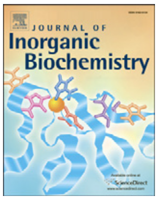
Journal of Inorganic Biochemistry 125 (2013) 50–63

Contents lists available at SciVerse ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio



Two copper complexes from two novel naphthalene-sulfonyl-triazole ligands: Different nuclearity and different DNA binding and cleavage capabilities

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ARTICLE INFO

Article history:
Received 25 October 2012
Received in revised form 12 April 2013
Accepted 12 April 2013
Available online 24 April 2013

Keywords:
1,2,4-Triazole ligands
Cu(II)-triazole complexes
Paddle wheel type copper acetate compound
DNA interaction
Nuclease activity

ABSTRACT

Two novel naphthalene-sulfonyl-triazole ligands, 5-amino-*N*1-(naphthalen-3-ylsulfonyl)-1,2,4-triazole (anstrz) and 3,5-diamino-*N*1-(naphthalen-3-ylsulfonyl)-1,2,4-triazole (danstrz), purposely designed to interact with DNA, have been prepared for the first time and then fully characterized by ^{1}H, ^{13}C NMR, and IR spectroscopy, mass spectrometry and elemental analysis. The crystal structures of two copper complexes of these derivatives, i.e. $[Cu(anstrz)_4(NO_3)_2]\cdot 4CH_3OH$ (**1**), mononuclear, and $[Cu(danstrz)(\mu\text{-}OAc)_2]_2\cdot 2(danstrz)$ (OAc = acetato) (**2**), dinuclear, have been determined by single-crystal X-ray diffraction. In both cases the ligand coordinates in a monodentate fashion *via* the *N*4 nitrogen atom of the triazole ring. Compound **2**, an example of *paddle wheel* type copper acetate, presents a Cu ··· Cu′ distance of 2.667(1) Å. As a result of strong stacking interactions and intense H-bonds, the structure of **2** constitutes a MOF (metal–organic framework). Besides, this dinuclear compound exhibits a very strong antiferromagnetic coupling ($J = -324$ cm^{-1}) and a silent X-band EPR at room temperature. The affinity toward DNA of **1** and **2** has been examined by fluorescence emission spectroscopy, thermal denaturation and viscosimetry assays. The apparent binding constant (K_{app}) values of 2.2×10^7 M^{-1} for **1** and 2.6×10^7 M^{-1} for **2** suggest important DNA interaction. The dinuclear compound (**2**) intercalates and produces a high change in the T_m. Both compounds promote DNA scission in the presence of H_2O_2/ascorbate (**1**) or ascorbate (**2**) through oxidative mechanism. The possible reasons for the higher DNA affinity and the more efficient DNA cleavage displayed by compound **2** in relation to compound **1** are discussed.

1. Introduction

The rational design of species capable of interacting with DNA in a specific way, in order to produce a controlled chemical cleavage, is an area of great interest due to its potential application as tools to manipulate the genetic material and as agents in chemotherapy. Transition metal complexes have been widely exploited for these purposes, not only because of their unique spectral and electrochemical signatures, but also because by changing the ligand environment the DNA binding and cleaving ability of a metal complex can be tuned [1–6]. Copper being a bio-essential transition metal ion, its complexes have been frequently used to obtain DNA cleavage agents in view of their biologically accessible redox potential and their relatively high affinity for nucleobases. Usually Cu(II) complexes under the proper conditions produce DNA scission by an oxidative pathway [1–4,7–10].

The ligand plays a crucial role in the design of the metallonucleases. The reactivity of the artificial nuclease is strongly enhanced when its structure comprises groups with high DNA affinity. Strategies which have shown to be effective to reach such goal are [1–3]: (i) the use of bimetallic agents, (ii) the introduction of functional groups positively charged, and (iii) the conjugation with DNA binding units such as groove binders or intercalators. Last feature taken into account, the planarity of the ligand and/or the presence of aromatic systems are factors that favor the intercalation; the design of these systems, however, is not trivial [1,2]. Sometimes the conjugation with an intercalator does not produce the results expected, since the advantage derived by the increased DNA affinity is canceled by the incorrect positioning of the reactive group [11–13]. The nature of the intercalator is also important; in this sense, J.K. Barton et al. and Burstyn et al., among other authors, have reported substantial differences in its reactivity [14,15]. As recent effort in this direction,

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http://dx.doi.org/10.1016/j.jinorgbio.2013.04.007

Kumbhar and co-workers have prepared Cu(II) complexes with naphthalene–phenanthroline ligands which intercalate, cleave plasmid pBR322 DNA and are highly active and selective toward cancer cells [9]. Kandaswamy et al. in a last study with a series of macrocyclic dinuclear Zn(II) complexes have shown that the introduction and extension of the aromatic moiety led to considerable changes in DNA binding mode. They concluded that the stacking interaction exhibited by the naphthalene fragment in one of the complexes is responsible for its stronger DNA intercalative binding and more effective DNA cleavage; furthermore, the naphthalene fragment seems to facilitate the transport of the complex across the cell membrane [16–20].

The coordination chemistry of 1,2,4-triazole ligands has been intensively investigated in the context of design and synthesis of novel coordination compounds with unique functionalities such as magnetic properties, spin-crossover, optic, sorption etc. [21–34]. The interest in these ligands often derives from their versatility to bridge metal centers in different motifs [21–23]. However, an area yet to explore is the use of the triazole systems as a way of obtaining metal complexes able of interacting with DNA and producing DNA cleavage. The potential of the triazole systems in the construction of metallonucleases lies in three aspects: (i) the 1,2,4-triazole ring resembles the biological imidazole ring; (ii) they exhibit the mentioned extensively documented ability to bridge metal ions to afford polynuclear compounds; (iii) diverse functional pieces (i.e. planar aromatic rings, hydrophobic/hydrophilic groups,…) can be easily introduced into the molecule as substituent of the triazole nucleus [21–23]. Few articles have been devoted to obtain copper-triazole complexes with nucleolytic capability. The dinuclear copper(II) triazole $[Cu_2L(\mu\text{-}SO_4)](PF_6)_2$ {L = 3,5-bis[bis(pyridine-2-ylmethyl)amino)methyl]-4-amino-1,2,4-triazole} complex has been described by Yan et al. to promote single and double strand DNA cleavage in both aerobic and anaerobic conditions [35]. Dalavalle et al. have reported on the antiproliferative activity toward neoplasic cells of a couple of mononuclear copper-triazole complexes of the ligands 4-amino-3-(2-pyridyl)-5-thioxo-1,2,4-triazole and 4-amino-5-methylthio-3-(2-pyridyl)-1,2,4-triazole [36]. For the last years our group has focused on the development of new copper-metallonucleases from 3,5-disubstituted-1,2,4-triazole ligands. We have reported a pair of related copper-triazole monomeric complexes, $[Cu(Hapt)]^{2+}$ and $[Cu(Hapt)_2]^{2+}$ (Hapt = 5-amino-3-pyridyl-1,2,4-triazole), which showed differences in their activity [37] and, most recently, the tetranuclear compound $[Cu_4(atc)_2(dien)_2(ClO_4)_2](ClO_4)_2 \cdot 2H_2O$ (H_2atc = acid 5-amino-l,2,4-triazole-3-carboxylic, dien = diethylenetriamine), which exhibited high affinity toward DNA due to favorable electrostatic interactions, and a good efficiency as nuclease [38]. Now basically we tried to expand the nucleolytic possibilities of these systems by incorporating new structural features in the basic 1,2,4-triazole ring.

In this work we have synthesized two new triazole ligands, 5-amino-*N*1-(naphthalen-3-ylsulfonyl)-1,2,4-triazole (**anstrz**) and 3,5-diamino-*N*1-(naphthalen-3-ylsulfonyl)-1,2,4-triazole (**danstrz**), which combine triazole, sulfonyl and naphthalene fragments. The naphthalene moiety has been integrated in the structure, as substituent of the triazole *via* sulfonylation, with the aim of favoring the intercalation of the compounds into the DNA. The sulfonyl group is related with the sulfonamide group, for which a wide family of metallonucleases has been reported by Borrás, Alzuet and co-workers [12,39–43]. By reaction of these ligands with copper(II) salts, two new complexes, one mononuclear, $[Cu(anstrz)_4(NO_3)_2]$ (**1**) and one dinuclear, $[Cu(danstrz)(\mu\text{-}OAc)_2]_2 \cdot 2(danstrz)$ (OAc = acetato) (**2**), have been isolated. The two new ligands and the two complexes have been fully characterized. The special structural and magnetic characteristics of **2** have been analyzed in the context of related acetato complexes. Then the DNA binding and nuclease properties of **1** and **2** are studied. Finally, the consequences both of the inclusion of the aromatic moiety and of the different nuclearity in the DNA affinity and cleavage properties will be discussed.

2. Experimental

2.1. Materials and methods

Copper salts, other chemicals and solvents were commercially available (Aldrich and Fluka) in high purity and used as received unless otherwise indicated. Plasmid pUC18 (0.5 μg/μL, 1500 μM in nucleotides) in TE (Tris 10 mM and EDTA 1 mM, pH = 8.0) was purchased from Fermentas. Calf thymus DNA (CT-DNA), type XV, was obtained from Sigma. Solutions of the metal complexes and other reagents for strand scission experiments were prepared fresh daily.

Elemental analyses for carbon, nitrogen, hydrogen and sulfur were performed with a CE Instrument EA 1110 CHNS analyzer. Infrared spectra were recorded as KBr disks using a Mattson Satellite FTIR spectrophotometer from 4000 to 400 cm^{-1}. UV–visible (UV–vis) spectra were recorded in 1 cm quartz cuvettes with an HP 8453 spectrophotometer. Fast atom bombardment mass spectrometry (FAB-MS) in positive mode spectra were performed on VG Analytical Micromass Instruments equipment. Low-resolution electrospray ionization mass spectrometry (ESI-MS) in positive mode spectra was performed on a Bruker Esquire 3000 plus LC–MS system. EPR spectra of molten crystals were collected with a Bruker ELEXSYS spectrometer operating at X-band frequency in the temperature range 10–300 K. Magnetic susceptibility measurements on polycrystalline samples were carried out with a superconducting quantum interference design (SQUID) magnetometer in the temperature range 1.9–300 K. Diamagnetic corrections of the constituent atoms were estimated from Pascal's constants. Experimental susceptibilities were also corrected for the temperature-independent paramagnetism [60×10^{-6} cm^3 mol^{-1} per copper(II)] and for the magnetization of the sample holder.

2.2. Synthesis and characterization of the ligands

2.2.1. Synthesis

*5-Amino-N1-(naphthalen-3-ylsulfonyl)-1,2,4-triazole (**anstrz**) and 3,5-diamino-N1-(naphthalen-3-ylsulfonyl)-1,2,4-triazole (**danstrz**)*

2-Naphthalenesulfonyl chloride (60 mmol, 13.5 g) was slowly added to a solution containing 5-amino-1,2,4-triazole (60 mmol, 5.04 g) (for **anstrz** synthesis) or 3,5-diamino-1,2,4-triazole (60 mmol, 5.95 g) (for **danstrz** synthesis) and pyridine (60 mmol, ρ = 0.978 g/mL, 4.85 mL) in 100 mL of acetonitrile (dry) under a N_2 atmosphere at 55 °C. Then, the temperature reaction was raised to 85 °C and the mixture stirred for 16 h. Afterwards, the mixture was brought to room temperature and later on placed in an ice bath for 1 h. The solid was filtered, washed with water and dichloromethane, and dried to constant weight.

2.2.2. Characterization

*5-Amino-N1-(naphthalen-3-ylsulfonyl)-1,2,4-triazole (**anstrz**):*

Yield: 14.16 g, 86%. Anal. Calc for $C_{12}H_{10}N_4SO_2$ (274.30): N, 20.43; C, 52.54; S, 11.69; H, 3.67%. Found: N, 20.07; C, 52.60; S, 11.59; H, 3.84%. Selected *FT*-IR data (KBr pellet) $\tilde{\nu}_{max}$ (cm^{-1}): [$\nu(N-H) + \nu(arC-H)$] 3465 m, 3302 w, 3227 w, 3112 m; [$\delta(N-H) + \nu(C{=}N)_{ring} + \nu(C{=}C)_{ring}$] 1693 sh, 1651 vs, 1571 m, 1521 s; [$\nu(SO_2)_{asym}$] 1383 s; [$\nu(SO_2)_{sym}$] 1179 vs. UV (DMSO): λ_{max} (nm) [ε_{max}] (M^{-1} cm^{-1}): 270 [5300]. FAB^+ mass spectra (methanol) (m/z): 275.4 $[M + H]^+$. ^1H RMN (300 MHz) (DMSO-d_6, δ/ppm): 8.8 (s, 1H, napht); 8.2 (d, 1H, *J* = 7.9 Hz, napht); 8.2 (d, 1H, *J* = 8.8 Hz, napht); 8.1 (d, 1H, *J* = 8.0 Hz, napht); 7.9 (dd, 1H, *J* = 6.8 and 2.0 Hz, napht); 7.8–7.7 (m, 2H, napht); 7.6 (s, 1H, CH-3trz); 7.4 (s, 2H, NH_2).^{13}C RMN (75 MHz) (DMSO-d_6, δ/ppm): 152.6 (1C, C-5trz); 135.1, 133.1, 131.3, 130.1, 130.1, 129.6, 129.6, 128.1, 127.9, 121.5 (10C, napht); C-3trz not observed. Solubility properties: water (low); ethanol (low); methanol (low); DMF (*N*,*N*-dimethylformamide) (soluble); DMSO (soluble).