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Original article

Picolinic acid based Cu(II) complexes with heterocyclic bases – Crystal structure, DNA binding and cleavage studies

Rabindra Reddy Pulimamidi [a,*], Raju Nomula [a], Raghavaiah Pallepogu [b], Hussain Shaik [a]

[a] Department of Chemistry, Osmania University, Hyderabad 500007, India
[b] School of Chemistry, University of Hyderabad, Hyderabad 500046, India

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ABSTRACT

In view of the importance of picolinic acid (PA) in preventing cell growth and arresting cell cycle, new PA based metallonucleases were designed with a view to study their DNA binding and cleavage abilities. Three new Cu(II) complexes [Cu(II)(DPPA)].4H_2O (**1**),[Cu(II)(DPPA)(bpy)].5H_2O (**2**) and [Cu(II)(DPPA)(-phen)].5H_2O (**3**), were synthesized using a picolinic acid based bifunctional ligand (DPPA) and heterocyclic bases (where DPPA: Pyridine-2-carboxylic acid {2-phenyl-1-[(pyridin-2-ylmethyl)-carbonyl]-ethyl}-amide; bpy: 2, 2′-bipyridine and phen: 1, 10-phenanthroline). DPPA was obtained by coupling 2-picolinic acid and 2-picolyl amine with L-phenylalanine through amide bond. Complexes were structurally characterized by a single crystal X-ray crystallography. The molecular structure of **1** shows Cu(II) center essentially in a square planar coordination geometry, while complex **2** shows an approximate five coordinated square-pyramidal geometry. Eventhough we could not isolate single crystal for complex (**3**), its structure was established based on other techniques. The complex (**3**) also exhibits five coordinate square pyramidal geometry. The complexes show good binding affinity towards CT-DNA. The binding constants (K_b) decrease in the order $1.35 \pm 0.01 \times 10^5$ (**3**) > $1.23 \pm 0.01 \times 10^5$ (**2**) > $8.3 \pm 0.01 \times 10^4$ (**1**) M^{-1}. They also exhibit efficient nuclease activity towards supercoiled pUC19 DNA both in the absence and presence of external agent (H_2O_2). The kinetic studies reveal that the hydrolytic cleavage reactions follow the pseudo first-order rate constant and the hydrolysis rates are in the range of (5.8–8.0) $\times 10^7$ fold rate enhancement compared to non-catalyzed double stranded DNA (3.6×10^{-8} h^{-1}).

1. Introduction

The interaction of metal complexes with DNA is of great interest for the development of artificial endonucleases followed by anticancer drug therapies. DNA offers several potential binding sites for transition metals, including the anionic phosphate backbone, electron-rich bases, and the major or minor grooves [1]. Transition metal complexes can associate with DNA mainly in two types of binding motifs: Covalent and non-covalent binding (intercalation, electrostatic interaction and major/minor groove binding). The well-studied first generation anticancer complex, cisplatin [cis-$Pt(NH_3)_2Cl_2$], binds covalently to the N^7-guanine of DNA, causing a distortion to the structure of DNA double helix leading to serious side effects and cell death [2–5]. Because of this, the usage of cisplatin is limited. Hence, other metals like copper [6–8], and ruthenium [9–11] are now regarded as promising alternatives to platinum in cancer therapy. Copper is most abundant bio-essential element with two oxidation states (+1 and +2) which are important in most aerobic organisms, is employed as a structural and catalytic cofactor, and consequently it is involved in many biological pathways [12]. The coordination flexibility and distortion ability of Cu(II) complexes contribute significantly to the structural diversity, which eventually play a vital role in DNA binding and cleavage.

Nitrogen ligands have been extensively used in coordination chemistry [13,14], especially to obtain derivatives that are able to mimic structural, spectroscopic and catalytic features of active sites of metallo-enzymes [15–18]. As a typical heterocyclic planar ligands, 1,10-phenanthroline and 2,2-bipyridine have attracted attention due to their diverse and wide-ranging antiviral, photochemical and photophysical properties, versatile coordination modes and the potential to form supramolecular aggregates through π…π stacking interactions. Thus, the design of their metal complexes have continuously increased since many of these materials may serve as models which mimic both the structure and reactivity of metal ion sites in complex biological systems and possess a broad spectrum of biological activity [19–21].

* Corresponding author.
E-mail addresses: rabi_pr@rediffmail.com, profprreddy@gmail.com (R. R. Pulimamidi).

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Many useful applications of the complexes require that the complexes bind to DNA through intercalative mode since it is one of the important binding modes that invariably lead to cellular degradation [22]. Considering the prominent role of intercalators in enhancing DNA binding, cleavage and biological applications, we have been focusing our attention in the development of new metallonucleases with increased ligand aromaticities [23–30]. Recently, it was shown that the treatment with picolinic acid disordered the cell growth and arrested cell cycle [31]. Picolinic acid also stimulates programmed cell-death (PCD) in cancer cells and efficiently interrupts the progress of HIV in vitro [32]. In view of this, we have synthesized a picolinic acid based ligand (DPPA), with phenylalanine and picolyl amine [33] and isolated its binary and ternary copper complexes. We reported (Fig. 1) herein the synthesis, characterization and X-ray structure (**1** & **2**). In the absence of crystal structure for (**3**), the geometry was established based on other techniques. Their interaction with CT-DNA was studied by employing various biophysical techniques such as thermal denaturation, electronic absorption, viscosity and fluorescence spectroscopy. The binding constants (K_b) for these complexes were determined ($1.35 \pm 0.01 \times 10^5$ (**3**), $1.23 \pm 0.01 \times 10^5$ (**2**) and $8.3 \pm 0.01 \times 10^4$ (**1**) M^{-1}). The DNA cleavage activity of the complexes was evaluated using gel electrophoresis technique. They bind and cleave DNA efficiently. The DNA hydrolysis rate constants were also determined. The ternary complexes (**2**, **3**) bind and cleave DNA more efficiently compared to binary complex (**1**).

Table 1
Important crystallography data of complexes **1** and **2**.

	1	**2**
CCDC	788099	811860
Formula	$C_{21}H_{18}$ Cu N_4 O_5.50	$C_{31}H_{36}$ Cu N_6 O_7
M	477.93	668.20
Cryst syst	Monoclinic	Monoclinic
T (K)	293(2)	293(2)
Wavelength/Å	0.71073	0.71073
Space group	C 1 2/c 1	P2(1)/c
a/Å	23.382 (5)	11.2413 (7)
b/Å	14.6449 (17)	23.3226 (15)
c/Å	13.904 (2)	13.3537 (9)
α (deg)	90	90
β (deg)	114.38 (2)	114.2790 (10)
γ (deg)	90	90
V/Å^3	4336.6 (12)	3191.4 (4)
Z	8	4
D_c/mgm^{-3}	1.464	1.391
Absorption coefficient/min^{-1}	1.050	0.740
F (000)	1960	1396
Crystal size/mm^3	0.42 × 0.30 × 0.14	
θ range for data collection (deg)	2.78 to 26.37	1.75 to 26.02
Reflections collected	9974	24640
Independent reflections	4435 [*R*(int) = 0.0412]	6258 [*R*(int) = 0.0495]
Completeness to $\theta = 26.37$	99.9	99.6
Max. and min. transmission	0.8670 and 0.6669	0.9297 and 0.8196
Data/restraints/parameters	4435/0/286	6258/9/446
Goodness-of-fit on F^2	0.914	1.262
Largest diff. peak and hole (e Å^{-3})	0.584 and −0.325	0.538 and −0.530

2. Experimental part

2.1. Materials

Picolinic acid, picolyl amine, phenylalanine-methyl ester, DCC (*N*,*N*′-dicyclohexyl carbodiimide), 2, 2′-bipyridine (bpy), 1, 10-phenanthroline (phen), $Cu(OAc)_2.H_2O$, $LiOH.H_2O$ and ethidium bromide (EB) were obtained from Sigma (99.99% purity) USA and were of analar grade. Solvents (MeOH, EtOH, CH_2Cl_2) were purchased from Merck, India. The CT-DNA was obtained from Fluka (Switzerland), supercoiled pUC19 DNA, agarose, tris-base and tris–HCl were obtained from Bangalore Genei (India). The chemicals were used as supplied.

2.2. Methods

Elemental analyses were obtained from the microanalytical Heraeus Carlo Elba 1108 elemental analyzer. The molar conductivity was measured on a Digisun digital conductivity bridge (model: DI-909) with a dip type cell. NMR spectra obtained from Bruker biospin Avance-III 400 MHz spectrometer. Infrared spectra were recorded on a Perkin–Elmer FT-IR spectrometer, in KBr pellets in the 4000–400 cm^{-1} range. Magnetic susceptibilities of the complexes were recorded at room temperature on a Faraday balance (CAHN-7600) using Hg[Co(NCS)$_4$] as the internal standard. Diamagnetic corrections were made by using Pascal's constants [34]. ESI mass spectra of the complexes were recorded using a Quattro Lc (Micro mass, Manchester, UK) triple quadruple mass spectrometer with Mass Lynx software. UV–vis spectra of the complexes were recorded on a Shimadzu 160A spectrophotometer (800–200 nm) and Jasco V-530 UV–**v**is spectrophotometer using 1-cm quartz micro-cuvettes. Gel pattern after the electrophoresis was photographed by Alpha-Innotec gel documentation system (USA).

2.3. X-ray crystallographic procedures

Reddish brown single crystals of **1** and **2** were grown by slow evaporation of reaction solution (aq.MeOH) for a week at room temperature. A crystal of the complex was mounted on a glass fiber and used for data collection. Crystal data were collected at 293 K, using Bruker-Nanious SMART APEX CCD single crystal diffractometer equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube ($\lambda = 0.71073$ Å) operated at 2.0 kW. The detector was placed at a distance of 6.0 cm from the crystal. Data were collected with a scan width of 0.3° in ω and an exposure time of 15 s/frame. The SMART software was used for data acquisition and

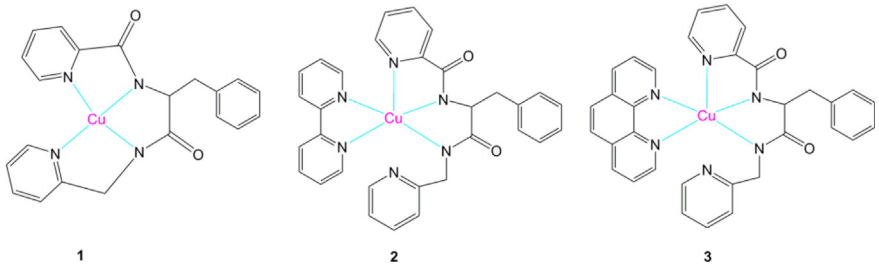

Fig. 1. Proposed structures of the complexes **1**–**3**.