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SYNTHESIS, CHARACTERIZATION AND DNA INTERACTION STUDIES OF COPPER(II) COMPLEX OF 4(3*H*)-QUINAZOLINONE-DERIVED SCHIFF BASE

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abstract: A novel copper(II) complex of quinolin-4(3H)-one derived Schiff base ligand was synthesized and characterized by elemental analyses, IR, 1H-NMR, MS, UV–Vis, magnetic moments, conductance measurements and thermal studies. The DNA binding and nuclease activities of copper(II) complex was performed.

key words: Copper(II) complex; spectral studies; DNA interaction studies.

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1. Introduction

Transition metal complexes of Schiff bases have attracted many researchers due to their potent biological activities such as antifungal, antibacterial, anticancer and herbicidal applications [1-5]. The complexation of metal to Schiff base ligand improves the antimicrobial and anticancer activities of the ligand. Investigations on the interaction between transition metal complexes and DNA has attracted many interests due to their importance in cancer therapy and molecular biology [6-9].

Schiff base metal complex is a kind of attractive metal based drug due to its special activities in pharmacology and physiology [10-13]. In our previous work, we have synthesized the Schiff bases of 3-amino-2-methyl-4(*3H*)-quinazolinone with different substituted aromatic aldehydes [14]. In this communication, we describe the synthesis, DNA binding and cleavage abilities of a copper(II) complex with Schiff base ligand derived from 3-amino-2-methyl-4(*3H*)-quinazolinone.

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2. Experimental

The chemicals and solvents were commercially available of high purity and used as such. CT-DNA (calf thymus DNA) was obtained from Genei laboratories, Bangalore. An elemental analysis was performed using a Perkin-Elmer 240 elemental analyzer. Infrared spectra were recorded with a Shimadzu FT-IR 8300 spectrophotometer from 4000-400 cm^{-1} using nujol mulls technique. Magnetic susceptibility measurement was made at room temperature were performed using Sherwood scientific MXI model Gouy magnetic balance. The UV-Visble spectra were recorded on Hitachi-3900 spectrophotometer. A Shimadzu TG-50H thermo analyzer was used to record simultaneous TGA and DTG curves in dynamic nitrogen atmosphere with a heating rate of 10 °C min^{-1} in the temperature range 20-700 °C using platinum crucibles.

Synthesis of $[Cu(C_{34}H_{32}N_6O_6)]2H_2O$ (1): A Schiff base ligand solution (0.618g, 2 mmol) was added to a hot methanolic solution of metal chloride (0.170 g, 1 mmol) and the resulting mixture was stirred under reflux for 4 h where upon the complex was precipitated. It was collected by filtration, washed with hot water, then diethyl ether and dried in air. The analytical and physical data of the complex were:

Yeild: 69; elemental analyses of $C_{34}H_{34}N_6O_8Cu$: Calcd. (found); C; 56.68 (56.77), H; 4.73 (4.81), N; 11.69 (11.74), Cu; 8.9 (8.97). Molar conductance: 23. 9 $\Omega^{-1}cm^2mol^{-1}$.

DNA binding studies

All the experiments involving the interaction of complexes with CT-DNA were conducted in Tris buffer (10 mm Tris-HCl-50 mm NaCl buffer, *p*H 7.4). The concentration of the DNA used for binding experiments was determined by measuring the absorption intensity at 260 nm with molar extinction coefficient value 6600 M^{-1} cm^{-1} [15]. The absorbance measurements were performed by keeping the concentration of the complex constant (1×10^{-4} M) while varying the DNA concentrations (1×10^{-4} M, 5, 10, 15, 20 and 25 µL).

Viscosity measurements

Viscosity titration experiments were carried on an Ostwald's viscometer at room temperature by varying the complex concentrations and a constant CT-DNA concentration (50 µM). Flow time was measured with a digital stop watch. Each sample was measured at least three times and average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ vs binding ratio ([complex]/ [DNA]), [16], where η was the viscosity value for DNA in presence of the Copper(II) complex and η_0 was the viscosity value of CT-DNA alone.

DNA cleavage

The cleavage of plasmid DNA was monitored using agarose gel electrophoresis. Supercoiled pUC19 (0.5 µg) in Tris-HCl buffer (50 mM) with 50 mM NaCl (pH 7.2) was treated with metal complex (10^{-3} M). The sample was incubated for 1 h at 37 °C. A buffer solution containing 25 % bromophenol blue, 0.25 % xylene cyanol and 25 % glycerol was taken in a electrophoretic unit (buffer tank) and electrophoresis was performed at 70 V for 2 h in TBE buffer using 1.0 µg/mL ethidium bromide in 1.0 % agarose gel [17]. 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 (Form I) to nicked circular form (Form II) or linear form (Form III).

3. Results and discussion

The formation of the copper(II) complex was achieved by reaction of the ligand with copper(II) salt in 1:2 [M:L] ratio. The conductance value indicates the non-electrolytic nature of complex [18]. The spectral, magnetic and thermal studies on copper complex are discussed in the following paragraphs.

IR spectra

Comparison of the infrared spectral data of the ligand and its copper(II) complex revealed that a significant shifts in the bands of the azomethine ν(CH=N) and lactam ν(C=O) groups were confirmed the complexation. The expected mode of interaction of copper(II) complex with the Schiff base via coordination of the azomethine nitrogen group and lactam oxygen atoms of the ligand. The IR spectra of the ligand showed the expected characteristic imine band in the region 1582 cm^{-1}, shifted to lower frequency (14 cm^{-1}) due to metal coordination [19]. A sharp band at 1650 cm^{-1} region in the ligand due to ν(C=O) was also been shifted to lower frequency (8 cm^{-1}) in the complex. Further, the complexation was confirmed by the appearance of additional weak bands in the region 474-445 and 565-525 cm^{-1} which were attributed to ν(M-O) and ν(M-N), respectively.

Magnetic susceptibility and Electronic spectra

The effective magnetic moment value (μ_{eff}) of copper complex was 1.80 B.M. which is consistent with the presence of one unpaired electron [20]. The electronic absorption spectrum of the complex **1** in DMF was recorded at room temperature. The complex exhibit an absorption band at 375 nm, which is assigned to charge transfer transition from the pπ-orbitals of the donor atoms to the d-orbitals of the metal. In addition, complex exhibit a d-d transition at 647 nm.

Thermal studies

The TG/DTG curves of complex **1** are illustrated in Fig.1. The TGA profiles over the temperature range 30-200 °C are usually due to loss of water of moisture, hydration and coordination. The first stage between 30 and 110 °C corresponds to the dehydration. The anhydrous complex is stable up to 200 °C. The observed weight loss indicates the loss of two coordinated water molecules present in the complex. In the second stage, continuous mass loss occurs in the range 200-400 °C (TG=42.1 %), suggesting the evaporation of ligand. The third step occurred between 400-510 °C, (TG=31.5 %). The loss in weight corresponds to the remaining organic ligand molecule leaving behind metal oxide as the end product [21].

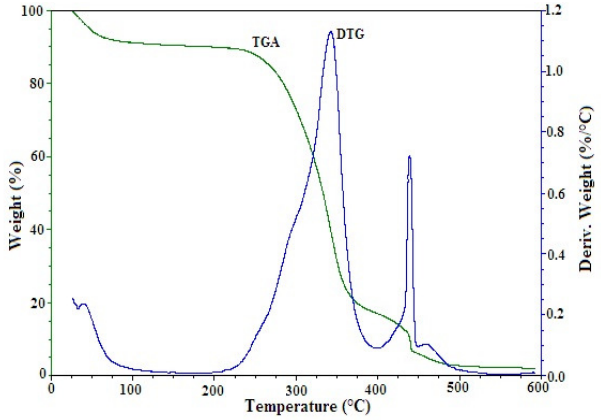

Fig. 1 *Thermogravimetric (TGA and DTG) curves of complex 1.*

Based on the above spectral and thermal studies, following structure has been assigned for the copper complex (Fig. 2):

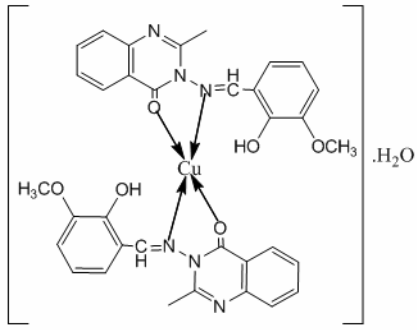

Fig. 2 *Structure of copper complex 1.*

Biology

DNA binding studies

The interaction between the complex and CT-DNA was evaluated by UV-Vis spectrometric titration. The absorption spectra of **1** in the presence of CT-DNA are shown in Fig. 3. From Fig. 3 it is clear that the hyperchromicity is observed on the addition of CT-DNA. This significant hyperchromism effect suggests that there exists a strong interaction between the complex **1** and CT-DNA.

Viscosity measurements

To further clarify the interaction between complex and DNA, viscosity measurements were carried out. A classical intercalation model results in lengthening of the DNA helix as base pairs to an increase of DNA viscosity [22]. The viscosity of CT-DNA is found to be increased in the presence of complex **1** (Fig. 4). This observation suggests that the mode of DNA binding by complex involved base pair intercalation.

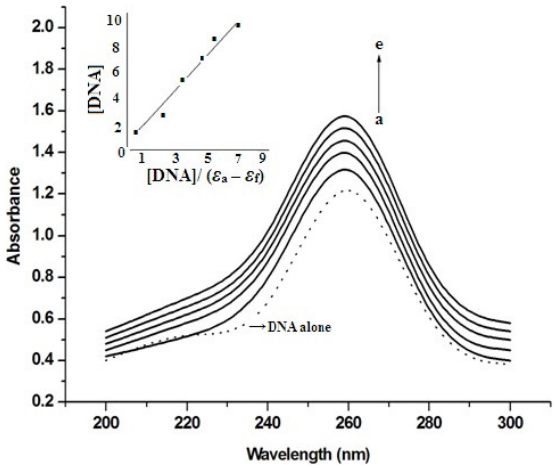

Fig. 3 *Absorption spectra of complex **1** in Tris-HCl buffer upon addition of DNA = 1×10^{-4} M, 0-25 μL.* Arrow shows the absorbance changing upon increasing the concentration of DNA.

The inner plot of [DNA]/ ($\varepsilon_a - \varepsilon_f$) versus [DNA] for the titration of DNA with complex **1.**

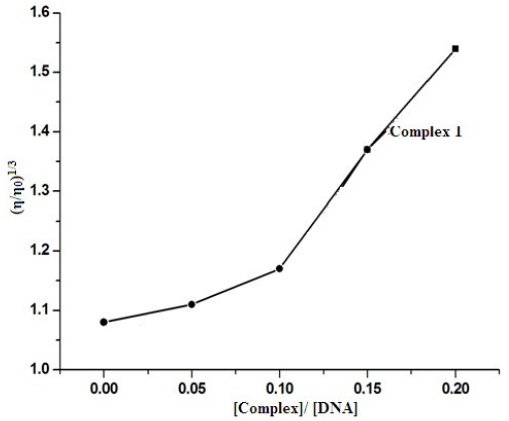

Fig. 4 *Effect of increasing amounts of complex 1 on the relative viscosity of CT-DNA.*

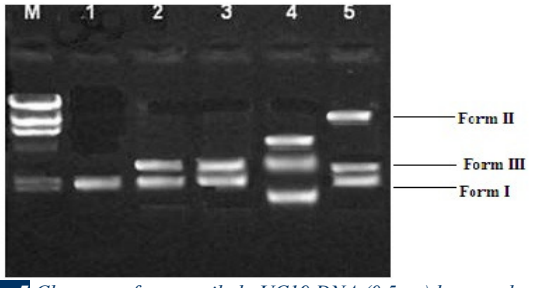

Fig. 5 *Cleavage of supercoiled pUC19 DNA (0.5 μg) by complex **1** in a buffer containing 50 mM Tris-HCl at 37 °C (30 min):*
lane M: marker; lane 1: DNA control; lane 2: DNA+H_2O_2;
lane 3: complex **1** (10^{-3} M) + DNA; lane 4: complex **1** (10^{-3} M) + DNA + H_2O_2.

Nuclease activity

The nuclease activity of **1** has been assessed by its ability to convert supercoiled pUC19 DNA from Form I to Form II and Form III by gel electrophoresis. Fig. 5 shows the cleavage pattern of plasmid DNA. The DNA cleavage efficiency of the complex was due to the different binding affinity of the complex to DNA. Complex **1** is able to cleave DNA to almost same extent in absence and presence of H_2O_2.

4. Conclusion

A novel Copper(II) complex was synthesized and characterized by analytical and spectral techniques. The results from these techniques confirmed the structure of the complex.

The DNA interaction studies suggested that the Copper(II) complex acts as avid binding and cleaving agent.

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