Synthesis, characterization, DNA binding and cleavage of new symmetrical acyclic copper (II) complexes

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Abstract— **New symmetrical acyclic binuclear copper (II) complexes $[CuL^{1-2}](ClO_4)_2$ 1 and 2 were synthesized by template method, from bicompartmental ligand 2,6-bis((E)-(2-(2-aminoethylamino)ethylimino)methyl)-4-methylphenol (L^1) and 2-((7E)-(2-((Z)-2-((naphthalen-1-yl) methyleneamino)ethylamino)ethylimino)methyl)-6-((9E)-(2-((Z)-2-((naphthalen-1-yl)methyleneamino) ethylamino)ethylimino) methyl)-4-methylphenol (L^2). These complexes were characterized by elemental analysis and spectroscopic techniques. The cyclic voltammogram of binuclear Cu(II) complexes exhibit two quasi-reversible reduction waves. The first reduction potential ranges from -0.6 V to -0.80 V and the second reduction potential lies in the range from -1.0 V to -1.25 V versus Ag/AgCl in Acetonitrile, 0.1 M TBAP. The DNA binding properties have been studied with the complexes 1 and 2 with calf thymus DNA. The complexes show good binding propensity to calf thymus DNA giving binding constant values in the range from 1.4×10^5 and $3.67 \times 10^6 M^{-1}$. The absorption, fluorescence and viscosity measurements spectral data suggests that the complexes are interacting strongly with DNA. These complexes display oxidative cleavage of supercoiled pBR322DNA in the presence of H_2O_2 at pH 7.2 and 37 ◦C using singlet oxygen as a reactive species. The binding affinities are in the following order: 2 > 1.**

Keywords- DNA binding and cleavage studies, copper (II) complexes DNA binding studies, intercalative binding mode and oxidative cleavage studies

I. INTRODUCTION

The chemistry of dinucleating ligands has received considerable attention largely due to their catalytic and bioinorganic relevance. Such complexes are important due to their potential biological activities such as antibacterial, antiviral, antifungal, antimalarial and antitumour drugs. Platinum (II) complexes has been used as anti-cancer drugs since long-time, among them cisplatin has proven to be a highly effective chemotherapeutic agent for treating various types of cancers. Cisplatin moves into the cell through diffusion and active transport. It causes platination of DNA, which involves interstrand and intrastrand cross-linking as well as formation of adduct and causes distortion which results in inhibition of DNA REPLICATION[1] and also serves as binding site for cellular proteins such as repair enzymes, histones, transcription factors and HMG-domain proteins. The clinical use of cisplatin is limited because of the **toxicity to the normal cells** and **drug resistance**, therefore, the study of other transition metal complexes is particularly important in order to broaden the chemotherapeutic arsenal, as they may be expected to exhibit different reactivity towards DNA and other cellular targets as compared to cisplatin. More recently, few Cu^{2+} complexes supported with amide-based macrocyclic ligands containing labile sites were shown to have good anticancer properties in man and animals[2]. This has thrown light on the present interrogation of the synthesis and characterization of the new symmetrical binuclear Copper (II) complexes. DNA binding studies were investigated for complexes 1and 2 using electronic absorption studies, fluorescence spectra, and the viscosity measurements method. The chemical nuclease activity of complexes 1 and 2 was examined using gel electrophoresis, reveals singlet oxygen plays a vital role in the nuclease activity and Complex 2 has good cleaving propensity compared to the complex 1.

II. EXPERIMENT

A. Materials and physical measurements

2,6-Diformyl-4-methylphenol prepared from literature methods[3]. Tetra(n-butyl)ammonium perchlorate (TBAP) was recrystallized from hot methanol, Copper perchlorate hexa hydrate, (Caution! All perchlorate salts are potentially explosive; hence, care should be taken in handling them) Diethylenetriamine and Triethylenetriamine was purchased from Fluka and the supercoiled pBR322 DNA was purchased from Bangalore Genei (India). Superoxide dismutase (SOD), ethidium bromide (EB) and L-histidine were obtained from Sigma (USA). Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution was prepared by using

deionized, sonicated triple distilled water. Elemental analysis was carried out with a Carlo Erba model 1106 elemental analyzer. FT-IR spectra was recorded in a Perkin Elmer FT-IR spectrometer with samples prepared as KBr pellets (4000–400cm^{-1}). UV–Vis spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer operating in the range 200–800 nm. Emission intensity measurements were carried out using a Perkin Elmer LS-45 fluorescence spectrometer. Cyclic voltammogram were obtained from the CH11008 Electrochemical analyzer using a three electrode set-up under oxygen free conditions. Electron spray ionization mass spectral (ESI-MS) measurements were made using a Thermo Finnegan LCQ-6000 Advantage Max-ESI mass spectrometer with acetonitrile as the solvent. Viscosity measurements were recorded using a Brookfield Programmable LV DVII+ viscometer.

B. Synthesis of binuclear symmetrical acyclic complexes 1 and 2.

An ethanolic solution of Copper (II) per chlorate hexahydrate was added dropwise to the 2,6-diformyl-4-methylphenol in 20ml of ethanol to this diethylenetriamine was added very slowly dropwise[4] in the ratio of 2:1:2 respectively the reaction mixture was allowed to stir in an Ice bath for 6 h. The resulting solution was then filtered immediately and allowed for slow evaporation at room temperature, washed with methanol and dried in vacuum. The corresponding dark blue solid (complex1) was recrystallized with acetonitrile yield: 75%**.** Further, the obtained complex 1 was condensed with naphthaldehyde in the stoichiometric ratio 1:2 respectively in ethanolic solution, was allowed to stir at room temperature for 12 h ,the above procedure was carried out to obtain the complex 2. yield 65%The dark greenish blue solid was recrystallized with acetonitrile and chloroform in the ratio of 5:1; Anal.Cal for **complex 1** $C_{17}H_{29}Cu_2N_6O$ (%): C, 44.33; H, 6.35; Cu, 27.60; N, 18.25; O, 3.47. Found C, 43.11; H, 5.27; Cu, 27.14; N, 16.21; O, 3.34. ESI-MS: displays a peak at m/z 461 (M+1) (calculated m/z 460). FT-IR (KBr,v/cm^{-1}): 1625(s), 1099 (s), 620(s). UV-visible in DMF [λ_{max} /nm(ε/M^{-1} cm^{-1})]: 582(110), 345 (124,000), 268 (210,000). Anal. Cal for **complex 2** $C_{39}H_{41}Cu_2N_6O$(%): C,63.37; Cu,17.25; N,11.40; H,5.61; O,2.17. Found C,63.21; Cu,17.19; N,10.15; H,5.48; O,2.11. ESI-MS: displays a peak at m/z 737 (M+1) (calculated m/z 736). FT-IR (KBr,v/cm^{-1}):1618 (s), 1077 (s), 629(s). UV-visible in DMF [λ_{max} /nm(ε/M^{-1} cm^{-1})]: 610 (140), 410 (158,000), 272 (250,000).

C. DNA binding and cleavage studies.

a. Absorption spectral studies

Electronic absorption spectrum of the complex was recorded before and after addition of CT-DNA in the presence of 50 mM Tris-HCl buffer (pH 7.5). A fixed concentration of metal complexes (10 µM) was titrated with incremental amounts of CT-DNA over the range (0 – 200 µM). The equilibrium binding constant (K_b) values for the interaction of the complex with CT-DNA were obtained from absorption spectral titration data using the following equation 1 [5].

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

Where ε_a is the extinction coefficient observed for the charge transfer absorption at a given DNA concentration, ε_f the extinction coefficient at the complex free in solution, ε_b the extinction coefficient of the complex when fully bound to DNA, K_b the equilibrium binding constant, and [DNA] the concentration in nucleotides. A plot of [DNA]/(ε_a - ε_f) versus [DNA] gives K_b as the ratio of the slope to the intercept. The non-linear least square analysis was performed using Origin lab, version 6.1.

b. Fluorescence spectral studies

Experiments were carried out at pH 7.2 in the buffer containing Tris – HCl buffer 50 mM by keeping EB-DNA solution containing [EB] = 4µM and [DNA] = 50 µM as constant and varying the concentration of complex (0 - 100µM). Fluorescence spectra were recorded using excitation wavelength of 510 nm and the emission range set between 500 and 750 nm. The quenching constant K_{sv} was deduced from Stern-Volmer method where the ratio of fluorescence of the compound alone (I_0) over the fluorescence of the compound in the presence of CT-DNA (I) is presented as a function of CT-DNA concentration.

$$I_o/I=1 + K_{sv}[r]. \quad (2)$$

Where I_0, is the ratio of fluorescence intensities of the complex alone, I is the ratio of fluorescence intensities of the complex in the presence of CT-DNA. K_{sv} is a linear Stern – Volmer quenching constant and r is the ratio of the total concentration of quencher to that of DNA, [M] / [DNA]. A plot of I_0 / I *vs.* [complex]/ [DNA], K_{sv} is given by the ratio of the slope to the intercept. 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 x 10^7 M^{-1} ([EB] = 3.3 µM) [6].

c. Viscosity measurements

The binding mode of the complex to CT-DNA, viscosity measurements were carried out on CT-DNA (0.5 mM) by varying the concentration of the complex (0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM). Data were presented as (η/η^{o}) versus binding ratio of concentration of complex to that of concentration of CT-DNA , where η is the viscosity of DNA in the presence of complex and η^{o} is the viscosity of DNA alone[7].

d. DNA cleavage experiment

The DNA cleavage experiments were performed by agarose gel electrophoresis. pBR322 DNA (0.1μg/μl) in Trisbuffer (pH 7.2) was treated with complex (50 μM) in the presence of additives. The sample was incubated for 3h at 37ºC and the reaction was quenched by 1 μl of loading buffer. pBR322 DNA bands were stained by EB, visualized under UV light and photographed. The extent of cleavage of SC DNA was determined by measuring the intensities of the bands using a UVITECH Gel Documentation System[8].

III. RESULT AND DISCUSSION

A. Electrochemical studies

The cyclic voltammograms of the complexes were recorded in acetonitrile. The electrochemical behavior in the negative potential range is sensitive to inductive (+I or –I) nature of the para-substituted benzene rings[9] and the steric effects of the ligand substituent. The first reduction potential ranges from -0.6 V to -0.80 V and the second reduction potential lies in the range from -1.0 V to -1.25 V versus Ag/AgCl in Acetonitrile. The ΔEp >60mV,the Epc and Epa values change with scan rate and the Ic and Ia peak currents were unequal, indicating the quasi reversible nature of electron transfer process.

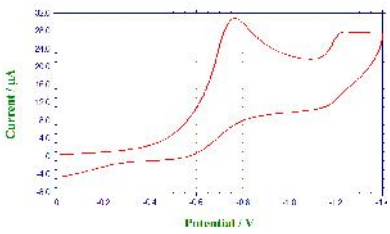

Figure 1: cyclic voltammogram of complex 2

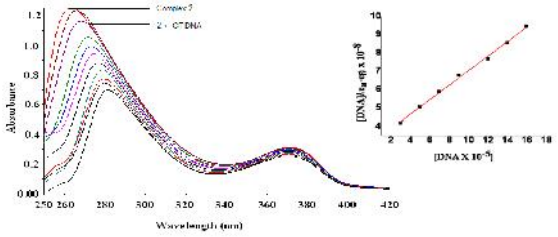

Figure 2: Absorption spectra of the complex 2 (10 μM) in the absence and presence of Increasing amount of CT-DNA (0 - 250 μM) in Tris-HCl buffer

B. Electronic absorption spectral titrations

The absorption spectral titration of the complexes with CT-DNA was followed by monitoring the CTbands. Both the complexes 1 and 2 show hypochromism and bathochromism due to intercalation involving a strong π-π* stacking interaction between aromatic chromospheres and the base pairs of DNA. The extent of hypocromism gives a measure of the strength of intercalative binding [10]. The presence of naphthalene ring in the complex 2 is responsible for more hypochromism indicating the interaction strength of the complex is much stronger more than the complex 1. The binding constants (K_b) of the complexes 1 and 2 have been calculated as 1.4×10^{5} and $3.67 \times 10^{6} M^{-1}$. The complex 2 possess better binding with CT-DNA than the complex 1, due to the presence of the planar aromatic naphthalene ring system.

C. Emission spectral studies

The extent of binding of the complexes 1 and 2 have been evaluated using competitive binding studies involving ethidium bromide (EB), which is known to emit strongly(λ_{ex} 510; λ_{em}, 610nm) due to its intercalative interaction with DNA. When the complexes 1 and 2 were added to CT DNA (1/R = 2), incubated with EB(1/R = 2), the emission of EB is quenched by both the complexes but to different extent, the following order is 1< 2. The complex 2 which is involved in a strong DNA intercalation would compete with the intercalative bound EB for DNA binding and quench the EB emission to a greater extent [11]. The DNA-bound complex 2 perturbs the DNA helix more than the enhanced planarity of the complex 1-ligand does, to displace the bound EB more efficiently. The Kapp values obtained for both the complexes 1 and 2 were 1.9×10^{6} and 3.2×10^{7} respectively

D. Viscometry measurements

The mode of the two complexes binding to DNA was explained by viscosity measurements. Optical photo-physical probes are necessary, but do not give sufficient clues to support a binding model. Hydrodynamic measurements that are sensitive to length change are regarded as the least ambiguous [12]. For complexes 2 and 1 the viscosity of DNA increases highly with the increasing, concentration of the complex which is similar to that of proven intercalator EB. Both complexes change the relative viscosity of DNA in the consistent manner

due to binding by intercalation mode shown in figure 3. This result also parallels the pronounced hypochromism and emission of both the complexes, whereas this result is comparable with proven classical intercalator EB. Viscosity of DNA increases with the increase of the concentration of EB. So these two complexes increase DNA helix length. On the basis of viscosity results, we infer that thecomplex 2 binds with DNA more prominently than complex 1through intercalation mode.

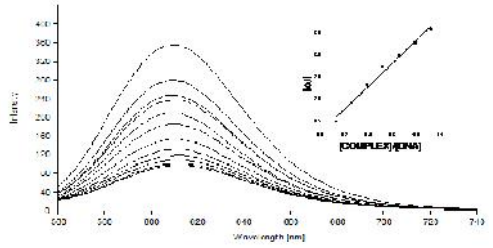

Figure 3: Emission spectra of EB bound to DNA in Tris – HCl buffer (pH-7.2) in the absence and presence of Complex 2 [EB] = 4µM, [DNA] = 20µM, [Complex 2] = 0 to 160µM, λ_{ex}= 510nm.

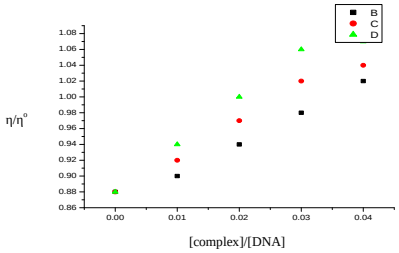

Figure 4: Changes in relative viscosity of CT-DNA on increasing amounts of complexes 1 and 2, B represents Complex 2, C represents complex 2 and D represents the EB,

E. Cleavage of plasmid DNA upon the complexes 1 and 2

The potential of the present complexes to cleave DNA was studied by gel electrophoresis by relation of supercoiled (SC) pBR322 DNA (form I) into nicked circular (form II) and linear (form III).if scission occurs on one strand (nicking), The SC will relax to generate a slower-moving open circular form (II) [13]. If both the strands are cleaved, a linear form (form III) will be generated that migrates between form I and II. The nuclease activity of the complex 1 and 2 is carried out in the presence of any radical scavengers and quenched, it may be due to presence of reactive oxygen species (ROS). The involvement of reactive oxygen species (hydroxyl radical, superoxide ion, singlet oxygen) for cleavage activity has been investigated by the inhibition of DNA cleavage in the presence of these scavengers. The hydroxyl radical scavenger (DMSO) and superoxide scavenger (SOD) do not inhibit the nuclease activities (Figure. 4,lanes 4 and 5) . So the hydroxyl radical and superoxide are ruled out for the cleavage mechanism process. L-histidine inhibit the nuclease activity as compared to other ROS quenchers. (Figure 4,lane 3)results suggested that singlet oxygen is responsible for the DNA cleavage scission. The supporting evidence is the addition of the EDTA (chelating agent) inhibit the cleavage mechanism which proves the good chelating ambience was provided for both the complexes 1 and 2. A similar mechanism was proposed for the oxidative cleavage reaction of the copper (II) complex reported earlier by our group [14]

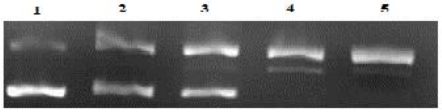

Figure 5: Lane 1, DNA control, Lane 2 complex2+ EDTA, Lane 3 Complex 2+L-Histidine, Lane4 Complex2+DMSO,Lane5 Complex2 + SOD

IV CONCLUSION

New symmetrical acyclic complexes 1 and 2 were synthesized and characterized. The DNA binding and cleavage activity were performed complex 2 has shown good binding propensity and nuclease activity K_b and K_{app} were calculated as $3.67 \times 10^6\ M^{-1}$ and $3.2\times10^7\ M^{-1}$ respectively.

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