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Synthesis, crystal structure and nuclease activity of a Cu(II) complex having two different co-ordination geometries in the same unit cell

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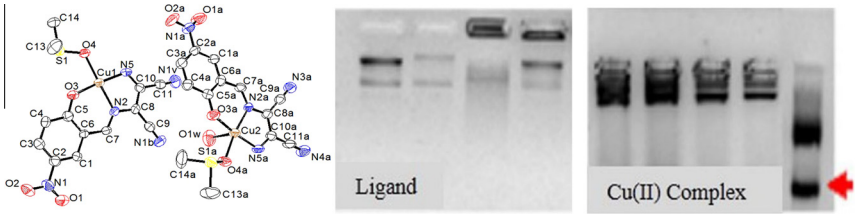
HIGHLIGHTS

- A new Cu(II) complex has been characterized through its single crystal X-ray studies.
- The same showed square planar and square pyramidal geometries in the same unit cell.
- The nuclease mimicking activity was exhibited by the same Cu(II) complex.
- The DNA binding was also investigated through UV–vis, fluorescence and CV studies.

GRAPHICAL ABSTRACT

The Cu (II) complex of a Schiff base ligand having N_2O donor set has been synthesized and fully characterized through its single crystal X-ray studies which showed two different coordination patterns *viz.* square planar and square pyramidal within the same unit cell. The same exhibited functional mimicking of nuclease activity by cleaving the plasmid pBR 322 DNA.



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ABSTRACT

A new Cu(II) complex of a Schiff base ligand having N_2O donor set has been synthesized and fully characterized through its single crystal X-ray studies. Two different co-ordination patterns viz. square planar and square pyramidal for Cu(II) were observed within the same unit cell. The DNA binding of copper(II) complex was investigated through UV–vis, fluorescence as well as cyclic voltammetric studies. The complex exhibited efficient functional mimicking of nuclease activity over the plasmid pBR322 DNA.

1. Introduction

The molecules able to perturb DNA irreversibly are of great interest due to their potential applications in understanding the structure and function of nucleases [1], as chemotherapeutic agents [2] as well as biological tool for genetic alterations, etc. [3]. Most of the naturally occurring nucleases e.g. P1 nuclease, hydrolases, etc. contain one or more metal ions at their active sites [4,5]. Inspired by the same many research groups have sought to develop low-molecular weight metal complexes having ability to mimic nucleases structurally, spectroscopically as well as functionally [6–10]. Since the first report of a synthetic Cu(II) system capable of inducing DNA cleavage by Sigman et al. in 1979 [11] a large number of Cu(II) and other metal complexes particularly of d-block have been synthesized, characterized and evaluated for their nuclease mimicking and anticancer activities [12–15]. These complexes have been proved to be the best alternatives to *cis*-platin

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[16] as many d-block elements particularly the 3d ones from d^5–d^{10} are well known for their significant biological functions particularly at the active sites of a large number of metalloenzymes [17,18]. The DNA binding and ultimately its cleavage are the two prerequisites for any substance to exhibit anticancer activity.

The mechanistic aspects of the same have been a genuine quest for chemists/biochemists working in the same field. Till now three different types of DNA cleavage mechanisms viz oxidative cleavage [19], photochemical cleavage [20] and hydrolysis [21] are known. The oxidative cleavage mechanism often involves the reduced oxygen species as reactive intermediates generated via redox active metal complexes. The interaction of these intermediates either with nucleic acid bases or sugar moiety can result in direct strand cleavage or in the formation of labile sites in the DNA. On the other hand photochemical cleavage mechanism involves either UV-induced DNA damage through dimerization of pyrimidine bases or DNA cleavage via the generation of reactive (oxygen) species by photochemical means. Thus cleavage mechanisms either oxidative or photochemical ones are quite similar to each other in terms of generation of reactive oxygen species which ultimately may lead to cytotoxic effects [22,23].

In spite of that, most of the studies dealing with the Cu(II) mediated DNA cleavage reported in literature have focused mainly on oxidative/photochemical cleavage processes [12,24–27]. There are only few reports of DNA cleavage activity of Cu(II) model compounds involving hydrolytic mechanism [28–31] as the phosphodiester linkage is among the most inert chemical functional group towards the hydrolysis [32]. The key role of the metal complexes in promoting hydrolysis of phosphate esters of DNA is supposed to be the intramolecular delivery of OH^- by reducing the pK_a of coordinated water molecule resulting into good amount of nucleophiles at neutral pH [32].

In continuation of our recent effort towards the development of mimicking systems for metallonucleases through simple synthetic protocol [33], we present here a new Cu(II) complex exhibiting the DNA cleavage property. In present communication we have also tried to establish the involvement of hydrolytic mechanism for the Cu(II) complex through an indirect route.

2. Experimental

2.1. Materials

All chemicals and reagents of analytical grade were purchased commercially and used without further purification unless otherwise mentioned. Calf thymus DNA (CT-DNA) was purchased from the Sisco Research Laboratories Pvt. Ltd. and was used as such. For the DNA cleavage experiment the plasmid pBR322 (4361 bp) was isolated and purified from the overnight grown *Escherichia coli* culture using the HiPura Plasmid DNA Miniprep purification spin kit (HiMedia, India) as per the manufacturer's instructions. The plasmid DNA thus purified was analyzed further on 1% agarose (Bangalore GeNei Ltd., India) in order to check the integrity and quality of the purified DNA and this DNA was used as a template for all cleavage setup reactions. Agarose (molecular biology grade) and ethidium bromide (EB) were purchased from Sigma (USA). Tris–HCl buffer solution was prepared using deionised, triple distilled water.

2.2. Apparatus

Melting points were recorded by open capillary method and are uncorrected. ^1H and ^{13}C NMR studies were performed on a JEOL AL 300 FT NMR and Bruker-400 Avance NMR Spectrometeres using TMS as an internal reference standard. ESI-MS was carried out on a MDS Sciex API 2000 LCMS spectrometer. The C, H and N elemental analysis were done on Model CE-440 CHN analyzer while UV–vis and emission spectra were recorded on a UV-1700 Pharmaspec spectrophotometer and Varian Cary Eclipse fluorescence spectrophotometer respectively with quartz cuvette (path length = 1 cm) at 298 K. The electrochemical measurements were performed with CHI 630C series (USA) electrochemical system. Cyclic voltammetric studies were performed in electrochemical cell with three electrode system including glassy carbon electrode acting as working electrode, and Ag/AgCl/3M KCl as reference electrode while Pt wire as counter electrode. The DNA bands were visualized under UV light and photographs were taken and analyzed by Alpha Digi Doc RT Gel documentation system (Alpha Innotech, CA, USA).

2.2.1. Synthesis of 2-amino-3-[(2-hydroxy-5-nitro-benzylidene)-amino]-but-2-enedinitrile, ligand (L1)

2-Amino-3-[(2-hydroxy-5-nitro-benzylidene)-amino]-but-2-enedinitrile (L1) was synthesized by adding 1.0 mM methenolic solution of diaminomaleonitrile to the equimolar methenolic solution of 2-hydroxy-5-nitrobenzaldehyde having one drop of conc. H_2SO_4 followed by constant stirring for three hours. A light yellow solid was precipitated which was filtered and washed with diethylether and finally dried under vacuum over anhydrous $CaCl_2$. L1 was characterized through IR, ^1H, ^{13}C NMR and mass spectral studies (ESI S1–S4).

Yield ~92%, m.p. ~290 °C UV–vis (λ_{max}) (DMSO): 291 nm, 317 nm, and 388 nm, EtOH 380 nm); IR ν_{max} (cm^{-1}): 3408, 3306, 3210, 3071, 2219, 1627, 1555, 1516, 1478, 1347, 1294, 1209, 1102, 964, 848, 667, 622, 497; ^1H NMR (300 MHz, DMSO-d_6, ppm): δ = 11.94 (s, 1H, –OH), 8.97–8.96 (d, 1H, ArH), 8.56 (s, 1H, >CH═N), 8.22–8.18 (m, 3H, –ArH and —NH_2), 7.12–7.10(d, 1H, ArH), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ = 102.8, 113.8, 114.3, 116.9, 121.9, 123.8, 127.3, 127.7, 140.3, 149.4, 163.1; Anal. (%): Calcd. for [$C_{11}H_7N_5O_3$] (L1): C, 51.37; H, 2.74; N, 27.23. Found: C, 50.49; H, 2.77; N, 27.62. M–H = 256.2 au, Calcd. for $C_{11}H_7N_5O_3$ = 257.2.

2.2.2. Synthesis of Cu(II)-L1 complex

To a solution of L1 (0.257 g, 1 mmol) in EtOH (25 mL), equimolar ethanolic solution of $CuCl_2{\cdot}2H_2O$ (0.147 g, 1 mmol) (10 mL) was added drop wise and the reaction mixture was left on stirring for overnight. A dark colored solid product was obtained which was filtered and washed with cold ethanol–water mixture (50/50, v/v) and dried to yield solid product. Yield = ~85%, m.p. >300 °C, UV–vis (λ_{max}) (DMSO): 278 nm, 348 nm (intra-ligand bands), 460 nm [metal to ligand charge transfer, (MLCT)], 518 nm (d–d transition), EtOH, 342 and 470 nm,] IR ν_{max} (cm^{-1}): 3483, 3375, 2242, 2180, 1598, 1531, 1469, 1415, 1326, 1287, 1196, 1105, 948, 761, 657, 527; Anal. (%): Calcd for [$C_{11}H_7CuN_5O_4$] (Cu(II)-L1): C, 39.23; H, 2.10; N, 20.80. Found: C, 40.20; H, 1.868; N, 19.57. M–H = 394 au, Calcd. For $C_{13}H_{11}CuN_5O_4S$ = 395.98 (ESI S5 and S6).

The above synthesis can be summarized through Scheme 1.

2.3. X-ray crystallography

Single-crystal X-ray data were collected at 100 K on a Bruker SMART APEX CCD diffractometer using graphite-monochromated Mo Kα radiation (k = 0.71073 Å). The linear absorption coefficients, scattering factors for the atoms, and the anomalous dispersion corrections were taken from International Tables for X-ray crystallography. The data integration and reduction were processed with SAINT [34] software. An empirical absorption correction was applied to the collected reflections with SADABS [35] using XPREP