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Original article

Two new neutral copper(II) complexes with dipicolinic acid and 3-amino-1*H*-1,2,4-triazole formed under different reaction conditions: Synthesis, characterization, molecular structures and DNA-binding studies

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ABSTRACT

Two Cu(II) complexes, $[Cu_2(\mu\text{-atr})(pydc)_2(H_2O)_4]\cdot 5H_2O$ (**1**) and $[Cu(atr)(pydc)(H_2O)]\cdot H_2O$ (**2**), with pyridine-2,6-dicarboxylic acid (H_2pydc) and 3-amino-1*H*-1,2,4-triazole (atr), have been synthesized and characterized. The interaction ability of the both complexes with native calf thymus DNA (CT-DNA) has been monitored as a function of the metal complex-DNA molar ratio. UV–vis spectrophotometry, circular dichroism (CD), thermal denaturation studies, cyclic voltammetry (CV) and viscosity measurements. The intrinsic binding constants K_b of complexes **1** and **2**, with CT-DNA obtained from UV–vis absorption studies were $4.7\,(\pm 0.1)\times 10^4$ and $9.5\,(\pm 0.1)\times 10^4\ M^{-1}$, respectively. Further investigation of interaction mode was performed using viscosity, cyclic voltammetry and T_m of CT-DNA studies as well as CD study, indicating complexes bind to DNA via an intercalation mode.

1. Introduction

Copper is a cofactor essential for the tumor angiogenesis processes [1]. Among the metal complexes, copper (II) complexes containing heterocyclic bases have been developed as a result of their diverse applications following the discovery of the "chemical nuclease" activity of the $[Cu(phen)_2]^{2+}$ complex [2]. Furthermore, copper complexes have shown strong interactions with DNA via surface associations or intercalation [3]. The factors that may affect their association with DNA include their size, the ligands, and the presence and the position of small lipophilic groups. On the other hand, copper complexes having a strong association with DNA are also capable of inducing a hydrolytic cleavage [4], while several copper-based synthetic nucleases have been reported [5–8]. Their importance is due to their capacity to cleave DNA via oxidative mechanisms resulting from copper's ability to adopt different oxidation states.

Heterocyclic ligands containing 1,2,4-triazole rings have attracted interest as versatile ligands with a variety of coordination modes with transition metal centers. 1,2,4-triazoles can bridge between two metal ions, yielding bi- or multi-nuclear species and abundant structural motifs [9–12]. In addition, 1,2,4-triazole derivatives display a broad range of biological activity, finding application as antimicrobial, antifungal, anticonvulsants, antiviral and antitumor agents [13–16].

In continuation of our research on synthesis of the transition metals complexes with pyridine-2,6-dicarboxylic acid (H_2pydc) in the presence of some amino compounds [17–22], here we present the preparation, spectroscopic characterization, thermal properties, crystal structure and DNA interaction of the first two Cu(II) complexes with pyridine-2,6-dicarboxylic acid (pydc) and 3-amino-1*H*-1,2,4-triazole (atr), $[Cu_2(\mu\text{-atr})(pydc)_2(H_2O)_4]\cdot 5H_2O$ (**1**) and $[Cu(atr)(pydc)(H_2O)]\cdot H_2O$ (**2**). To the best of our knowledge, there is

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no report in the literature of any copper(II) complex with mixed pyridine-2,6-dicarboxylate and 3-amino-1,2,4-triazole ligands.

2. Experimental section

2.1. Materials

The reagents and chemicals were purchased from commercial sources and used as received without further purification. Calf thymus DNA (CT-DNA) was obtained from Sigma. The stock solution of CT-DNA gave a ratio 1.8–1.9 in UV absorbance at 260 and 280 nm (A_{260}/A_{280}) to check DNA purity, indicating that the DNA was sufficiently free of protein contamination [23]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ($\varepsilon = 6600\ M^{-1}\ cm^{-1}$ at 260 nm) [24,25]. The stock solutions were stored at 5 °C and used over no more than 4 days. All the experiments involving interactions of the compounds with DNA were carried out in doubly distilled water buffer containing 5 mM Tris–HCl [tris (hydroxymethyl)-aminomethane] and 50 mM NaCl, and adjusted to pH 7.4 using hydrochloric acid.

2.2. Physical measurements

The UV–vis spectra were recorded on a Varian Cary 100 UV–vis spectrophotometer using a 1 cm path length cell. Infrared spectra (4000–400 cm^{-1}) of solid samples were taken as 1% dispersions in KBr pellets using a FTIR Spectra Bruker Tensor 27 spectrometer. TGA/DTA measurements were performed at heating rate of 10 °C min^{-1} in the temperature range of 25–800 °C, under nitrogen flow of 20 mL min^{-1} on instrument Rheometrics STA 1500. Elemental analyses were performed using a Costech ECS 4010 CHNS analyzer. The T_m spectra were recorded on a Varian BioCary-100 UV–vis spectrophotometer using a 1 cm path length cell. Circular dichroism measurements were carried out on a Jasco-810 spectropolarimeter at room temperature with a rectangular quartz cell of 1 cm path. Cyclic voltammetry experiments with a conventional three-electrode electrochemical cell, and using a Metrohm Autolab Potentiostats/Galvanostats. The three-electrode system used in this work consists of a gold electrode as working electrode, an Ag/AgCl reference electrode and a Pt foil auxiliary electrode. The solution was prepared by dissolving the complex in water buffer containing 5 mM Tris–HCl (pH 7.3) and 50 mM NaCl concentrations. The viscosity was determined using a digital Brookfield Circulating Bath Ultra DV III Viscometer maintained at 25.0 ± 0.1 °C in a circulating water-bath. Data were analyzed as $(\eta/\eta_0)^{1/3}$ versus ratio of the concentration of Cu (II) complex and DNA, where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone.

2.3. Synthesis of complexes **1** and **2**

Preparation of $[Cu_2(atr)(pydc)_2(H_2O)_4]\cdot 5H_2O$ (**1**): pyridine-2,6-dicarboxylic acid (0.167 g, 1 mmol) and sodium hydroxide (0.08 g, 2 mmol) were dissolved in distilled water (15 mL) and stirred for 30 min at room temperature. 3-Amino-1*H*-1,2,4-triazole (0.084 g, 1 mmol) and $Cu(NO_3)_2\cdot 6H_2O$ (0.241 g, 1 mmol) were added to above solution and the resulting mixture was refluxed for 8 h. The reaction mixture was filtered and the filtrate was kept at 4 °C to give blue single crystals suitable for X-ray diffraction studies. Anal. Calcd. for $C_{16}H_{26}Cu_2N_6O_{17}$: C, 31.70; H, 2.53; N, 16.27%. Found: C, 32.40; H, 2.34; N, 16.59%. IR (KBr) (cm^{-1}): 3080–3400 (b), 1653 (s), 1430 (s), 1375 (s), 1345 (s), 1267 (m), 1178 (m), 1082 (m), 911 (w), 770 (m), 741 (m), 678 (m).

Preparation of $[Cu(atr)(pydc)(H_2O)]\cdot H_2O$ (**2**): pyridine-2,6-dicarboxylic acid (0.167 g, 1 mmol) and sodium hydroxide (0.08 g, 2 mmol) were dissolved in water (15 mL) and stirred for 30 min at room temperature. 3-Amino-1*H*-1,2,4-triazole (0.084 g, 1 mmol) and $Cu(NO_3)_2\cdot 6H_2O$ (0.241 g, 1 mmol) were added to the above solution. The reaction mixture was placed in a Parr-Teflon lined stainless steel vessel, sealed and heated at 130 °C for 8 h. The reaction mixture was filtered off, and the filtrate was kept at 4 °C to obtain purple single crystals suitable for X-ray diffraction studies. Anal. Calcd. for $C_9H_{11}CuN_5O_6$: C, 30.96; H, 3.15; N, 20.07%. Found: C, 30.74; H, 3.04; N, 19.99%. IR (KBr) (cm^{-1}): 3290–3490 (b), 1682 (s), 1630 (s), 1582 (s), 1427 (s), 1363 (s), 1345 (s), 1278 (m), 1078 (m), 778 (m), 685 (m).

2.4. Single-crystal structure determination

The selected crystals of complexes **1** and **2** were covered with perfluorinated oil and mounted on the top of a glass capillary under a flow of cold gaseous nitrogen. The orientation matrix and the unit cell dimensions were determined from 6000 (**1**, Stoe IPDS I) and 6100 (**2**, Stoe IPDS I) reflections (graphite-monochromated Mo-Kα radiation ($\lambda = 71.073$ pm)). The intensities were corrected for Lorentz and polarization effects. In addition, absorption corrections were applied for all compounds (numerical). The structures were solved by direct methods for all compounds using SHELXS-97 for complex **1** and SIR-92 for complex **2** and were refined against F2 by full-matrix least-squares using the program SHELXL-97. The hydrogen atoms H1–H11 were calculated for ideal positions and were refined with a common displacement parameter, while the hydrogen atoms in complex **2** were freely refined atoms Programs used were SIR-92 [26], SHELXL-97 [27], SHELXTL-Plus [28] and PLATON [29]. **1** was refined as twin with the X-Area software of Stoe (0.56/0.44). In addition, the central heterocyclic is disordered around a C2-axis. This means that C8 and N3 are occupied 0.5/0.5 in Fig. 1. The crystal parameters, data collection and refinement results for **1** and **2** are summarized in Table 1.

3. Results and discussion

3.1. Characterization of complex

The treatment of $Cu(NO_3)_2\cdot 3H_2O$ with pyridine-2,6-dicarboxylic acid in the presence of 3-amino-1*H*-1,2,4-triazole and sodium

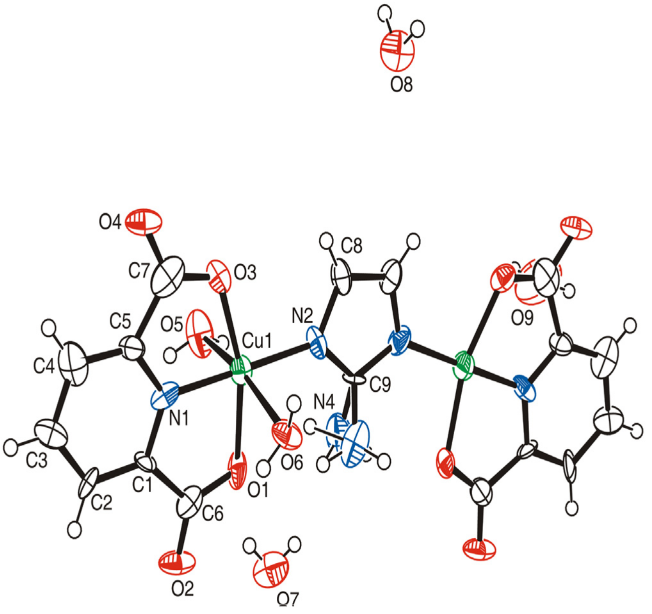

Fig. 1. Molecular structure of **1** (40% probability level).