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New mono and binuclear unsymmetrical Cu(II) bioactive complexes: Synthesis, Characterisation, Electrochemical and interaction studies with CT-DNA

K.R. Krishnapriya and M. Kandaswamy*
Department of Inorganic Chemistry, University of Madras,
Maraimalai Campus - Guindy, Chennai 600 025, India.
mkands@yahoo.com & krkrishnapriya@yahoo.com

Abstract:- **A new series of mono and binuclear copper(II) complexes, [CuL1-3] and [Cu2L1-3]+, where, L = precursor complex prepared from condensation of salicylaldehyde, pyridine and various amines namely ethylene, propylene and butylene diamines with copper(II) perchlorate. All the complexes have been synthesized and characterized by FT IR, UV-Vis spectral and electrochemical methods. Cyclic voltammogramms of mono and binuclear copper(II) complexes exhibit one single and two quasi-reversible reduction waves in the cathodic region (0 to - 1.5 V) respectively. Electrochemical changes in the presence calf thymus DNA (CTDNA) with these complexes were also studied over this cathodic potential range (0 to - 1.5 V). DNA binding properties of the complexes were studied by UV-Visible and fluorescence spectral methods. All the complexes show good binding propensity to CT DNA. The binding constant data suggest a DNA intercalative binding nature of the complexes. DNA cleavages of all these complexes were studied by gel electrophoresis method. All the complexes show efficient hydrolytic cleavage of supercoiled pBR322-DNA.**

Keywords: Salicylaldehyde; Mononuclear Cu(II) complexes; Binuclear Cu(II) complexe; DNA cleavage.

I. Introduction

Studies pertaining to DNA cleavage by synthetic reagents are of considerable interest because of their utility as tools in molecular biology. This has resulted in the development of both sequence specific DNA cleavers and DNA foot printing agents [1, 2]. In recent years, our group has shown substantial interest in understanding the binding properties of metal complexes, particularly binuclear complexes of copper, with biomolecules like DNA [3–5].

So far, copper(II) complexes having extended aromatic planar bidentate ligands have been extensively studied. On the other hand, copper(II) complexes having acyclic, unsymmetrical, end off remote donar set concerned, only a few are reported [6]. In this paper, we report the synthesis and characterization of new series of acyclic-end off, unsymmetrical mono and binuclear copper(II) complexes as shown in **Scheme I**. The DNA binding and cleavage studies have been carried out for the complexes.

II. Experimental

The melting points were determined in an open glass capillary and were uncorrected. The FT-IR spectra were obtained on a Perkin Elmer FTIR spectrometer (Perkin–Elmer 2000) with samples prepared as KBr pellets. Absorption spectra in the 200–750 nm range were recorded on a Perkin Elmer Lambda 35 spectrophotometer. Fluorescence spectra were recorded on a Perkin Elmer LS-45 Fluorescence spectrometer. Cyclic voltammograms were run on a CHI model 600 A electrochemical analyzer in DMF solutions containing tetra(n-butyl)ammonium perchlorate (TBAP) as the supporting electrolyte. UV spectroscopic titration, fluorescence spectroscopic experiments were carried out following the literature procedure [4]. The experiments on the cleavage of supercoiled pBR322 DNA (0.5 μg/μL) by the Cu(II) complexes (5.0–100 μM) in *Tris*–HCl/NaCl (5:50 mM) buffer (pH 7.2) were performed using agarose gel electrophoresis [3-5].

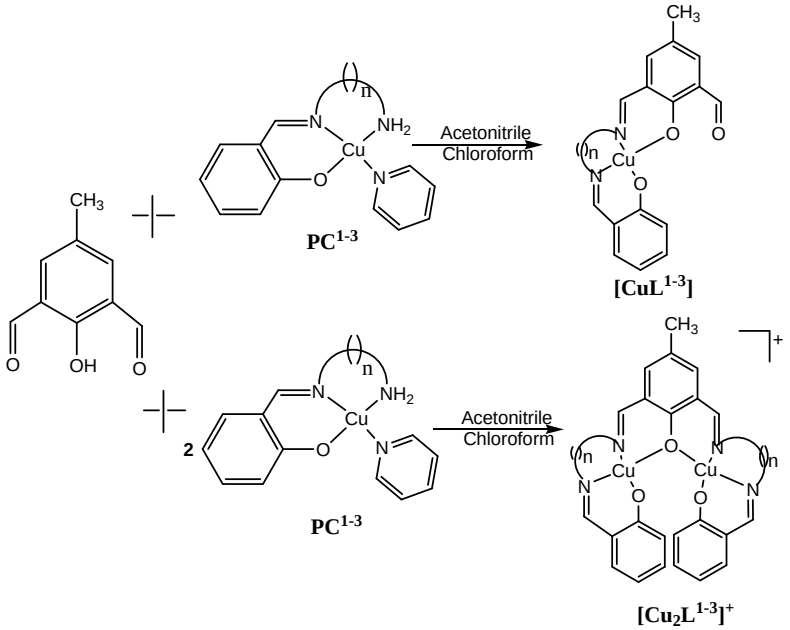
III. Synthesis

A. Synthesis of the precursor complexes: PC^{1-3}

The precursor complexes PC^1, PC^2 and PC^3 were prepared by the condensation of salicylaldehyde, pyridine and various amines namely ethylene, propylene and butylene diamines respectively with copper(II) perchlorate by following the reported procedure [7].

B. Synthesis of the Mono and binuclear copper(II) Complexes: [CuL^{1-3}] and [Cu_2L^{1-3}]$^+$

The mononuclear, [CuL^{1-3}] and binuclear complexes, [Cu_2L^{1-3}]$^+$ were prepared by adding the solution of 2, 6 diformyl-4-methyl phenol [8] (0.41 g, 0.0025mol) in Chloroform to the solutions of 0.0025 mol and 0.005 mol of precursor complexes **PC^1, PC^2** and **PC^3** respectively in Acetonitrile with constant stirring for an half an hour followed by refluxing for six hours **(Scheme I)**. The resulting brown colored solutions were evaporated, to get the corresponding complexes as brown colored solids and recrystallised by using Acetonitrile.



(where, n = 1,2 and 3 for en,pn and bn)

(Scheme I)

IV. Results and discussion

A. Characterization and general properties

FTIR spectra of new mono and binuclear complexes show a strong absorptions band in the region 1615-1625 cm^{-1} due to azomethine (C=N) group. This shows that Schiff's base condensation of aldehyde group with amine group of precursors. A band observed in the region 3400 – 3500 cm^{-1} can be assigned to weakly coordinate as lattice hydroxy molecules present in the complexes. The presence of uncoordinated perchlorate anions in the complexes the infrared from single broad band's at 1100 cm^{-1} (asymmetric stretching) and a band at 650 cm^{-1} (antisymmetric bending).

The electronic spectra of the complexes in CH_3CN solutions showed three main transitions. A weak band observed in the range 530–560 nm is due to a d–d transition of the copper(II) ion. This strongly suggests that the coordination geometry around the metal ion in the mono and dicopper(II) complexes might be distorted square pyramidal geometry [9]. A moderately intense band observed in the near-UV region 360–370 nm due to the overlap of the transition of the azomethine **π-π*** transition with the charge transfer band from bridging phenolate to the vacant d orbital of Cu(II) ion. The strong band observed in the range 260–270 nm due to an intraligand charge transfer transition.

B. DNA-Absorption Spectral Titration

The DNA-UV absorption titrations were performed by keeping the concentration of the complexes fixed while varying the DNA concentration. Complex-DNA solutions were allowed to incubate for 30 min at room temperature before measurements were made. Absorption spectra were recorded using cuvettes of 1 cm path length at room temperature. Upon addition of DNA to the solution of the complexes, the peak around 270 nm and 365 nm decreased. Hypochromism in the band reaches as high as 37% after adding DNA, indicating that the interaction mode between CT DNA and the complex is intercalative [5]. In order to quantitatively investigate the binding strength of the complex with CT DNA, the intrinsic binding constant, K_b was obtained by monitoring the changes in

absorbance at 270 nm for the complexes as increasing concentration of CT DNA.

The DNA binding constants for complexes tabulated as shown in Table 1. Comparing the intrinsic binding constant of the mononuclear complexes **[CuL^{1-3}]** with those of binuclear **[Cu_2L^{1-3}]$^+$** complexes, we can deduce that the binuclear complexes binds to DNA by strong intercalation.

Table 1. Electronic spectral data for complexes (Table caption)

S.No.	Complexes	λ max(nm)/ ϵ mol^{-1} dm^3 cm^{-1}		K_b x 10^3 M^{-1}
		LCTransition	LMCTransition	
1.	**[CuL^1]**	268(81000) & 369(38000)	617(250)	9.6
2.	**[CuL^2]**	267(150000) & 369(340000)	570(120)	10.2
3.	**[CuL^3]**	267(180000) & 366(50000)	566(150)	11.5
4.	**[Cu_2L^1]$^+$**	267(1,60,000) & 369(80,000)	616(170)	10.9
5.	**[Cu_2L^2]$^+$**	270(175,000) & 361(80,000)	597(80)	11.5
6.	**Cu_2L^3]$^+$**	268(70,000) & 370(30,000)	607(110)	12.7

C. Electrochemical studies of complexes

The electrochemical properties of all the complexes were studied by cyclic voltammetry recorded in DMF in the cathodic region of 0 to -1.5 V and the data are summarised in Table 2. The mononuclear complexes show one quasireversible (ΔE > 59 mV) waves in the range -0.85 to -0.92V. All the binuclear complexes show two reduction waves at different potential indicating the two single step one electron transfer processes. The reduction process at the electrode surface could be assigned.

$$Cu^{II}Cu^{II} \rightleftharpoons Cu^{II}Cu^{I} \rightleftharpoons Cu^{I}Cu^{I}$$

Table 2. Electrochemical data[a] of all the copper(II) complexes in DMF medium (Table caption)

Complex	E^1_{pc} (V)	E^1_{pa}(V)	$E^1_{1/2}$(mV)	ΔE^1(mV)	E^2_{pc}(V)	E^2_{pa}(V)	$E^2_{1/2}$(V)	ΔE^2(mV)
[CuL^1]	-0.94	-0.79	-0.865	150	_	_	_	_
[CuL^2]	-0.72	-0.61	-0.665	110	_	_	_	_
[CuL^3]	-0.63	-0.50	-0.565	130	_	_	_	_
[Cu_2L^1]$^+$	-0.70	-0.61	-0.655	90	-1.2	-1.05	-1.125	150
[Cu_2L^2]$^+$	-0.98	-0.84	-0.91	140	-1.13	-1.04	0.085	90
[Cu_2L^3]$^+$	-0.58	-0.45	-0.515	130	-1.14	-0.89	-1.015	250

[a] Measured by CV at 100 mV/s. E versus Ag/AgCl conditions: GC working and Ag/AgCl reference electrodes; supporting electrolyte TBAP; concentration of complex $1x10^{-3}$ M, concentration of TBAP $1x10^{-1}$ M.

D. DNA-binding-Cyclic voltammetry study

The cyclic voltammograms of the complexes in the absence and presence of CT DNA for **[CuL^1]** and **[Cu_2L^2]$^+$** are shown in Fig. 1 and 2. The presence of DNA in the solution at the same concentration of complexes causes shift in $E_{1/2}$ and ΔE values. The value of i_{pc} /i_{pa} also decreases with the increase of the DNA concentration. The decrease in peak currents can be explained in terms of an equilibrium mixture of free and DNA-bound copper (II) complex to the electrode surface. This may be due to that the complex intercalates into the base pairs of DNA by the aromatic planar group. Because of the intercalation, the complexes are not readily accessible to the electrode, thus causing the peak currents of the CV waves to diminish. Moreover, the obvious positive shifts of peak potentials also indicate that this interaction mode may be intercalation between complexes and DNA [10].

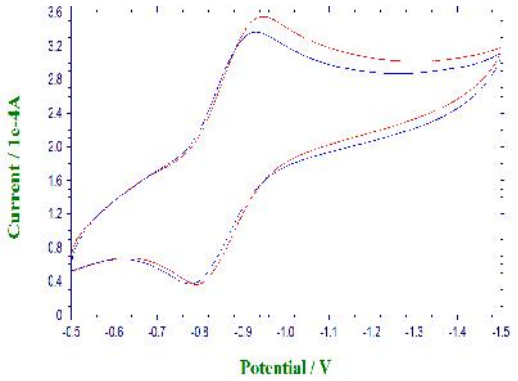


Figure. 1. Cyclic voltammograms of the [CuL^1]complex in the absence (__) and presence (__) of CT-DNA in 50 mM Tris–HCl/50 mM NaCl buffer solution (pH = 7.2), scan rate: 0.1 V/s (Figure caption)

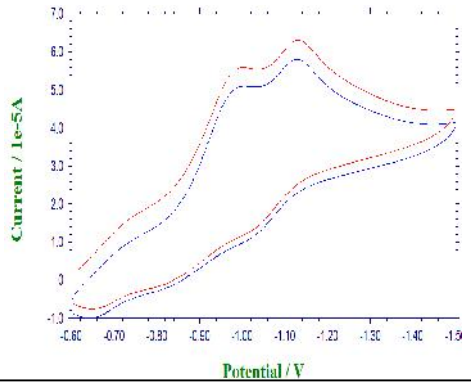


Figure. 2. Cyclic voltammograms of the [Cu_2L^2]$^+$ complex in the absence (__) and presence (__) of CT-DNA in 50 mM Tris–HCl/50 mM NaCl buffer solution (pH = 7.2), scan rate: 0.1 V/s (Figure caption)

E. DNA-Fluorescence spectroscopic studies

In order to investigate the binding mode of the Cu(II) complexes and DNA, the fluorometric competitive binding experiment was carried out using ethidium bromide (EB) as a probe. Fluorescence quenching experiments were performed by adding a solution of the complex at different concentrations to EB-bound CT DNA solution (1.5 mL). All experiments were carried out using cuvettes of 1 cm path length at room temperature. Samples were excited at 510 nm, and emission was recorded at 550–700 nm.

In this study, when the Cu(II) complexes were added to the EB-DNA system, the emission intensity was reduced. The emission spectra of EB bound to CT DNA in the absence and presence of the complex are given in Fig. 3. The significant reduction in fluorescence intensity of the EB-DNA solution on addition of the complex suggests that the Cu(II) complex can displace the EB and therefore bind to the DNA. Such a characteristic change is often observed in the intercalative DNA interaction [10]. The binding of the complex to DNA can be determined according to the classical Stern–Volmer equation. The quenching constant, K obtained for the complex is obtained by the plotting the slope of the Stern–Volmer quenching plot of EB bound to DNA by Cu(II)complexes as K_{app} are 3.12 x10^5, 3.10 x10^5, 3.2 x10^5, 3.4x10^5, 3.2 x10^5 M^{-1} for the complexes [**CuL^1], [CuL^2], [CuL^3]**and [**Cu_2L^1]**$^+$, [**Cu_2L^2]**$^+$ and [**Cu_2L^3]**$^+$ respectively. These constants are similar to those of DNA-intercalative complexes [11], hence, we deduce that the Cu(II) complexes binds to DNA with a moderate intercalative mode showed remarkable cleavage (about 80 %).

F. DNA cleavage activity

The cleavage of supercoiled pBR322 DNA was studied in a medium of 50 mM Tris-HCl/NaCl Buffer (pH = 7.2) in the presence of mercaptoethanol as a reducing agent. All the complexes showed about 80% DNA cleavage. All the samples were incubated for 4 hrs at 37°C followed by its addition to the loading buffer containing 25 % bromophenol blue, 0.25 % Xylene cyanol, 30 % glycerol (3 µL). The solution was finally loaded on 0.8 % agarose gel containing 1.0 µg / mL^{-1} of Ethidium bromide. Fig 4. shows the results of the gel electrophoretic separations of plasmid pBR322 DNA by the complexes [Cu_2L^{1-3}]$^+$ and in the presence of mercaptoethanol. Under same conditions, free mercapto ethanol or complexes no cleavage of pBR322 DNA. (Lane 1-2). All supercoiled (Form I) DNA was cleaved to Form II in the concentration (20 µmol L^{-1}) of all the three binuclear complexes. (Lane 3-5).

Conclusion

We have synthesized the new unsymmetrical acyclic, mono and binuclear copper(II) complexes. All the complexes were subjected to spectral, electrochemical, DNA binding, and cleavage studies. The DNA binding experiments results suggests that the interaction of the complexes with DNA is an intercalative mode. All the binuclear complexes showed about 80% DNA cleavage.

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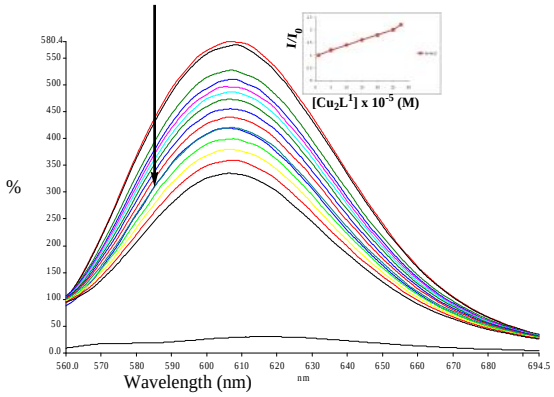

Figure 3. Emission spectrum of EtBr bound to DNA in the presence of $[Cu_2L^1]$[EB] = 1.2 µM, [DNA] = 20 µM, [complex] = 0-2.5 x 10^{-4} M, λ_{ex}=510 nm). Arrow shows the absorbance changing upon increasing $[Cu_2L^1]$ concentrations. **Inset : Stern–Volmer quenching plot of EB bound to DNA by Cu(II)complex. I_0 is the emission intensity of EB-DNA in the absence of complex; I is emission intensity of EB-DNA in the presence of complex. (Figure caption).**

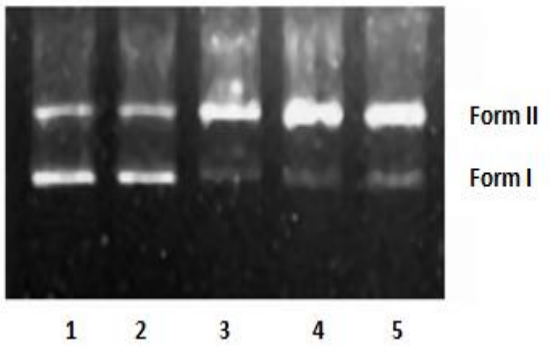

Figure 4. Electrophoresis diagram. Cleavage of SC pBR322 DNA (0.2 µg, 30 µM) by binuclear $[Cu_2L^{1-3}]^+$ complexes (50 to 100 mM) in the presence of reducing agent mercaptoethanol (0.71 mM) in 50 mM Tris-HCl / NaCl buffer (pH 7.2).
{ lane 1, DNA control; lane 2, DNA + Mercaptoethanol;
lane 3, DNA + Mercaptoethanol + $[Cu_2L^1]^+$;
lane 4, DNA + Mercaptoethanol + $[Cu_2L^2]^+$;
lane 5, DNA + Mercaptoethanol + $[Cu_2L^3]^+$}.(Figure caption).

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