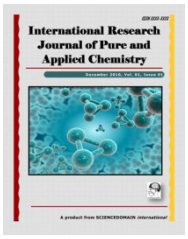

International Research Journal of Pure & Applied Chemistry
1(2): 42-57, 2011

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New Copper(II) Complexes of Peptides: DNA Binding and Photocleavage Studies

C. N. Sudhamani[1], H. S. Bhojya Naik[1*], D. Girija[1] and T. Aravinda[1]

[1]Department of Studies and Research in Industrial Chemistry, School of Chemical Sciences, Kuvempu University, Shankaraghatta-577451, India.

Research Article

Received 28th March 2011
Accepted 28th April 2011
Online Ready 3rd May 2011

ABSTRACT

Peptides were synthesized by the reaction of Fmoc-amino acid chloride and aminoacid ester hydrochloride in DCM. The obtained peptide ligands were characterized by elemental analysis, NMR, IR and mass spectra. Three copper complexes of peptides Fmoc.Ala-val-OH (F-AVOH), Fmoc-Phe-Leu-Ome (F-PLOMe) and Z-Ala-Phe-CO-NH_2 (Z-APCONH_2) and have been synthesized and characterized by elemental analysis, IR and mass spectral data. The IR spectral results shows that the –NH group of peptides chain involved in bonding with metal ion. The binding of the complexes with calf thymus DNA have been investigated using absorption spectroscopy, viscosity measurements and thermal denaturation methods. The results suggest that the complexes bound to CT-DNA by partial intercalation with binding constant ≈ 3.9 - 4.8x10^4 M^{-1}. The photonuclease activities of the complexes were assayed on pUC19 DNA and they exhibit efficient photonuclease properties.

Keywords: pUC19 DNA; binding; intercalation; photonuclease activities;

1. INTRODUCTION

Metal-containing amino acids and peptides are key components for the study of photo-induced energy and electron transfer processes (Wuttke et al., 1993; Tsai and Chang, 1998;

**Corresponding author: Email: hsb_naik@rediffmail.com;*

Gretchikhine and Ogawa, 1996), as well as for the development of chemosensors and labeling reagents (Torrado and Imperiali, 1996; Pogozelski and Tullius, 1998).

Amino acid and peptide-based transition metal complexes without having any photoactive group are used as synthetic hydrolyses and chemical nucleases (Ren et al., 2000; Herebian and Sheldrick, 2002; Cheng et al., 2001; Roger et al., 2003). Amino acids and peptides tethered with photoactive organic molecules are known to cleave DNA on photoirradiation at UV light (Mahon et al., 2003; Saito and Takayama, 1995). Widespread clinical applications of platinum complexes in cancer chemotherapy resulted in many publications on the platinum(II/IV) and palladium(II) complexes of peptide ligands (Saito and Takayama, 1995; Shi et al., 1999; Watabe et al., 1999, 2000; Haas et al., 1999).

The bioactive copper(II) complexes of histidine-containing small peptides have a striking interest as mimicking the structure of various metalloenzymes. The most important results obtained from the copper(II) complexes of the terminally free peptides have already been reviewed (Kozlowski et al., 1999). Copper complexes, which possess biologically accessible redox potentials and demonstrate high nucleobase affinity, are potential reagents for cleavage of DNA both hydrolytically (Hegg and Burstyn, 1998) and oxidatively (Sigman, 1986). The ability of copper complexes to cleave DNA upon photoactivation under physiological conditions has also received considerable attention because of their possible utility in highly targeted photodynamic therapeutic applications (Armitage, 1998; Dhar et al., 2003; Bradley et al., 2004). Studies with a copper tripeptide, suggested that it displayed some anti-tumor activity. It is clear from these studies that simple peptides are very efficient and versatile ligands for the complexation with divalent copper ions and show good biological activity.

Here, the present work stems from our continuous interest (Prabhakara and Bhojya naik (2008); Prakash Naik et al. (2004)) in defining and evaluating binding ability of metal complexes containing different biologically active ligands to DNA, We synthesized different peptides and their Cu(II) complexes and studied their DNA binding and photocleavge activities.

2. MATERIALS AND METHODS

All reagents and solvents required in this study were of AR grade, purchased commercially. Amino acids were purchased from Himedia (India) and used without further purification. The solvents were purified by distillation and used. The $CuCl_2.2H_2O$ and *Tris*-HCl were purchased from Merck (India), calf thymus (ds)DNA and Super coiled (SC)pUC19 DNA were purchased from Bangalore Genie (India), Agarose (molecular biology grade) ethidium bromide were purchased from Himedia (India). *Tris*-HCl buffer solution used for binding was prepared using deionised double distilled water. Elemental analyses were done on Perkin-Elmer Model 240-C CHN analyzer. The molar conductivities of the complexes in dimethylformamide (DMF) solution (10^{-3} M) at room temperature were measured using Equip-tronic digital conductivity meter model No. EQ-660A. Magnetic susceptibilities of the solid complexes were measured employing Gouy balance at room temperature ($28\pm2\ ^{o}C$). The electronic spectra of the complexes in the UV-visible region were measured using Shimadzu spectrophotometric model UV-1650 PC double beam spectrophotometer. Viscosity measurements were carried out at $25\pm1°C$ using semimicro dilution capillary viscometer at room temperature. IR spectra were recorded in 4000-250 cm^{-1} region using KBr pellets on Shimadzu FTIR-8400S.

The DNA binding experiments were carried out in *Tris*-HCl buffer (*Tris*-HCl buffer containing 50 mM NaCl, pH 7.2) in absorption, viscosity measurements and phosphate buffer (1 mM Phosphate, pH 7.2 mM NaCl) was used for thermal denaturation. The calf thymus (ds) DNA in the buffer medium gave a ratio of UV absorbance at 260 and at 280 nm of ca.1.9:1 suggesting the DNA apparently free from protein. The concentration of DNA was estimated from its absorption intensity at 260 nm with a known molar absorption coefficient value of 6600 dm^3 mol^{-1} cm^{-1} (Reichmann et al., 1954). Absorption titration experiment was performed by varying the concentration of the CT DNA with the metal complex concentration. The absorption data were analyzed for an evaluation of the intrinsic binding constant K_b using reported procedure (Wolf et al., 1987). Viscosity measurements were carried out using a semimicro dilution capillary viscometer at room temperature. Each experiment was performed three times and an average flow time was calculated. Data were presented as $(\eta|\eta_o)$*Vs* binding ratio, where η is the viscosity of DNA in the presence of complex and η_o is the viscosity of DNA alone. Thermal denaturation experiments were carried out by monitoring the absorption of CT DNA in 50 μM concentration for the nucleotides at 260 nm with different temperature in the presence (10 μM complex) and the absence of each complex. The melting temperature (T_m, the temperature at which 50% of double stranded DNA becomes single stranded) and the curve width (σ_T, the temperature range between which 10 and 90% of the absorption increases occurred) were recorded.

The cleavage of DNA was monitored using agarose gel electrophoresis. (Chakravarty, 2006). Supercoiled pUC19DNA (0.5 μg) in *Tris*-HCl buffer (50 mM) with 50 mM NaCl (pH 7.2) was treated with metal complex (40 and 60 μM) followed by dilution with *Tris*-HCl buffer to a total volume of 20 μl. The samples were incubated for 1h at 37 °C. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol were added and electrophoresis was performed at 50 V for 3 h in TBE buffer using 0.8% agarose gel containing 1.0 μg/ml ethidium bromide. The samples were irradiated for 60 min inside the sample chamber. Bands were visualized using UV light and photographed. The cleavage efficiency was measured by determining the ability of the complex to convert the supercoiled DNA (SC) to nicked circular form (NC) and linear form (LC).

2.1 Synthesis of Peptides

The peptides such as (F-AVOH), (F-PLOMe) and (Z-APCONH$_2$) were synthesized according to the method reported earlier (Gopi and Suresh Babu, 1998) purity of the peptides was checked by TLC, HPLC and their structure was elucidated by IR, mass and ^{1}H NMR spectra.

2.2 General Procedure for the Synthesis of the Complexes [Cu(F-AVOH)$_2$](I), [Cu(F-PLOMe)$_2$](II) and [Cu(Z-APCONH$_2$)$_2$](III)

Methanolic solution of 2 mmol of Fmoc.Ala-val-OH (0.792 g) /Fmoc-Phe-Leu-Ome (0.5 g)/ Z-Ala-Phe-CO-NH$_2$ (0.710 g) initially reacted with NaOH (1.0 mmol) and it was added drop wise in to a methanolic solution of $CuCl_2.2H_2O$ (0.17 g, 1.0 mmol) with magnetic stirring for 0.5-1 hrs. Then, the reaction mixture was stirred at room temperature for 10 h. The resulting solution was filtered and precipitated the complex by addition of ammonium hexafluorophosphate. The obtained complex was isolated and washed with cold methanol.

3. RESULTS AND DISCUSSION

3.1 Characterization of the Peptides and Their Copper(II) Complexes

The elemental analysis data, IR, molar conductance and magnetic moment data of the new complexes are summarized in the Table 1 and 2. The obtained elemental analysis data are in agreement with the theoretical values within the limits of experimental error. The elemental analysis data suggest that, they have the molecular formula ML_2 (where M is copper(II) ion, L = (F-AVOH), (F-PLOMe) or (Z-APCONH$_2$)) with the metal to ligand ratio of 1:2.

The complexes showed good solubility in DMF, DMSO and less solubility in methanol, ethanol and water. But their salt form obtained by the addition of ammonium hexaflurophosphate was found to be soluble in buffer solution. The conductometric measurement values of the complexes observed in the range of 15-22 mhos cm^2mol^{-1} in DMF at 10^{-3} M concentration indicate they are non-electrolytic in nature.

Table 1. Elemental analysis data of peptide and their Cu(II) complexes

Compounds	Stochiometry	Analysis % calc. (found)			
		C	H	N	M
F-AVOH		67.30	6.38	6.82	
		(67.26)	(6.43)	(6.81)	
F-PLOMe		72.35	6.66	5.44	
		(72.29)	(6.70)	(5.46)	
Z-APCONH$_2$		64.21	5.96	11.82	
		(64.27)	(5.89)	(11.83)	
I	ML_2 (1:2)	47.04	4.46	4.77	5.41
		(47.09)	(4.39)	(4.82)	(5.38)
II	ML_2 (1:2)	53.86	4.96	4.05	4.60
		(53.79)	(4.99)	(4.10)	(4.59)
III	ML_2 (1:2)	42.89	3.98	7.90	5.97
		(42.94)	(3.92)	(7.86)	(6.02)

The IR spectra of three peptides (F-AVOH), (F-PLOMe) and (Z-APCONH$_2$) clearly exhibit bands in the region 2637-2870 cm^{-1}, 1689-1748 cm^{-1} and 1251-1276 cm^{-1} corresponding to ν(N-H), ν(N-C=O) and ν(O-C=O) group, respectively. In IR spectra of complexes, the bands due to ν(N-C=O) and ν(O-C=O) were observed in the same region as their respective peptides, suggesting the non-involvement of the C=O in coordination with Cu(II) ion. Whereas the weakening and shift of ν(N-H) bands to lower energy of 30-40 cm^{-1} indicates the –NH group involved in bonding with metal center through amide nitrogen atom. This view is further supported by the appearance of new band corresponding to the metal-nitrogen stretching vibration in the region 554-579 cm^{-1} in the complexes (Tümer et al., 1999). In addition, the IR spectrum of the PF_6 salt of each complex showed a strong band in the 837-839 cm^{-1} region ascribable to the counter anion.

The observed magnetic moments of the Cu(II) complexes lies in the range of 1.72–1.78 BM at room temperature, corresponding to one unpaired electron. Hence, the magnetic moment

of copper complexes confirms the mononuclear nature of the complexes having an octahedral geometry (Gudasi et al., 2007).

The Electronic spectra of the copper complexes in DMF show a broad band at 651 and 660 nm which can be assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition of the Cu(II) ion with octahedral geometry (Lever, 1984). The UV-Vis absorption spectra of peptides show characteristic peaks in the region 270-350 nm. This is due to intense intra-ligand (π - π*) and charge transfer (CT) bands. The spectra of complexes showed two additional peaks due to d-d transition in the region of 390-500, 370-400 and 450-500 nm. .

Table 2. IR and electronic spectral data of copper(II) peptide complexes

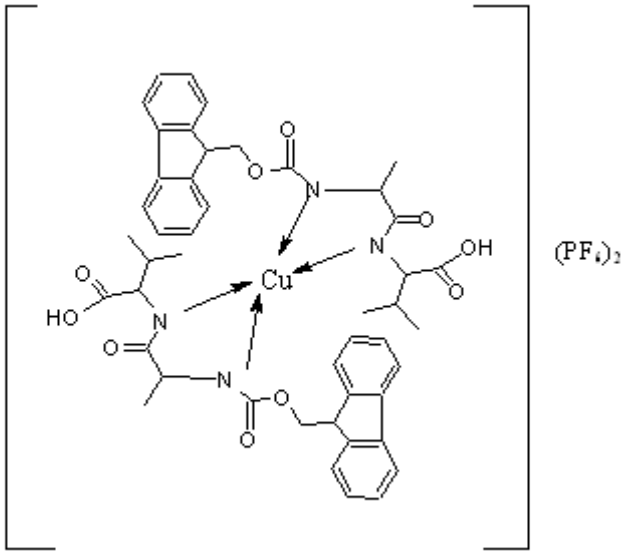
Compound	IR, cm^{-1}				λ_{max}, nm	Ω_M mohs cm^2 mol^{-1}	μ_{eff} BM
	ν(N-H)	ν(N-C=O)	ν(C-O)	ν(M-N)			
F-AVOH	2870	1708	1251		270, 340,		
F-PLOMe	2637	1748	1261		240, 275		
Z-APCONH$_2$	2745	1689	1276		290, 310, 325		
I	2815	1708	1261	544	368, 420	22	1.76
II	2531	1748	1262	567	245, 308	15	1.78
III	2715	1693	1275	579	305, 325, 405	21	1.72

The magnetic moments of the Cu(II) complexes indicates their paramagnetic nature hence it is not amenable to ^{1}H NMR spectral study. The ^{1}H NMR spectral data of the peptides are given in the Table 3.

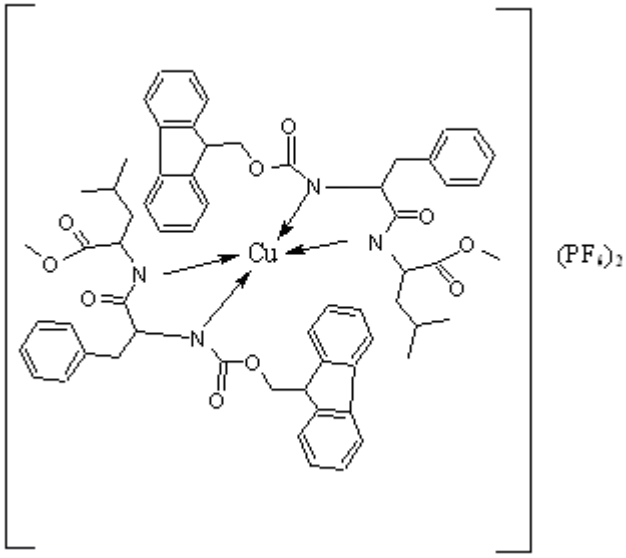
Table 3. Some important ^{1}H NMR spectral data of peptides

Compounds	^{1}H NMR(δ) ppm
F-AVOH	11.2(s,1H, OH), 7.3-7.89(m, 8H,ArH), 7.5(s,1H,NH), 7.30(s,1H, NH), 4.25(d, 2H, CH_2-O-), 4.15(m, 1H, CH), 2.8(d,1H,CH), 2.5(t,1H,CH), 1.22(d, 3H,CH_3), 1.2(d, 6H, 2CH_3)
F-PLOMe	7.2-8.05(m, 13H, ArH), 5.8(s,1H, NH,), 5.5(d,2H, CH_2), 4.9(s,1H, NH), 4.5(t,1H, NH), 5.8(s,1H,-Co-NH) 5.5(d, 2H,CH_2), 4.9(1s,1H, NH), 4.5(t,1H, CH), 4.0 (m,1H,-CH), 3.0(s, 3H, OCH_3), 1.5(t, 2H, CH_2), 1.4(d, 2H, CH_2), 1.2(d, 6H, 2CH_3)
Z-APCONH$_2$	7.2-8.0(m, 10H, ArH), 5.9(s, 2H, NH_2), 5.4(s, 2H, 2NH), 4.8(d, 2H, O-CH_2), 3.2(d, 2H, CH_2), 1.2(d, 3H, CH_3)

Based on the analytical data and physicochemical properties, the following structure is proposed in which the metal ion is coordinated through the –NH group.



Complex I



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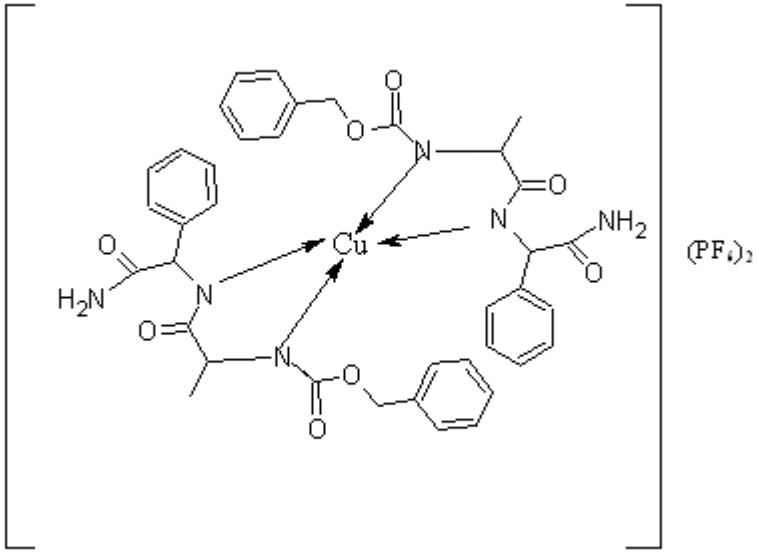


Complex III

Fig. 1. Structure of complex – I, II and III

3.2 DNA Binding Studies

The ability of complexes to bind the DNA was investigated by absorption titration, viscosity and thermal denaturation methods.

3.2.1 Absorption spectral features of DNA binding

The binding of the complexes to the calf thymus (CT) DNA has been studied by electronic absorption spectral technique. Binding of complexes to DNA through intercalation usually results in hypochromism and red shift (bathochromic shift). The extent of the hypochromism in the charge transfer band is commonly consistent with the strength of intercalative binding (Ambroise et al., 2000). The absorption spectral traces of the complexes of peptides with increasing concentration of CT DNA are shown in Figs.2 (a) and (b). The binding results show that the bathochromic shift of 2–4 nm along with significant hypochromicity observed in addition of DNA to complex solution.

When the amount of CT DNA is increased, decrease of intensity in the charge transfer band at about 65% was observed. Intense absorption bands observed near 300 nm for I, II and III complex are attributed to the LMCT transition. In order to compare the binding strength of the complexes, their intrinsic binding constants (K_b) with CT-DNA have been determined from the decay of the absorbance.

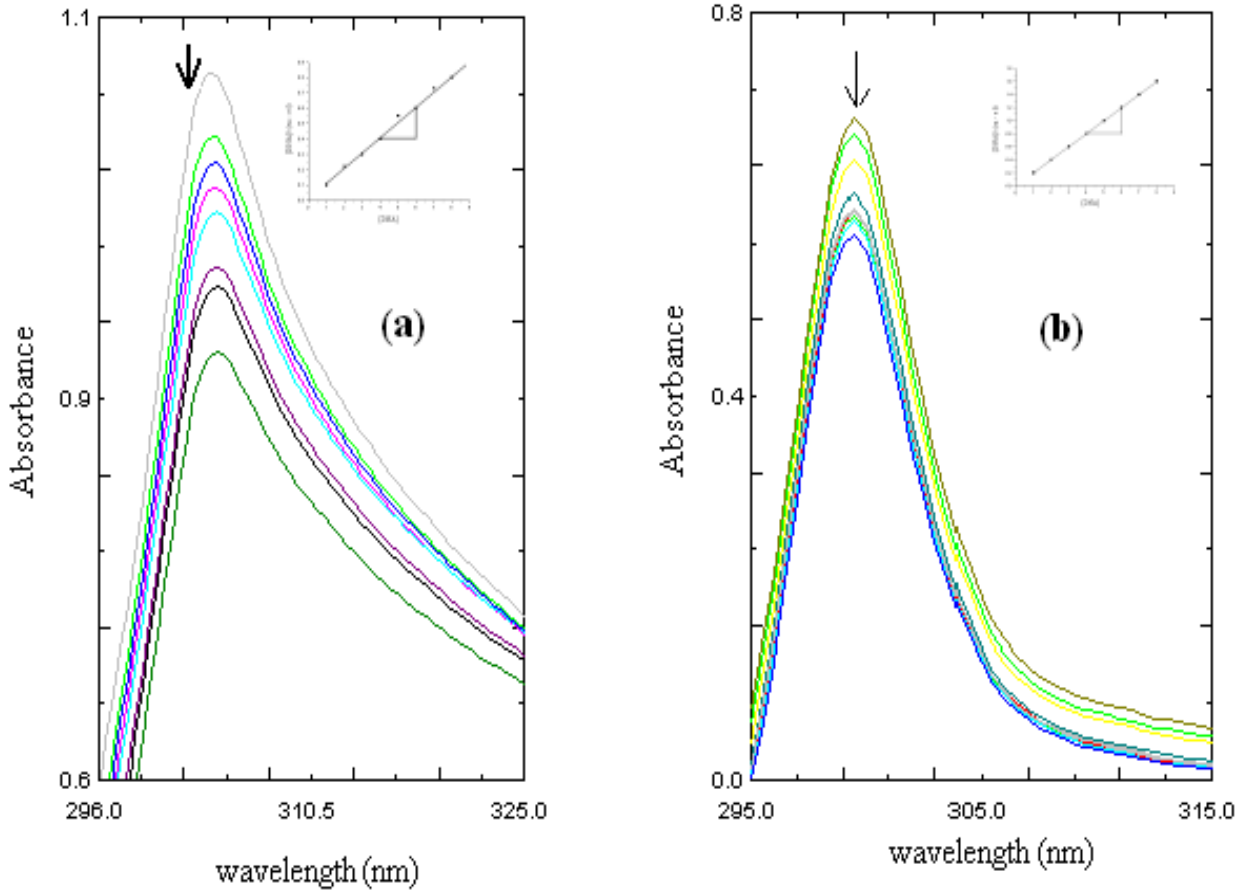


Fig. 2. (a) Absorption spectra of I and (b) complex III in *Tris*-HCl buffer upon addition of DNA. [Complex] = 0.5 μM, [DNA] = 0.1 μM. Arrow shows the absorbance changing upon increase of DNA concentration. The inner plot of [DNA]/ε_a-ε_f *vs* [DNA] for the titration of DNA with complex

To compare quantitatively the affinity of these complexes toward DNA, the intrinsic binding constants K_b of the these complexes to CT-DNA were determined by monitoring the changes of absorbance at 325 nm for I, 308 nm for II and 305 nm for III. The observed intrinsic binding constants K_b of I, II and III complexes are $4.8x10^4$ M^{-1}, $4.2x10^4$ M^{-1} and $3.9x10^4$ M^{-1}, respectively. The complexes I and II having extended fused aromatic rings, as a result they show significantly high binding propensity to DNA compared to complex III. The intrinsic binding constants are almost equal to the other reported intercalators (K_b , 1.8 x 10^6 M^{-1}, [VO (saltrp)(B)], saltrp = N- salicylidene-L-methionate, B = N,N-donor heterocyclic base); $[Co(bpy)_2(PIP)]^{3+}$(K= 1.9 x 10^5M^{-1}), $[Co(bpy)_2(HNOIP)]^{3+}$(K= 2.06 x 10^5M^{-1}).

Table 4. Effect of CT-DNA on the absorbance bands and binding constant values of the copper peptide complexes

Complex	λ_{max} (nm)	Δλ(nm)	H%	Change in Abs.	$K_b(M^{-1})$
I	306	2	21	Hypochromism	$4.8x10^4$ M^{-1}
II	303	3.5	19	Hypochromism	$4.2x10^4$ M^{-1}
III	300	1	12	Hypochromism	$3.9x10^4$ M^{-1}

3.2.2 Viscosity measurement studies

A classical intercalation model demands that the DNA helix lengthen as base pairs are separated to accommodate the binding ligand, leading to an increase in DNA viscosity. In contrast, a partial, non-classical intercalation of compound could bend (or kink) the helix, reducing its effective length and concomitantly, its viscosity (Xiong et al., 1999; Sathyanarayana et al., 1993). In the present study, effect of complexes **I, II** and **III,** complex on the viscosity of DNA was investigated. The values of relative viscosity (η/η_0) (where η and η_0 are the specific viscosities of DNA in the presence and absence of the complexes) are plotted against [Cu]/[DNA]. Viscosity experimental results clearly shows that the viscosity of DNA decreased gradually by the addition of various concentration of complex solution as shown in Fig 3. The results supports that the complexes can intercalate through partial intercalation between adjacent base pairs of DNA.

3.2.3 Thermal denaturation study

Thermal behavior of DNA in the presence of metal complexes can give insight into their conformational changes when temperature is raised and information about the interaction strength of the complexes with DNA. The double- stranded DNA tends to gradually dissociate to single strands on increase in the solution temperature and generates a hyperchromic effect on the absorption spectra of DNA bases (at 260 nm). In order to identify the thermal behaviour of DNA, the melting temperature T_m which is defined as the temperature where half of the total base pairs get non-bonded was studied. Intercalation of synthesized organics and metallointercalators generally results in considerable increase in melting temperature (T_m) (Sudhamani et al., 2009). In the present study melting temperature T_m of DNA alone was found to be 60 ± 1 °C. Under the same set of experimental conditions, addition of complexes I, II and III increased the melting temperature T_m (± 1 °C) by 4, 5 and 4 °C, respectively. While σ_T value (± 1 °C) for DNA alone was 21 °C, the corresponding values in the presence of I, II and III were found to be 23, 24 and 22 °C, respectively (Fig. 4). This experimental data indicates that the Cu(II) complexes of peptides has interaction with double helix CT-DNA.

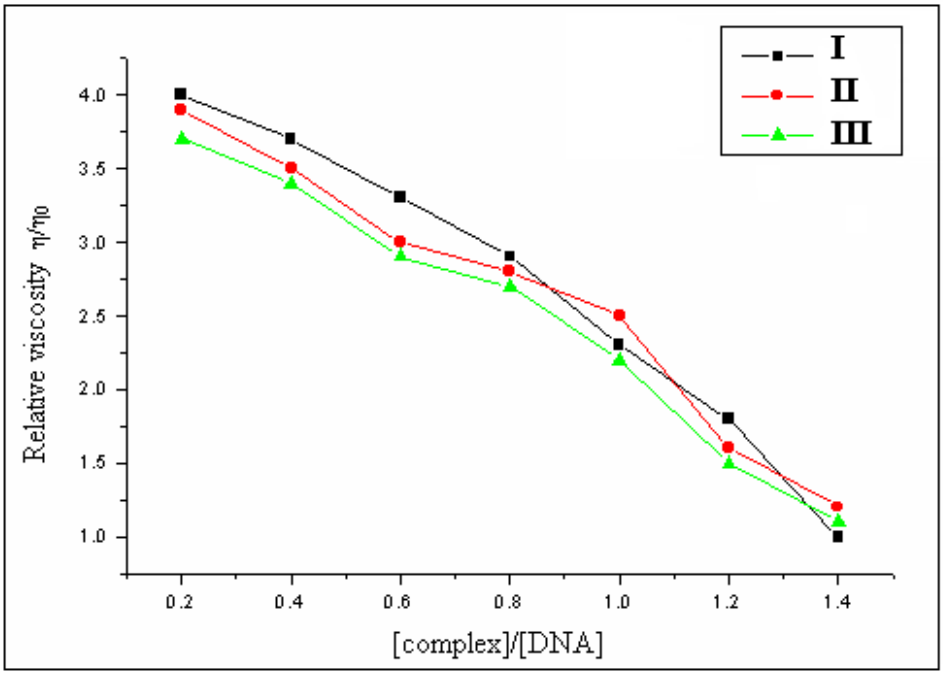


Fig. 3. Plot of relative viscosity vs [Complex]/[DNA]. Effect of complex I, II and III on the viscosity of CT-DNA at 25 ^{0}C. Complex = 0-100 μM, [DNA] = 50 μM

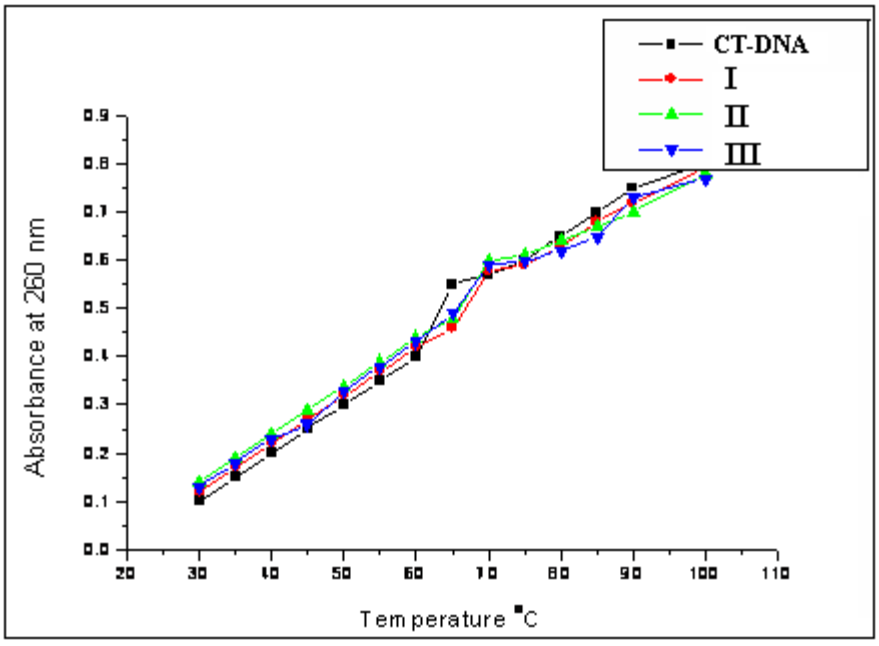


Fig. 4. Thermal denaturation of CT-DNA in the absence and presence of complexes. [DNA]=50 μM, [complex] = 10μM, Buffer: Phosphate

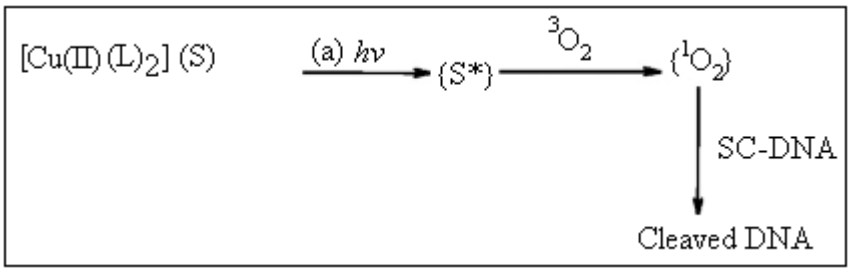
Table 5. Results of thermal melting experiments

Compound	T_m °C (σ_T)
DNA	60(21)
I	64(23)
II	65(24)
III	64(22)

3.3 Photonuclease Activity

The DNA cleavage reactions was carried out in the presence of different additives to understand the mechanistic pathways involved in the photocleavage reactions (Scheme 1) (Chakravarty, 2006).Generally, there is a necessity of molecular oxygen or hydroxyl radical for cleaving DNA on photoactivation. The DNA cleavage reactions involving $^3O_2$ could proceed via two major mechanistic pathways. (i) The singlet excited electronic state of the complex through efficient inter system crossing could generate an excited triplet state of the complex, that can activate molecular oxygen from its stable triplet ($^3O_2$, $^3\sum_g^-$) to the reactive singlet ($^1O_2$,$^1\Delta g$) state (Kelly at el., 1985). (2) In an alternate pathway, the excited-state complex could reduce molecular oxygen to generate reactive hydroxyl radical.

The photo-induced DNA cleavage activity of the complexes I, II and III at 40 µM in the presence of various 'inhibitors' for 1 hour exposure at 365 nm was studied (Fig. 5-7). Complexes show significant cleavage of SC DNA (FORM-I) to NC DNA (FORM-II) (lane 2). The cleavage efficiency of the complexes follows the order: III > II > I. The photocleavage activity of the complexes may be due to the presence of free NH_2 and having more aromatic rings in peptides involving ligand n-π^* and π–π^* transitions by metal assisted photo-excitation process. The results show that, the complex generates the reactive singlet oxygen species by the absorption of UV light at 365 nm which responsible for cleave the SC-DNA. The cleavage of DNA by the singlet oxygen confirmed in gel electrophoresis experiment. Hydroxyl radical scavenger like DMSO has no apparent effect on the cleavage activity. So, the result exclude the possibility of any hydroxyl radical pathway for the DNA photocleavage reaction (lane 4). A significant inhibition of DNA cleavage is, however, observed in the presence of singlet oxygen quencher NaN_3 (lane 3), which significantly inhibits the photoinduced DNA cleavage activity of the complexes. Formation of singlet oxygen is also indicated from the reaction in D_2O, showing enhancement of the cleavage activity due to the longer lifetime of $^1O_2$ in this medium (Khan, 1976; Ashwini Kumar et al., 2009). The addition of D_2O leads to an enhancement of the DNA cleavage (lane 5). Thus, the data suggest the formation of the singlet oxygen ($^1O_2$) as the reactive species, which explained as below. The cleavage data are given in the Table 6.



Scheme 1. Mechanistic pathways for the cleavage of DNA on photoirradiation

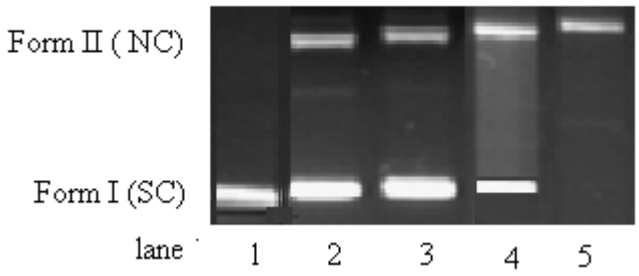


Fig. 5. Gel electrophoresis diagram of the control experiments using SC pUC19DNA (0.5 µg), complex I (40 µM), and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA control; lane 2, DNA+I; lane 3, DNA+I+NaN_3; lane 4, DNA+ I +DMSO; lane5, DNA+ I +D_2O

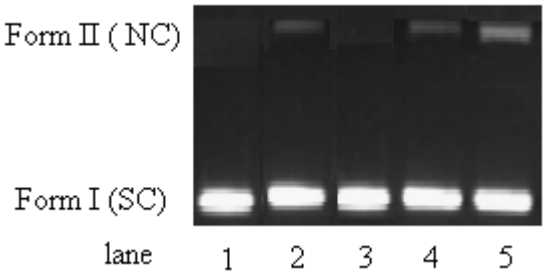


Fig. 6. Gel electrophoresis diagram of the control experiments using SC pUC19DNA (0.5 µg), complex (2) (40 µM), and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA control; lane 2, DNA+II; lane 3, DNA+II+NaN_3; lane 4, DNA+II+DMSO; lane5, DNA+II+D_2O

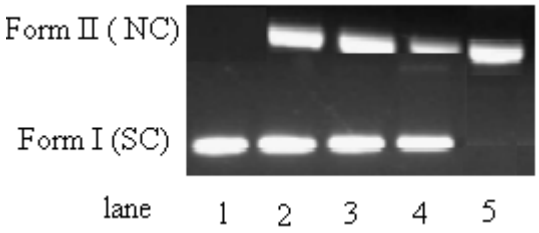


Fig. 7. Gel electrophoresis diagram of the control experiments using SC pUC19 DNA (0.5 µg), complex (3) (40 µM), and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA control; lane 2, DNA+III; lane 3, DNA+III+NaN_3; lane 4, DNA+III+DMSO; lane5, DNA+III+D_2O

Table 6. Photoinduced cleavage of pUC19 DNA (0.5 µg)

Reaction condition	Form-I (%)	Form-II(%)
DNA+**I**	80	20
DNA**+I**+ NaN_3	95	5
DNA+**I+**DMSO	70	30
DNA+**I**+ D_2O	55	45
DNA**+ II**	60	40
DNA+**II**+ NaN_3	70	30
DNA+**II+**DMSO	50	60
DNA+**II+**D_2O	10	90
DNA**+III**	45	55
DNA+**III**+ NaN_3	65	35
DNA+**III+**DMSO	55	45
DNA+**III+**D_2O	10	90

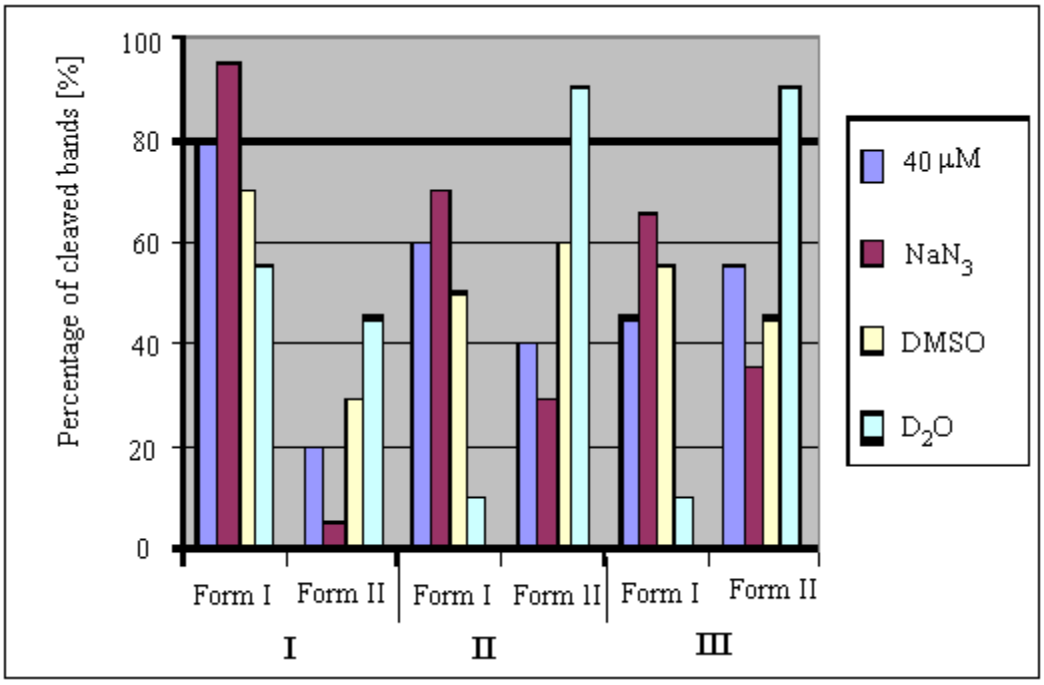


Fig. 8. Cleavage of SC pUC19 DNA (40 μM) by complexes I, II and III in the presence of various additives upon photo irradiation at 365 nm in 50 mM *Tris*-HCl / NaCl buffer (pH 7.2)

4. CONCLUSION

The Copper(II) complexes containing peptide as ligands were prepared and structurally characterized. The DNA binding results showed reduction in peak intensities and bathochromic shifts in the absorption titration experiments, an increase in the T_m value in the thermal denaturation experiments and the negative changes of viscosity. This is indicated that the complexes are involved in partial intercalation with DNA. Being more extended π-system, complex $[Cu(F\text{-}AVOH)_2](PF_6)_2$ (1), is obviously better intercalator than $[Cu(F\text{-}PLOMe)_2](PF_6)_2$ (2) and $[Cu(Z\text{-}APCONH_2)_2](PF_6)_2$. The photocleavage reaction involves singlet oxygen type-II pathway. The complex $[Cu(Z\text{-}APCONH_2)_2](PF_6)_2$ (3) showed better nuclease activity than $[Cu(F\text{-}AVOH)_2](PF_6)_2$(1) and $[Cu(F\text{-}PLOMe)_2](PF_6)_2$ (2) .

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