	Zone of inhibition (mm)			
	Complex 1	Complex 2	Copper nitrate	Ciprofloxacin
Bacteria				
Escherichia coli	25	28	13	19
Enterococcus faecalis	27	26	19	29
Staphylococcus aureus	29	22	32	33
Micro organisms	**Zone of inhibition (mm)**			
	Complex 1	**Complex 2**	**Copper nitrate**	**Amphotericin-B**
Fungi				
Aspergillus fumicatus	18	19	16	15
Mucor sps	16	16	14	14

against bacteria and the fungus. Our group recently, reported that aminoacid containing complexes have good antimicrobial activity [21]. Copper complexes show considerable activity against the bacteria, the copper(II) complexes with L-phenylalanine has exhibited considerable activity against some human pathogens. In our biological experiments, using copper(II) complexes, we have observed antibacterial activity antifungal activity. The complex 1 has shown higher antibacterial activity (Table 1) against *Staphylococcus aureus* and complex 2 has shown higher activity against *Escherichia coli*. Complex 1 and 2 have shown high antifungal activity against *Aspergillus fumigatus*. It may be concluded that our complexes 1 and 2 inhibit the growth of bacteria and fungi to a greater extent.

4. CONCLUSION

Ternary copper(II) complexes having heterocyclic bases and L-valine are prepared and characterized. The copper(II) complexes with heterocyclic base in CuN_4O coordination geometry shows DNA binding ability. The combination of UV absorption, fluorescence, viscosity, and cyclic voltammetry showed that the copper(II) complexes could bind to CT-DNA in intercalative (1) mode and partial intercalation (2) mode. The results might be provide insights into copper(II) complexes antimicrobial activities.

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Bibliographical Sketch

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