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Oxidative DNA cleavage, cytotoxicity and antimicrobial studies of L-ornithine copper (II) complexes

P.R. Chetana [a,*], Ramakrishna Rao [a], Sounik Saha [b], R.S. Policegoudra [c], P. Vijayan [d], M.S. Aradhya [e]

[a] Department of Chemistry, Central College Campus, Bangalore University, Bangalore 560001, India
[b] Department of Inorganic & Physical Chemistry, Indian Institute of Science, Sir C.V. Raman Avenue, Bangalore 560012, India
[c] Department of Biotechnology, Defense Research Laboratory, Tezpur 784001, India
[d] JSS College of Pharmacy, Ooty 643001, India
[e] Department of Fruit and Vegetable Technology, CFTRI, Mysore 570020, India

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ABSTRACT

New ternary copper (II) complexes, [Cu(L-orn)(B)(Cl)](Cl·2H_2O) (**1**–**2**) where L-orn is L-ornithine, B is an *N*,*N*-donor heterocyclic base, viz. 2,2′-bipyridine (bpy, **1**) and 1,10-phenanthroline (phen, **2**), were synthesized and characterized by various spectroscopic techniques. Complex **2** is characterized by the X-ray single crystallographic method. The complex shows a distorted square-pyramidal (4 + 1) CuN_3OCl coordination sphere. Binding interactions of the complexes with calf thymus DNA (CT-DNA) were investigated by UV–Vis absorption titration, ethidium bromide displacement assay, viscometric titration experiment and DNA melting studies. Complex **2** shows appreciable chemical nuclease activity in the presence of 3-mercaptopropionic acid (MPA). The complexes were subjected to *in vitro* cytotoxicity studies against carcinomic human alveolar basal epithelial cells (A-549) and human epithelial (HEp-2) cells. The IC_{50} values of **1** and **2** are less than that of cisplatin against HEp-2 cell lines. MIC values for **1** against the bacterial strains *Streptococcus mutans* and *Pseudomonas aeruginosa* are 0.5 mM.

1. Introduction

Metal complexes constitute a growing field in drug design and have been considered as promising antitumor agents in recent decades by the virtue of their unique spectroscopic and electrochemical signatures. The interest in the role of metal complexes in cancer therapy was triggered by the discovery of the potent epithelial ovarian cancer drug cisplatin [1]. Metal complexes with tunable coordination environments and versatile physicochemical properties offer scope for designing and developing highly sensitive diagnostic agents for medicinal applications [2–17]. Metal complexes with polypyridyl phenanthroline bases have attracted great attention by virtue of their binding propensity to nucleic acids under physiological conditions. In addition to the rich coordination chemistry of the metal ions, they have great potential in constructing metal complexes with diverse structures and redox potentials [18–22]. Coordination compounds of the bioessential element copper have been extensively used in metal mediated DNA cleavage through the generation of hydrogen abstracting activated oxygen species (ROS). Sigman et al. investigated the chemical nuclease activity of a bis-(1,10-phenanthroline) copper (I) complex, which on activation with H_2O_2 induced oxidative strand scission [2]. The active oxo species attack the deoxyribose sugar proton of the nucleotide, which is in the vicinity of the copper (I) bisphen species in the minor groove, oxidatively initiating a series of free radical chain reactions to induce DNA strand scission [23,24]. The strong binding affinity of the copper bis phen complex and the redox behavior of the copper center play an important role in inducing oxidative DNA cleavage. Chakravarty and co-workers recently explored amino acid transition metal based chemistry towards cleavage of DNA under physiological conditions by oxidative as well as photochemical means on charge transfer or d–d band excitation [25–39]. The copper complex of L-lysine structure is closely resembles the copper–ornithine structure, which shows efficient chemical nuclease activity [25]. The present work stems from our interest to explore chemical nuclease activity, cytotoxicity against A-549 and HEp-2 cancer cells and antimicrobial activities of L-ornithine copper (II) complexes.

Compounds with α-amino acids containing a terminal amine moiety easily penetrate through cell walls due to their ionic character. L-Ornithine is a non-protein, basic amino acid and it is most potent for stimulating, production and release of growth hormones, in maintaining arterial flexibility and defeating hypertension [40–42]. Ornithine decarboxylase activity is higher in rapidly growing tumors than in non-malignant tumors [43–45]. This activity may be reduced by chelating the carboxylate group of ornithine. Due to these activities of L-ornithine, we were inter-

* Corresponding author. Tel./fax: +91 80 22961353.
E-mail address: pr.chetana@gmail.com (P.R. Chetana).

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ested in exploring the chemical nuclease activity of L-ornithine copper complexes.

Herein, we report the two new ternary copper (II) complexes [Cu(L-orn)(B)(Cl)](Cl·2H_2O) (**1**–**2**), where L-orn is L-ornithine, B is *N*,*N*-donor heterocyclic base, viz. 2,2′-bipyridine (bpy, **1**) and 1,10-phenanthroline (phen, **2**), which were synthesized and characterized by various spectroscopic techniques. Complex **2** is characterized by single X-ray crystallographic method. Studies have been made to explore the role of a DNA binder and amino acid with a terminal amine group along with the mechanistic pathways involved in the chemical nuclease activity.

2. Experimental

2.1. Materials and methods

The reagents and chemicals were purchased from commercial sources and used as received without further purification. The solvents used were purified by standard procedures [46]. Supercoiled (SC) pUC19 DNA (cesium chloride purified) was purchased from Bangalore Genie (India). CT-DNA, agarose (molecular biology grade), distamycin-A, catalase, superoxide dismutase (SOD) and ethidium bromide (EB) were from Sigma (USA). Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer was prepared using deionized and sonicated triple distilled water. The elemental analysis was done using a Thermo Finnigan FLASH EA 1112 CHNS analyzer. The infrared and electronic spectra were recorded on Perkin Elmer Lambda 35 and Perkin Elmer spectrum one 55 spectrophotometers respectively at 25 °C. Magnetic susceptibility data for polycrystalline samples of the complexes were obtained using a Model 300 Lewis-coil-force magnetometer of George Associates Inc. (Berkeley, USA) make and Hg[$Co(NCS)_4$] was used as a standard. Experimental susceptibility data were corrected for diamagnetic contributions [47]. Molar conductivity measurements were carried out using a Control Dynamics (India) conductivity meter. Cyclic voltammetric measurements were made at 25 °C on a EG&G PAR 253 Versastat potentiostat/galvanostat using a three electrode configuration consisting of a glassy carbon working, a platinum wire auxiliary and a saturated calomel reference (SCE) electrode.

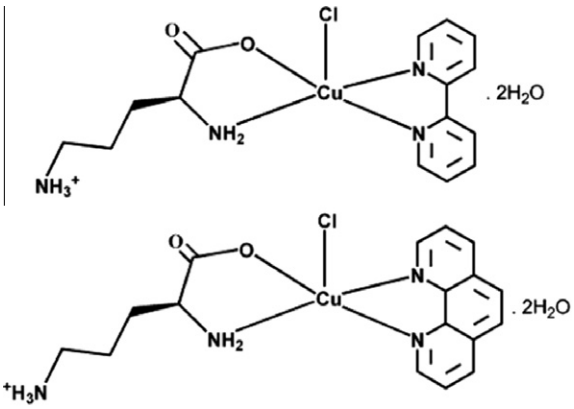
2.2. Synthesis

2.2.1. Preparation of [Cu(L-orn)(B)(Cl)](Cl) (**1**–**2**) (L-orn = L-ornithine and B = bpy, **1**; phen, **2**)

An aqueous solution of $CuCl_2·3H_2O$ (0.48 g, 2 mM) was reacted with L-ornithine which was pre-treated with NaOH (0.08 g, 2.0 mM) in water (10 mL) and stirred for 2 h at room temperature. A 20 mL methanolic solution of the heterocyclic base [bpy (0.28 g), phen (0.35 g)] was added dropwise using a syringe at room temperature. The solid that formed was filtered and the filtrate, on slow concentration, yielded a blue crystalline solid of the complexes (Scheme 1).

Anal. Calc. for $C_{15}H_{24}CuN_4O_4Cl_2$ (**1**): C, 39.23; H, 5.23; N, 12.20. Found: C, 39.64; H, 5.24; N, 12.73%. FT-IR, cm^{-1} (KBr disc): 3107br, 3051w, 1608s, 1560m, 1494m, 1471s, 1442vs, 1315s [br, broad; vs, very strong; s, strong; m, medium; w, weak]. UV–Vis in water [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 312 (7590), 588 (75). Magnetic moment at 298 K [μ_{eff}/BM]: 1.56. Λ_M (Ω^{-1} cm^2 M^{-1}) in water at 25 °C: 143. ESI-MS in methanol *m*/*z*: 417.41 $[M-Cl]^+$.

Anal. Calc. for $C_{17}H_{24}CuN_4O_4Cl_2$ (**2**): C, 42.36; H, 4.99; N, 11.63. Found: C, 42.32; H, 4.94; N, 11.61%. FT-IR, cm^{-1} (KBr disc): 3252br, 3446br, 3247m, 3128w, 1172w, 1622vs, 1514m, 1421m, 848m, 721m. Magnetic moment at 298 K [μ_{eff}/BM]: 1.79. UV–Vis in water [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 789 (13), 626 (72), 610 (65), 376 (903), 232 (1356), 208 (1168). Λ_M (Ω^{-1} cm^2 M^{-1}) in water at 25 °C: 137. ESI-MS in methanol *m*/*z*: 443.44 $[M-Cl]^+$.



Scheme 1. Schematic representation of complexes **1** and **2**.

2.3. X-ray crystallographic procedures

Single crystals of **2** were grown by slow evaporation of water/methanol mixture. A block shaped single crystal was mounted on a glass fiber with epoxy cement. The X-ray diffraction data were measured in frames with increasing ω (width of 0.3° per frame) and with a scan speed of 15 s/frame on a Bruker SMART APEX CCD diffractometer, equipped with a fine focus 1.75 kW sealed tube X-ray source. Empirical absorption corrections were carried out using the multi-scan program [48]. The structure was solved by the heavy atom method and refined by full matrix least-squares using the SHELX system of programs [49]. All non-hydrogen atoms were refined anisotropically and the hydrogen atoms were refined isotropically. The hydrogen atoms attached to the hetero atoms were in their calculated positions and refined according to the riding model. The perspective view of the complex was obtained by ORTEP [50].

2.4. DNA binding and cleavage experiments

DNA binding experiments were performed in Tris–HCl/NaCl buffer (5 mM Tris–HCl, 5 mM NaCl, pH 7.2) using an aqueous solution of the complexes. CT-DNA (ca. 250 μM NP) in Tris–HCl buffer medium gave a ratio of the UV absorbances at 260 and 280 nm of ca.1.9:1, indicating the purity of DNA which is apparently free from protein [51]. The concentration of DNA was calculated from its absorption intensity at 260 nm with the known molar absorption coefficient value of 6600 M^{-1} cm^{-1} [52]. UV–Vis absorption titration experiments were performed by varying the concentration of CT-DNA keeping the metal complex concentration constant (50 μM) with due correction for the absorbance of CT-DNA itself. Samples were allowed to get equilibrated to bind sufficiently to CT-DNA before recording each spectrum. The intrinsic equilibrium binding constant (K_b) and the binding site size (*s*) of the complex were determined from a non-linear fitting of the plot of $\Delta\varepsilon_{af}/\Delta\varepsilon_{bf}$ versus [DNA] using the McGhee–von Hippel (MvH) method. The expression of Bard and coworkers: $\Delta\varepsilon_{af}/\Delta\varepsilon_{bf} = (b - (b^2 - 2K_b^2C_t[DNA]/s)^{1/2})/2K_b$, $b = 1 + K_bC_t + K_b[DNA]/2s$ was used to evaluate K_b and *s*, where K_b is the microscopic equilibrium binding constant for each site, C_t is the total concentration of the metal complex, *s* is the site size of the metal complex interacting with the DNA, [DNA] is the concentration of DNA in nucleotides, ε_f, ε_a and ε_b are respectively the molar extinction coefficients of the free complex in solution, complex bound to DNA at a definite concentration and the complex in the completely bound form with CT-DNA