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SYNTHESIS AND CHARACTERIZATION OF NEW UNSYMMETRICAL MACROCYCLIC BINUCLEAR COPPER(II) COMPLEXES: ELECTROCHEMICAL BEHAVIOUR AND DNA BINDING STUDIES

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ABSTRACT

A new series of unsymmetrical macrocyclic binuclear Copper (II) complexes have been synthesized and characterized by elemental and spectral techniques. A cyclic voltammetric investigation of these binuclear Cu (II) complexes evidenced that two successive quasi-reversible one electron transfer reduction waves (E^1_{pc} = -0.54 to -0.67 V, E^2_{pc} = -0.80 to _0.85 V) are obtained. In the positive potential region (+0.60 to +1.2 V) two quasi-reversible oxidation couples are observed for all the complexes. The first one electron oxidation is observed around (E^1_{pc}) +0.59 to +0.79 V and the second around (E^2_{pc}) +0.81 to + 1.17 V. The ESR spectra of all the binuclear copper complexes showed a single broad-band resonance at ca. g = 1.98 to 2.11 with the half field signal at 1500G (M = ±2), which suggest magnetic interactions between two Copper ions through the phenolate bridge. DNA binding experiments show that the macrocyclic Cu^{II} complexes display better DNA interactions with increasing order of the chain length.

Keywords: Copper (II) complexes, Elemental and Spectral techniques.

INTRODUCTION

The design of small molecules that bind and react at specific sequences of DNA under physiological conditions via oxidative and hydrolytic mechanisms has been attracting great interest in the field of bioinorganic chemistry [1–3]. Intercalators are small molecules that contain a planar aromatic heterocyclic functionality which can insert and stack between the base pairs of double helical DNA [4]. As both spectroscopic tags and functional models for the active centers of proteins, metal complexes have helped elucidate the mechanisms by which metalloproteins function. Binucleating ligands are particularly suited for the synthesis of such complexes as they are more stable and the two metal ions are fixed in close proximity, which has important implications for metal–metal interactions and their reactivity [5]. These properties have turned chemical nucleases into useful tools as adjuvants in PCR diagnos-tics [6,7], nucleic acid attacking and cleaving agents [8]. It is known that binuclear copper(II) complexes have greater cleaving effi-ciency or DNA interaction than the mono nuclear complexes [7,9]. Transition metal complexes with tunable coordination environ-ments and versatile spectral and electrochemical properties offer a great scope of design for species that are suitable for catecholase, DNA binding and cleavage activities [10]. The present work stems from our interest in designing new binuclear copper(II) complexes that are capable of cleaving DNA in the presence of mercap-toethanol under physiological conditions. Copper(II) complexes are known to show ‘‘chemical nuclease’’ activity in the presence of a reducing agent like thiols [11]. Recently, we have reported that binuclear Cu(II) complexes of an aromatic diamine condensed Schiff base macrocyclic ligand display significant DNA cleavage activity (in the presence of mercaptoethanol), greater than the ali-phatic diamine condensed Cu(II) analogs [12].

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A variety of propos-als have been put forth describing the reactivity of DNA with copper phenanthroline and bipyridyl complexes [13,14]. It is remarkable to notice that the macrocyclic binuclear transition metal complexes show greater cleaving efficiency or DNA interaction than their mononuclear analogues [16,17]. The efficient DNA binding and cleavage ability of the binuclear com-plexes may be due to a synergy between the metal centers and the presence of the 4-methyl phenol group in the macrocyclic ring [15]. This work focuses on the synthesis of binuclear Cu^{II} complexes.

MATERIALS AND METHODS

Physical measurements

Elemental analysis was carried out on a Carlo Erba model 1106 elemental analyzer. FT-IR spectra were obtained on a Perkin Elmer FT-IR spectrometer with samples prepared as KBr pellets. UV–Vis spectra were recorded using a Perkin Elmer Lambda 35 spectro-photometer operating in the range 200–800 nm with quartz cells and e are given in M^{-1} cm^{-1}. Electrospray ionization mass spectral measurements were made using a Thermo Finnigan LCQ-6000 Advantage Max-ESI mass spectrometer. Cyclic voltammograms were obtained on a CH1100 Electrochemical analyzer using a three- electrode set-up comprising of a glassy carbon working, platinum wire auxiliary and a saturated Ag/AgCl reference elec-trode under oxygen-free conditions. The concentration of the complexes was 10^{-3} M. TBAP (Tetra (n-butyl) ammonium perchlorate) (10^{-1} M) was used as the supporting electrolyte. EPR spectra were recorded on powdered samples of the complexes using a JEOL TES 100 ESR spectrometer. Low temperature measurements were made using a liquid nitrogen Dewar.

Materials

2, 6-Diformyl-4-methylphenol [18] was prepared by following the literature methods. Tetra (*n*-butyl) ammonium perchlorate (TBAP) was purchased from Fluka and recrystallized from hot methanol. (Caution! TBAP is potentially explosive; hence care should be taken in handling the compound.) All the solvents were purified by reported procedures. [19] CT-DNA and pBR322DNA were purchased from Bangalore Genie (India). All other chemicals and solvents were of analytical grade and used as received.

General methods

DNA binding experiments

The DNA binding experiments were performed in Tris–HCl/NaCl buffer (50 mM Tris HCl/1 mM NaCl buffer, pH 7.5) using DMF (dimethylformamide) solution (10%) of the Copper (II) complexes. The concentration of calf thymus (CT) DNA was determined from the absorption intensity at 260 nm with € value [20] of 6600 M^{-1} cm^{-1}. Absorption titration experiments were made using different concentrations of CT DNA, keeping the concentration of the complexes constant. The fluorescence spectral method using ethidium bromide (EB) as a reference was used to determine the relative DNA binding properties of the complexes to calf thymus (CT) DNA (50 mM Tris HCl/1 mM NaCl buffer, pH 7.5). Fluorescence intensities of EB at 610 nm with an excitation wave-length of 510 nm were measured at different complex concentra-tions. Reduction in the emission intensity was observed with addition of the complexes. The apparent binding constant (K_{app}) was calculated using the equation $K_{EB}[EB]/K_{app}[complex]$, where the complex concentration was the value at a 50% reduction of the fluorescence intensity of EB and $K_{EB} = 1.0 \times 10^7$ M^{-1} ([EB] = 1.3 µM) [21].

Synthesis of mono and binuclear Copper complexes

To a solution of 2,6 diformyl 4-methyl phenol in warm DMF at 90^0c added the various diamines (2:1 molar ratio) like 1,2 diamino ethane, 1,3 diamino propane and 1,4 diamino butane respectively in the presence of one mole of $Cu(OAc)_2.nH_2O$. Mono nuclear complex were synthesized (CuL). To obtain the binuclear copper complex acetonitrile solution of $M(ClO_4)_2.nH_2O$ was added to the hot solution of Mono nuclear complex[ML] in acetonitrile. Then add guanidine diamine hydrochloride in acetonitrile (1:1molar ratio). The solution reflux for 12 h. obtains a clear solution. The resulting solution was then filtered at hot condition and allowed to cool at room temperature . The resulting solid that separated out on evaporating the solution at room temperature and the resulting compound was washed with ether and dried. All the compounds were recrystallized from acetonitrile solution.

For Complex 1 a -[Yield: -80%].Anal. Calc. for $C_{32}H_{26}Cu_2N_8O_2$ Exact Mass : 680.08 , Mol.Wt : 681.69 : C, 56.38; H, 3.84; Cu, 18.64; N, 16.44; O, 4.69. Found: C, 56.8; H, 3.89; Cu, 18.04; N, 15.40; O, 4.29

RESULTS AND DISCUSSION

Spectral studies

The IR spectrum of the precursor compound shows a peak at 1650–1680 cm^{-1} due to the presence of a CHO group. The forma-tion of an imine group (C = N) in the macrocyclic complexes by Schiff base condensation between CHO and NH_2 is evidenced by the appearance of a strong peak at 1610–1630 cm^{-1}. The strong peak around 1110–1120 cm^{-1} may due to the sulfate ion. The elec-tronic spectra of all the complexes were measured in CH_3CN at 298 K for the range 300–800 nm. They all show a weak band in the range 570–600 nm due to metal centered d–d transitions [22]. Moderately intense charge transfer bands appear in the range 350–450 nm. These bands are due to charge transfer from the azomethine nitrogen and phenoxide oxygen to Cu^{II} ion.

A strong peak in the range 250–290 nm is due to a intraligand transition ($p–p^-$). The absorption maxima for the d–d transition suggest that the geometry around the Copper ion is approximately square pyramid. Further, the complexes reported in the present work show that on moving from 1a to 1c increase in k_{max} (red shift) of the d–d transition of the Cu^{II} ion is observed. It indicates that the coordination geometry of complex 1a is less distorted than that of the later complexes 1b and 1c. ESI mass spectral data of the binuclear Cu^{II} complexes confirm the proposed formula of the complexes. The EPR spectra of the so-lid complexes 1a–1c were recorded at liquid nitrogen temperature (LNT). All the binuclear complexes showed a single broad-band resonance (Figure. 1) at ca. g = 1.98–2.04 with the half field signal at 1500 G (M = ±2), which suggest magnetic interactions be-tween two Copper ions through the phenolate bridge.

Cyclic voltammetric studies

The electrochemical properties of complexes were studied by cyclic voltammetry. The cyclic voltammogram of 1a recorded in acetonitrile is given in Figure. 1. The electrochemical data are summarized in Table 1. For all the complexes two quasi-reversible waves were observed involved two –one electron transfer. Based on these observations, it is reasonable to suggest that the reduction process may involve the stepwise redox processes depicted below:

$$Cu^{II}\,Cu^{II} \leftrightarrow Cu^{II}\,Cu^{I} \leftrightarrow Cu^{I}\,Cu^{I}$$

In the positive potential region (+0.50 to +1.00 V) two quasi-reversible oxidation couples are observed for all the complexes. The cyclic voltammograms of the complexes are shown in Figure. 1 and the data are summarized in Table 1.

DNA binding studies

Absorption spectra studies

In the present investigation the interaction of the macrocyclic binuclear Copper(II) complexes 1a, 1b and 1c in DMF solutions (10%) with CT DNA have been investigated by UV absorption spectroscopy. Absorption titration experiments of Cu^{II} complexes in buffer were performed by using a fixed complex concentration to which increments of the DNA stock solution were added. The binding ability of the complexes with CT DNA was studied by UV spectroscopy by following the intensity changes of the intraligand $d–d^-$ transition band 200–220 nm. The binding of complexes to duplex DNA led to a decrease in the absorption intensities (Figure. 4) with a small amount of red shift in the UV–Vis absorption spectra. For the complexes 1a, 1b and 1c the intrinsic binding constant K_b has been determined from the spectral titration data (Figure. 5) using the following equation 1

$$[DNA] / (\varepsilon_a-\varepsilon_f) = [DNA] / (\varepsilon_b-\varepsilon_f) + 1 / K_b (\varepsilon_b-\varepsilon_f) \text{ ------ (1)}$$

Here, in equation 1 , e_a, e_f and e_b correspond to $A_{obsd}/[Cu^{II}$ complex], the extinction coefficient for the free complex, and the extinction coefficient for the complex in the fully bound form, respectively. The binding constants (K_b) of complexes 1a, 1b and 1c are calculated as 2.97 X 10^4, 4.23 X10^4 and 5.14 X 10^4 M^{-1}, respectively.

Fluorescence spectra studies

Fluorescence measurements were performed to study the metal interaction with DNA using a Perkin Elmer spectrofluorimeter. Ethidium bromide (EB) is one of the most sensitive fluorescence probes that can bind with DNA. The fluorescence of EB in-creases after intercalating into DNA. If the metal intercalates into DNA, it leads to a decrease in the binding sites of DNA available for EB, resulting in a decrease in the fluorescence intensity of the EB–DNA system. The emission spectra of EB bound to DNA in the absence and presence of complex 1a are shown in Figure. 3. The addition of the complex to DNA pretreated with EB causes an appreciable reduction in fluorescence intensity. This indicates that the complex 1c binds with DNA. The reduction of the emission intensity gives a measure of the DNA binding propensity of the complex and the stacking interaction (intercalation) between the adjacent DNA base pairs [32]. The fluorescence quenching curves of DNA-bound EB by complexes 1d and 1e illustrates that the quenching of EB bound to DNA by the complexes are in good agreement with the linear Stern–Volmer equation. In the linear fit plot of I_0/I versus [complex]/[DNA], K is given by the ratio of the slope to intercept. The K values for the complexes 1a and 1b are 4.02 (R = 0.989) and 5.12 (0.991), respectively (I_0 is the emission intensity of EB–DNA in the absence of complex; I is the emission intensity of EB–DNA in the presence of complex). The concentrations of the complexes are taken when a 50% reduction of emission intensity of EB is observed [33]. From the data in Figure. 3, we know that 50% of the EB molecules were replaced from DNA-bound EB at a concentration ratio of [Cu^{II} complex]/[EB] of 7.39 for 1a and 8.64 for 1b. By taking a DNA binding constant of 1.0 X 10^7 M^{-1} for EB, apparent DNA binding constants of 1.0 X 10^6 and 1.2 X10^6 M^{-1} were derived {K_b(EB)/([Cu^{II} complex]

Table 1.Electrochemical data of the binuclear Cu^{Ii} complexes in the anodic potential region

Complex	E^1_{pc} (V)	E^1_{pa} (V)	$E_{½}$ (V)	ΔE_p (mV)	E^2_{pc} (V)	E^2_{pa} (V)	$E_{½}$ (V)	ΔE_p (mV)
1a	0.64	0.55	0.59	70	0.85	0.77	0.81	60
1b	0.66	0.56	0.61	80	0.87	0.75	0.81	80
1c	0.65	0.59	0.62	80	0.83	0.78	0.80	30

Scheme 1. Synthesis of Unsymmetrical Macrocyclic Binuclear Copper (II) Complexes

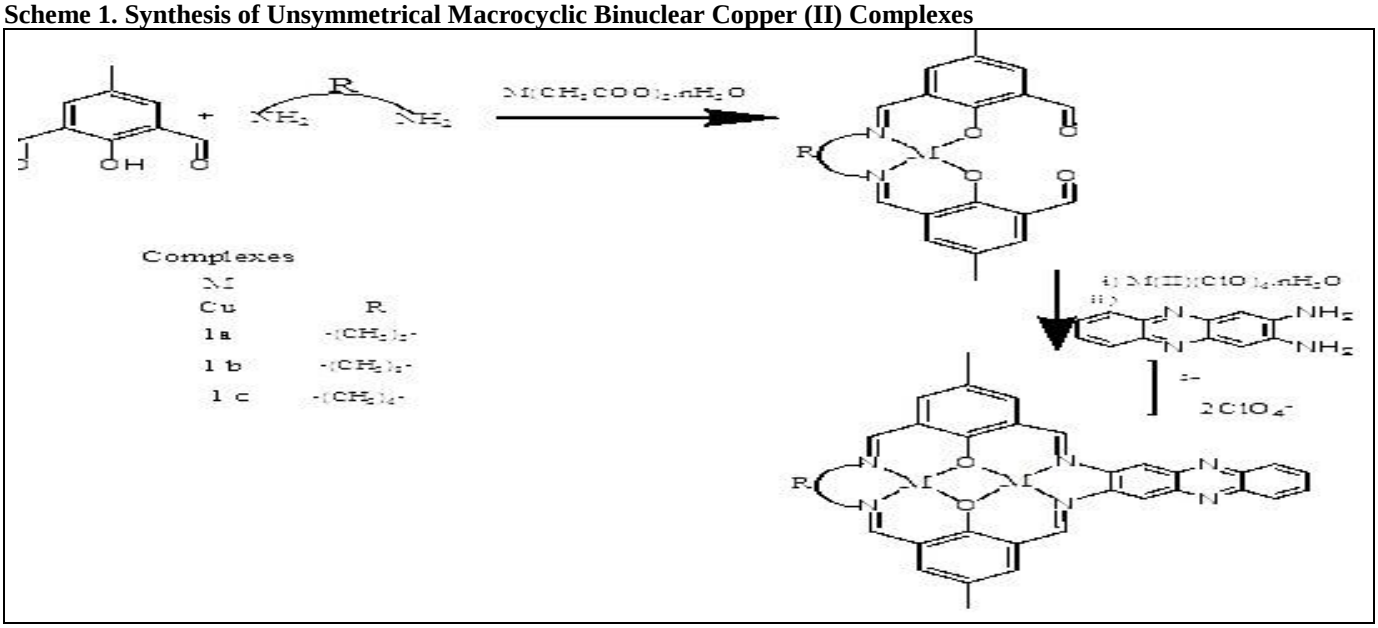


Figure 1. Cyclic voltammograms of the macrocyclic binuclear Copper(II) complexes 1a, 1b and 1c (10^{-3} M in DMF)

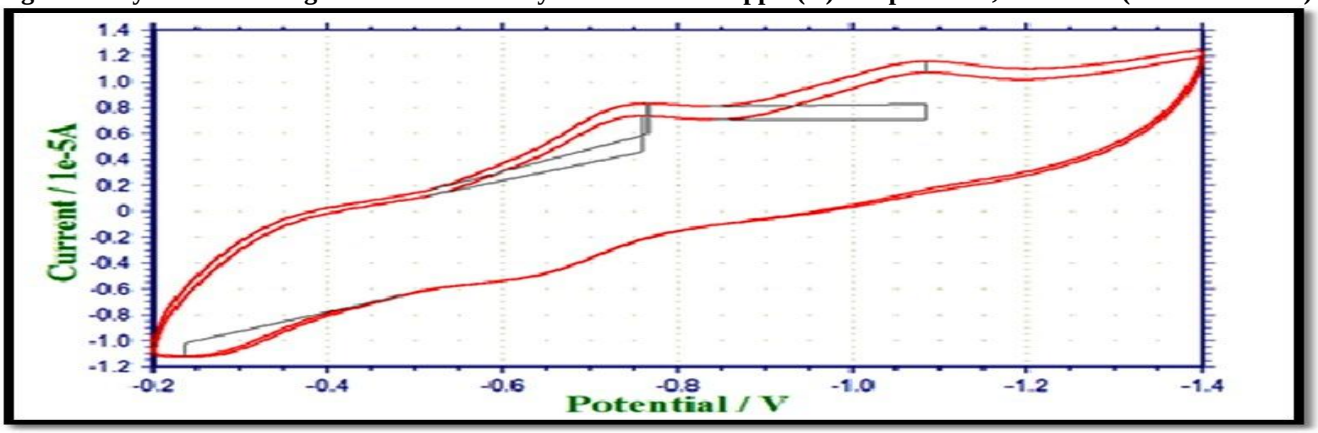


Figure 2. Absorption spectra of the complex 1a (10^{-5} M) in the absence (a) and presence of increasing amounts (b) of CT DNA (0–2.5 _ 10^{-6} M) at room temper-ature in 50 mM Tris–HCl/NaCl buffer (pH 7.5). The arrow indicates decreasing absorbance with increasing DNA concentration. Insets shows the saturation in absorption intensity hypochromism is indicated by the plot of A_0/A versus [DNA]

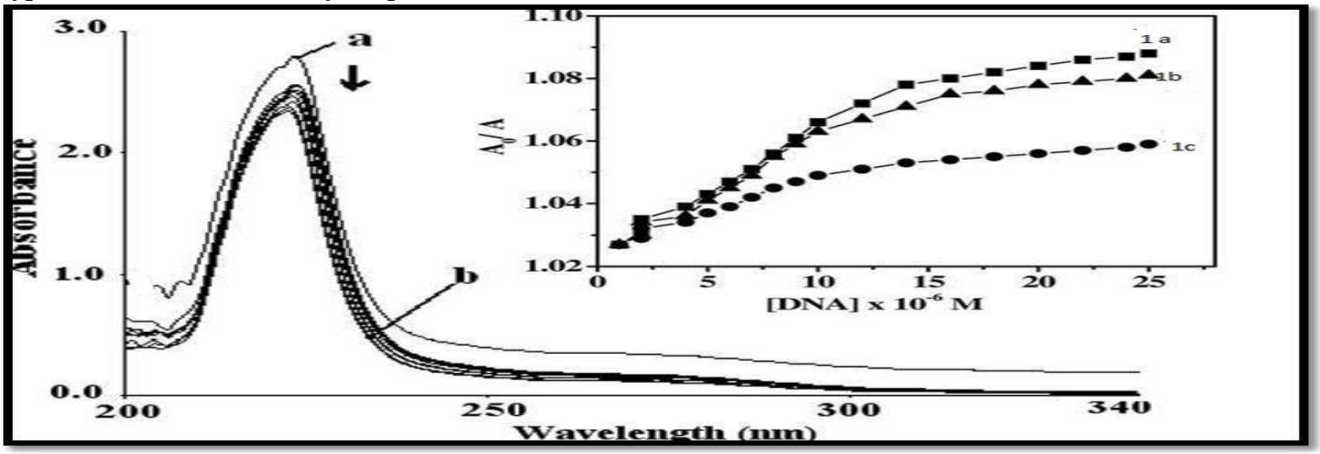
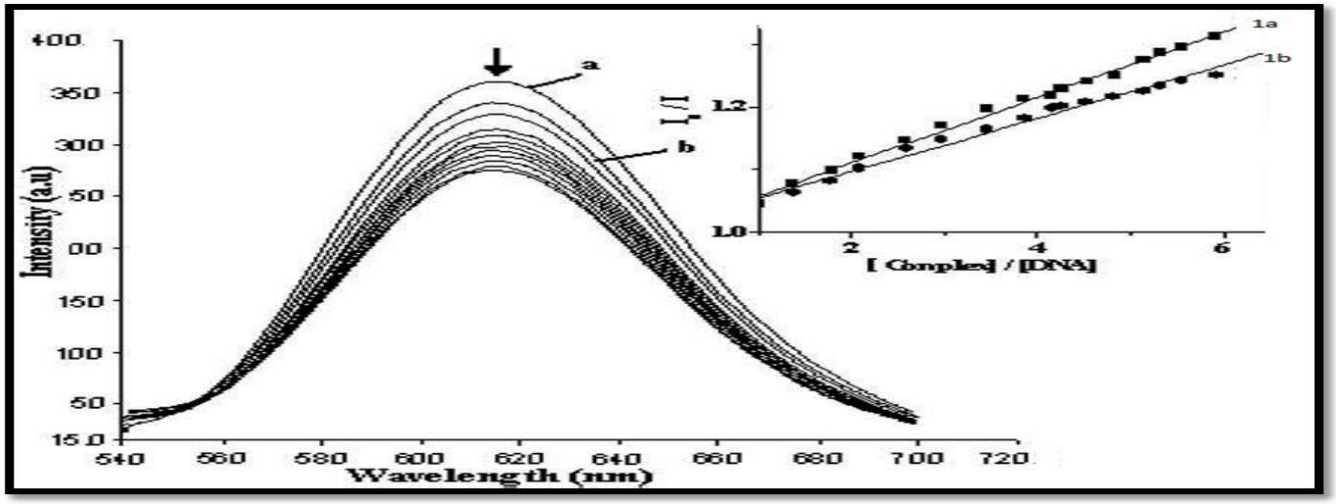


Figure 3. Emission spectra of EB bound to DNA in the presence of 1a ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0–25 µM, k_{ex} = 510 nm). (a) Emission spectrum of DNA-bound EB. (b) The arrow indicates decreasing absorbance with increasing Copper(II) complex (1a) concentrations. Inset shows the plots of emission intensity I_o/I versus [DNA]/[complex 1a]



CONCLUSION

In conclusion, it has been observed that the small variation in the chain length of the imine compartment: (i) shift the reduction potential anodically on increasing the chain length, (ii) shift the oxidation potential to a more positive potential as the chain length increases, (iii) aromatic diimine containing unsymmetrical macro-cyclic binuclear Cu^{II} complexes show better DNA binding ability than the aliphatic diimine containing complexes,

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