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Synthesis, structural characterization and DNA interaction studies on a mononuclear copper complex: Nuclease activity via self-activation

Kaushik Ghosh *, Pramod Kumar, Nidhi Tyagi, Udai P. Singh, Nidhi Goel

Department of Chemistry, Indian Institute of Technology, Roorkee, Roorkee-247667, Uttarakhand, India

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ABSTRACT

Mononuclear copper(II) complex [Cu(Pyimpy)(Cl)(ClO_4)] has been synthesized from a tridentate ligand Pyimpy (Pyimpy = 1-phenyl-1-(pyridin-2-yl)-2-(pyridin-2-ylmethylene)hydrazine) and characterized by elemental analysis, electronic absorption and IR spectroscopy. Molecular structure of **1** was determined by single crystal X-ray diffraction. Structural index parameter (τ) calculation supported distorted square pyramidal geometry around the metal centre. The stabilization of copper(II) centre was examined by electrochemical studies. DNA interaction studies were investigated by absorption spectral studies and EthBr displacement assay. Nuclease activity studies afforded cleavage of pBR322 plasmid DNA via self-activating mechanism. Investigation of mechanism indicated the possible role of reactive oxygen species in DNA cleavage.

There has been considerable interest in the recent years for design and synthesis of DNA cleaving reagents for their applications in biotechnology and medicine [1–3]. Transition metal ions show diverse structural features, variable oxidation and spin states and redox properties in different complexes. These properties could be exploited to discover novel artificial nucleases. Among the first row transition elements, copper has got a special interest in this regard since the discovery of first chemical nuclease by Sigman et al. [4]. Biologically relevant copper has high affinity for the nucleobases and copper complexes possess biologically accessible redox properties [5]. There are several reports of copper complexes and their nuclease activity studies, however few reports [6,7] are there in the literature where copper complexes are acting as artificial nucleases without the addition of any oxidizing or reducing agent. This type of nuclease activity could be possible via a self-activating mechanism [6] and/or through a hydrolytic pathway [7]. Our quest was to synthesize such type of molecule and herein we report the synthesis and characterization of a novel copper complex [Cu(Pyimpy)(Cl)(ClO_4)] (**1**) derived from tridentate ligand Pyimpy. Complex **1** was successful in cleaving DNA in absence of any oxidizing or reducing agents. Molecular structure of **1** was determined by single crystal X-ray diffraction. Redox property of the metal centre was examined by electrochemical studies. DNA interaction with calf thymus DNA (CT DNA) and nuclease activity with pBR322 plasmid were investigated and results of our mechanistic studies will be scrutinized in this communication.

Schiff-base ligand Pyimpy (Scheme 1) was synthesized by the condensation of pyridine 2-aldehyde and 2-(1-phenylhydrazinyl) pyridine [8]. Pyimpy was reacted with $Cu(ClO_4)_2 \cdot 6H_2O$ in methanol and a green solution was obtained. Addition of one equivalent of Et_4NCl to the above solution gave rise to [Cu(Pyimpy)(Cl)(ClO_4)] (**1**) (shown in Scheme 1). In IR spectra of **1**, splitting of peaks near 1090 cm^{-1} indicated the presence of coordinated perchlorate ion. However, solution conductivity measurement gave rise to 1:1 electrolyte [8]. Hence we speculate the dissociation of perchlorate ion in solution. Complex **1** afforded strong charge transfer band at 384 nm in UV–vis spectra. Oxidation state 2+ for copper in complex **1** was confirmed by magnetic susceptibility measurements at room temperature.

Molecular structure of complex [Cu(Pyimpy)(Cl)(ClO_4)] (**1**) is depicted in Fig. 1 and the matrix parameters are described in Table S1. In crystal structure, two pyridine nitrogen (N_{Py}) and one azomethine nitrogen (N_{Im}) bind to the metal centre in *mer* fashion. The ligand has three six-membered rings, among them, two pyridine rings are in the same plane whereas the other phenyl ring is roughly perpendicular (82.92°) to the ligand binding plane. The structural index parameter [9] (τ) was determined to describe the geometry around penta coordinated metal centre. In complex **1** the τ value was calculated to be 0.174 and the geometry of the complex around the metal centre was described as distorted square pyramid (Scheme S1, details are given in the supporting information). In the complex the square plane consisted of two N_{Py} and one N_{Im} donors along with a Cl^- ligand whereas ClO_4^- ligand occupied in axial position. The copper centre was 0.13 Å above the plane generated by N1, N2, N4 and Cl1. Cu–N_{Py} distances (~2.00 Å) and Cu–N_{im} distance ~1.97 Å are consistent with the reported data [10]. The equatorial Cu–Cl bond distance (2.21 Å) is

* Corresponding author. Tel.: +91 1332 285547; fax: +91 1332 2735601.
E-mail address: ghoshfcy@iitr.ernet.in (K. Ghosh).

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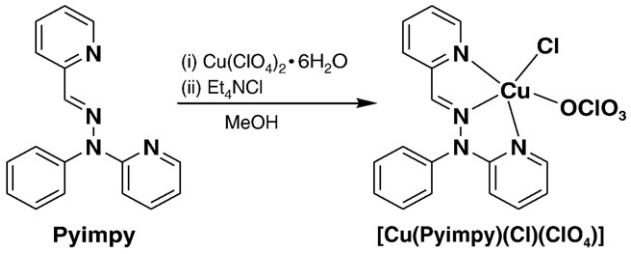

Scheme 1. Schematic conversion of Pyimpy into [Cu(Pyimpy)(ClO_4)(Cl)] (**1**).

smaller than the values (2.34 Å–2.43 Å) reported in the literature [5,11] and even shorter than the values (2.26 Å and 2.28 Å) reported [12] for the equatorial Cu–Cl bond. The axial Cu–O_{ClO4} bond distance was close to the value reported by Palaniandavar et al. [7a].

The redox behavior of Cu(II) in complex **1** was investigated by cyclic voltammetry at a glassy carbon electrode using Ag/AgCl reference electrode. Cyclic voltammogram of **1** in DMF solution at 298 K is shown in Fig. S2. The complex showed irreversible cyclic voltammogram with a cathodic peak near −0.060 V possibly due to Cu^{II}/Cu^{I} couple. The anodic peak near + 0.488 V vs. Ag/AgCl probably satisfy the condition for self activation predicted by Reedijk et al. [6b].

We have done all the DNA interaction studies in phosphate buffer (0.1 M, pH 7.2) and there was negligible change in absorbtion spectra of **1** after one month. Electronic absorption spectroscopic techniques were used to investigate the binding of DNA with metal complexes. To achieve this, the absorption spectra of **1** in the absence and presence of CT-DNA at different concentrations were measured (Fig. 2). The binding constant K_b for **1** has been found to be $5.2\times10^3\ M^{-1}$. The observed binding constant was similar to be values obtained for other copper complexes [13] but much smaller than the classical intercalators and metallointercalators where binding constants were reported to be in the order of $10^7\ M^{-1}$ [14]. During the titration with DNA we found some interesting spectral changes. The spectral changes showed a blue shift (37 nm) for the charge transfer band and concomitant formation of an isosbestic point at 358 nm. This type of spectral changes were possibly due to new species generation in presence of DNA [15]. Fate of **1** with the variation of pH was

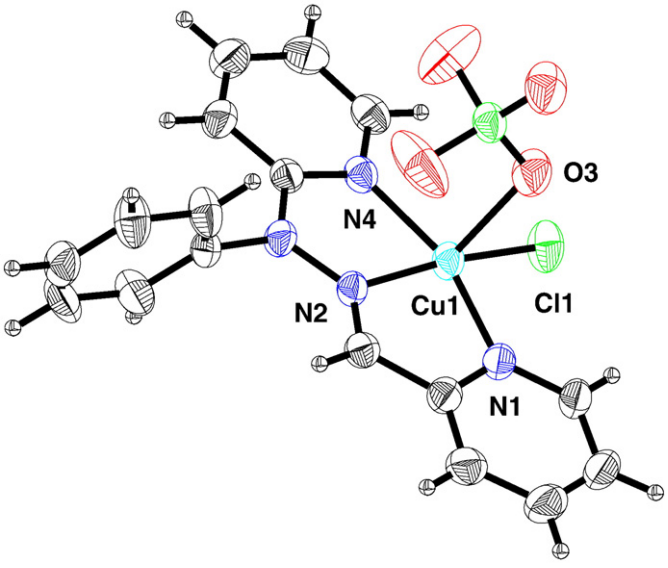

Fig. 1. ORTEP plot of [Cu(Pyimpy)(Cl)(ClO_4)] showing the atom numbering scheme. Thermal ellipsoids are drawn at 30% probability. Selected bond lengths (Å) and angles (deg): Cu(1)–N(1) 2.004(4), Cu(1)–N(2) 1.973(3), Cu(1)–N(4) 1.996(4), Cu(1)–Cl(1) 2.2133(15), Cu(1)–O(3) 2.524, N(1)–Cu(1)–N(2) 79.88(15), N(1)–Cu(1)–N(4) 159.59 (15), N(1)–Cu(1)–Cl(1) 99.03(12), N(2)–Cu(1)–N(4) 79.95(14), N(2)–Cu(1)–Cl(1) 170.05(10), N(4)–Cu(1)–Cl(1) 100.30(11).

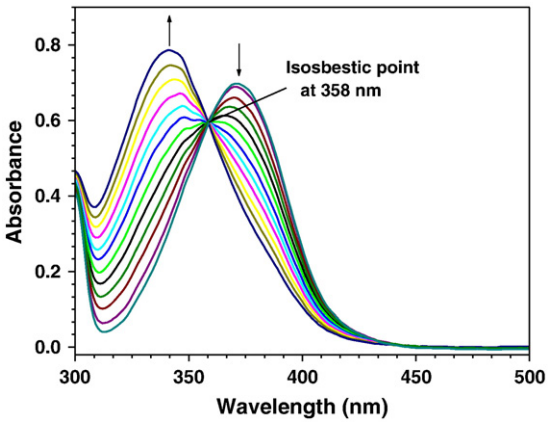

Fig. 2. Absorption spectra of complex **[1]** = 50 μM, in 0.1 M phosphate buffer (pH 7.2) containing 5% DMF in the presence of increasing amounts of [DNA] = 0–90 μM. Arrows show the absorbance changes upon increasing DNA concentration.

investigated and we found no change in UV–visible spectra in the pH range 3–10 [15]. We have invesigated ESI-MS spectral studies of complex **1** in water. The data clearly expressed the presence of copper complex in aqueous solution (detailed are shown in supporting information). We performed the titration experiment in H_2O (MilliQ) instead of that in buffer. The change in absorption spectra of complex **1** in H_2O (MilliQ) afforded spectral changes similar to the data depicted in Fig. 2. These data indicated that phosphate group was not involved for such changes. It may be possible that copper in presence of DNA got attached to the nucleic acid base(s) because of its high affinity [3,16] and a new species was generated during DNA interaction. More insight into this DNA binding event is under progress.

We have examined the competitive binding of ethidium bromide (EthBr) vs complex **1** with CT DNA using fluorescence spectral studies to get better insight into DNA binding events. Fluorescence quenching spectra of **1** were recorded (Fig. 3) and Stern–Volmer constant K_{sv} was found to be $2.45\times10^4\ M^{-1}$. K_b and K_{sv} values for complex **1** indicated moderate interaction with DNA [13,17].

We extended our DNA interaction studies by examining the nuclease activity of the complex **1**. The cleavage of supercoiled pBR322 DNA by **1** and formation of nicked (NC) and linear (LC) DNA were studied by several gel electrophoresis experiments in Tris–boric acid–EDTA buffer (TBE). Following important observations were obtained from Fig. 3. First, nuclease activity of **1** was observed (Fig. 4, lane 3–9) in absence of any oxidizing or reducing agents and

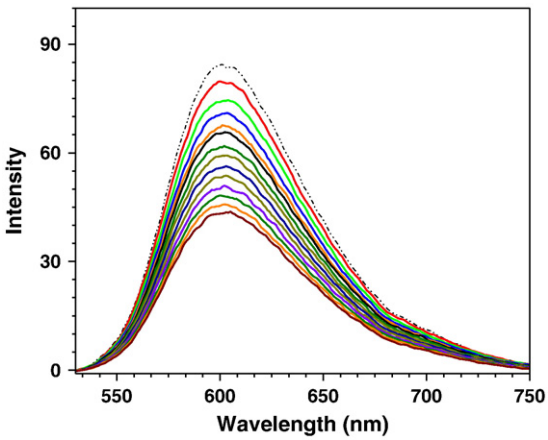

Fig. 3. Fluorescence emission spectra of the EB–DNA in 0.1 M phosphate buffer (pH 7.2) containing 5% DMF in the absence (dashed line) and presence (solid line) of complex **1**. [EB] = 5 μM, [DNA] = 25 μM, [Cu(Pyimpy)(Cl)(ClO_4)] = 0–14.38 μM, λ_{ex} = 250 nm.

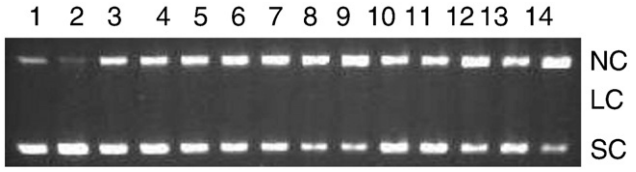

Fig. 4. Gel electrophoresis separations showing the cleavage of supercoiled pBR322 DNA (100 ng) by complex **1**. Incubated at 37 °C for 1.5 h. lane 1, DNA control; lane 2, DNA + 10% DMF; lanes 3–9, DNA + **1** = 10, 25 40, 50, 60, 80, 100 μM respectively; lanes 10–14, DNA + **1** (100 μM) + incubation time 5, 15, 30, 60 and 90 min respectively.

the amount of NC DNA was found to be increased with the increase in concentration of **1** (10–100 μM) without the formation of LC form of DNA. Second, enhancement of DNA cleavage activity was observed due to variation of incubation time (Fig. 4, lane 10–14).

In this next experiment depicted in Fig. 5, we examined the DNA cleavage activity in presence of oxidizing or reducing agent. Nuclease activity of **1** in presence of H_2O_2 or 2-mercaptoethanol (BME) increased the cleavage activity as compared to the previous experiment done by complex itself. Moreover, it has been found out that in the presence of H_2O_2 or 2-mercaptoethanol 50 μM concentration of **1** showed the complete conversion of SC form to NC and LC form of DNA (Fig. 5, lane 7, 8). The results of our control experiment were described in Fig. S4. These data clealy indicated that Pyimpy itself was not responsible for DNA cleavage activity.

Investigation of the mechanism becomes very much important when copper complexes exhibit nuclease activity in absence of any external reagents. There are several reports in the literature where such type of activity was described, however, following reports will be of our interest at this point. In certain reports authors explained such type of DNA clevage activity via hydrolytic pathway because nuclease activity was not inhibited by radical scavengers [7]. Later on religation experiment was examined to prove hydrolytic cleavage. If nuclease activity was inhibited by the presence of radical scavengers we could speculate the possible role of reactive oxygen species (ROS) in nuclease activity. Hence we decided to perform first the investigation of nuclease actvity in presence of radical scavengers (Fig. 6).

Involvement of reactive oxygen species (hydroxyl radical, superoxide ion, singlet oxygen and hydrogen peroxide) in nuclease activity could be diagnosed by monitoring the quenching of DNA cleavage in the presence of radical scavengers in solution [5]. The hydroxyl radical scavengers like DMSO, ethanol and urea showed the complete inhibition of nuclease activity (Fig. 6, lane 4–6). These results suggested that hydroxyl radicals may be involved in the cleavage process. Addition of singlet oxygen scavengers like L-histidine and NaN_3 (Fig. 6, lane 9,10) showed complete inhibition of nuclease. So these results suggested that $^1O_2$ or any other singlet oxygen-like entity may participate in the DNA strand scission. However, we did not observed enhancement of nuclease activity in presence of D_2O [18] (Fig. 6, lane 8). Probable participation of hydrogen peroxide was excluded due to enhancement of nuclease activity upon addition of catalase (Fig. 6, lane 7). On the basis of above observations we predict

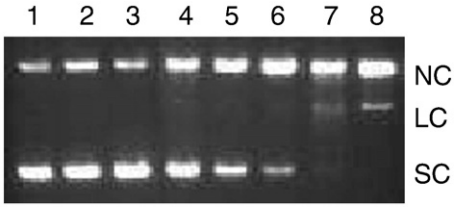

Fig. 5. Gel electrophoresis separations showing the cleavage of supercoiled pBR322 DNA (100 ng) by complex **1** in presence of H_2O_2 and BME. Incubated at 37 °C for 1.5 h. (a) lane 1, DNA control; lane 2, DNA + H_2O_2 (400 μM); lane 3, DNA + BME (400 μM); lanes 4–6, DNA + **1** = 25, 50, 100 μM; respectively, lane 7, DNA + **1** (50 μM) + H_2O_2 (200 μM); lane 8, DNA + **1** (50 μM) + BME (200 μM).

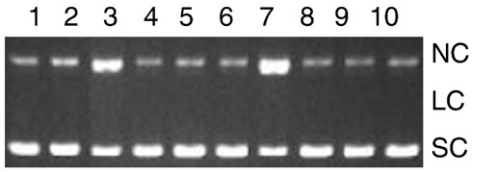

Fig. 6. Gel electrophoresis separations showing the cleavage of supercoiled pBR322 DNA (100 ng) by complex **1** (50 μM) in presence of radical scavengers (20 mM). lane 1, DNA; lane 2, DNA + $Cu(ClO_4)_2{\cdot}6H_2O$ (100 μM); lane 3, DNA + **1** (50 μM); lane 4–10, DNA + **1** (50 μM) + DMSO, ethanol, urea, catalase (1 U), D_2O, L-histidine, NaN_3, respectively.

that complex **1** generated reactive oxygen species (ROS) which were responsible for nuclease activity. Hence we found that **1** is a novel example of a copper complex by which nuclease activity happened via self-activation and to the best of our knowledge the single example of such type of DNA cleaving agent available in the literature was by Reedijk et al. [6]. However, our results derived from machenistic investigation was not similar to the data reported by Reedijk and coworkers.

In conclusion, mononuclear copper(II) complex [Cu(Pyimpy)(Cl)(ClO_4)] (**1**) derived from tridentate ligand Pyimpy has been synthesized and characterized. Molecular structure of complex **1** was determined by X-ray crystallography. DNA interaction by absorption spectral studies showed formation of a new species during interaction. This molecule exhibited nuclease activity in absence of any oxidizing or reducing agents. The cleavage efficiency was dependent both on the complex concentration and on the incubation period. Inhibition of nuclease activity was observed in the presence of radical scavengers and possible role of reactive oxygen species was speculated. Details of mechanistic investigation on this interesting activity of this complex, bilogical activity studies and reactivity studies on other related complexes are currently in progress.

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Appendix A. Supplementary material

The experimental and crystal structure details are in supporting information. The CCDC No. for [Cu(Pyimpy)(Cl)(ClO_4)] is 773744. The data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html. Supplementary data to this article can be found online at doi:10.1016/j.inoche.2011.01.008.

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