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Research paper

Cu(II) complexes with hydrazone-functionalized phenanthrolines as self-activating metallonucleases

Julian Heinrich, Jessica Stubbe, Nora Kulak *

Institut für Chemie und Biochemie, Freie Universität Berlin, Fabeckstraße 34/36, 14195 Berlin, Germany

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ABSTRACT

Cu(II) phenanthroline complexes are able to cleave DNA and show cytotoxic activity against cancer cells. Also, the biological activity of hydrazone-based compounds has been reported in the literature. This motivated us to combine these two systems. Hydrazone-functionalized phenanthroline ligands with acetyl and benzoyl substituents were synthesized and characterized. The DNA cleavage activity of the corresponding Cu(II) complexes **3** and **4** was compared to the one of the well-known $[Cu(phen)_2]^{2+}$ complex (**1**) and $[Cu(phenD)_2]^{2+}$ complex (**2**, phenD = 1,10-phenanthroline-5,6-dione, the precursor of hydrazone-functionalized phenanthrolines). In the presence of ascorbate, but also in its absence, oxidative cleavage of DNA was proven by quenching therein involved reactive oxygen species (ROS), specifically hydrogen peroxide and superoxide anion radicals. Only complexes **2**–**4** were active in the absence of ascorbate, but not $[Cu(phen)_2]^{2+}$ (**1**). The surprising occurrence of ROS in the absence of any activating agent indicates that **2**–**4** represent self-activating artificial metallonucleases. Thereby, self-activation was most prominent for the novel hydrazone-based complexes **3** and **4**. Cyclic voltammetry and circular dichroism spectroscopy were applied to get an insight in redox behavior and DNA binding characteristics of the Cu(II) complexes and thus to explain their different nuclease activity.

1. Introduction

In the last few decades 1,10-phenanthroline (phen) has played an important role in coordination chemistry due to its broad field of applications. This ligand has received enormous interest due to its rich functionalization chemistry, its chelating properties towards several metal ions and biological activity of the corresponding metal complexes [1]. The derivatization of phenanthroline in positions 2 and 9 as well as 5 to hydrazones is well investigated. Thereby hydrazone-functionalized phenanthrolines can coordinate several metal ions, e.g. Re(I), Pb(IV), Sn(IV), Gd(III), La(III) and Cu(II) at either the phenanthroline moiety or both the phenanthroline and hydrazone moieties [2–5]. To the best of our knowledge, coordination of phenanthrolines with hydrazone functionalization in position 5 to Cu(II) ions over the phenanthroline scaffold has not been described before.

The Cu(II) complex of the unsubstituted phenanthroline, $[Cu(phen)_2]^{2+}$, is still up to date one of the most efficient artificial metallonucleases since its discovery in the '70 s [6–8]. On the other hand, hydrazone-based compounds have been exploited for their versatile biological activity [9]. Combining both systems to form a Cu(II) complex with hydrazone-functionalized phenanthrolines could lead to novel artificial nucleases with enhanced biological activity. Such an approach is of general interest in biological/medicinal inorganic chemistry, but it also bears potential for the development of well-tolerated anticancer drugs. The most successful metal-based anticancer drug, cisplatin, suffers from causing severe side effects. Whereas a compound comprising copper as an endogenous metal, instead of platinum, could guarantee lower systemic toxicity [10].

In general there are two common pathways, in which artificial Cu(II) metallonucleases degrade DNA: oxidative and hydrolytic cleavage. The latter cleavage mechanism involves the phosphate backbone of DNA, and is enzymatically reversible [11]. The oxidative cleavage mechanism implies a double-strand break subsequent to the generation of reactive oxygen species (ROS) [12,13]. The latter cleaving mechanism is irreversible, which is important for the potential application of such metallonucleases as chemotherapeutic agents. In general, the oxidative DNA cleavage is common for complexes, which can be activated by a reductant. They can then initiate the generation of ROS in an aerobic environment while being re-oxidized themselves [14,15]. Besides the initiation of redox processes, another possibility for the activation

* Corresponding author.
E-mail address: nora.kulak@fu-berlin.de (N. Kulak).

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of metallonucleases is using light as a trigger. This has been broadly covered in the literature [16]. Some of the rarest DNA cleaving agents are the self-activating metallonucleases. In this case, the ligand should be redox active replacing the external reducing agent. On the one hand for Cu(II) complexes, the ligand could be oxidized – as seen with prodigiosins or hydrazones – to create a reduced Cu(I) species, which in turn generates ROS in a cascade manner as described above [17–19]. On the other hand, the reduced form of the ligand can be stabilized – as seen with hydroxy-salens – to gain a rare Cu(III) species, which can bind molecular oxygen for the generation of superoxide anion radicals without the need for any reducing agent [20].

Only a few examples of self-activating metallonucleases based on Cu(II) complexes with either phenanthroline or hydrazone ligands, where DNA is cleaved by generation of ROS in the absence of a reducing agent, are described in literature [20–22]. Thus, the aim of this work was the synthesis of novel Cu(II) complexes with hydrazone-functionalized phenanthrolines and the evaluation of their biological activity regarding the DNA cleavage properties in a self-activating manner.

2. Experimental part

2.1. Materials and methods

All chemicals and solvents were purchased from Acros Organics, Alfa Aesar, Fisher Scientific, Carl Roth or Sigma Aldrich and were used without further purification. ^{1}H NMR spectra of the ligands were recorded in $CDCl_3$ or DMSO d_6 on a Jeol ECX 400 spectrometer at room temperature. Chemical shifts are given relative to TMS with positive δ values indicating a low field shift. The characterization of the ligands and their Cu(II) complexes was performed *via* elemental analysis on a vario EL CHNS, UV/Vis spectroscopy on an Agilent Cary 100 instrument and electrospray ionization mass spectrometry (ESI-MS) on an Agilent 6210 ESI-TOF using methanol and ethanol, respectively, for the ligands and acetonitrile for the complexes (flow rate 10 μL/min).

2.2. Synthesis of the ligands

2.2.1. 1,10-Phenanthroline-5,6-dione (phenD)

The ligand phenD was prepared by oxidation of commercially available phen according to the literature [23].

A mixture of potassium bromide (29.750 g, 0.250 mol) and phen (5.000 g, 0.028 mol) in 96% sulfuric acid (75 mL) and 65% nitric acid (37.5 mL) was refluxed and stirred for 2 h. The reaction mixture was diluted with water (1000 mL) and neutralized with sodium bicarbonate. The mixture was then extracted three times with dichloromethane (3 × 300 mL). The organic phase was dried over sodium sulfate for 30 min and the solvent was evaporated under reduced pressure. The obtained crude product was recrystallized in methanol to obtain phenD as a yellow powder. Yield: 30%. *Anal.* Elemental analysis: Calc.: $C_{12}H_6N_2O_2$·0.2 CH_3OH (%): C, 67.65; H, 3.16; N, 12.93. Found: C, 67.28; H, 2.95; N, 13.23. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59 (dd, 2H^{phen}, J = 4.68 Hz, J = 7.87 Hz); 8.50 (dd, 2H^{phen}, J = 1.85 Hz, J = 7.87 Hz); 9.12 (dd, 2H^{phen}, J = 1.84 Hz, J = 4.70 Hz) ppm. HR ESI-MS: m/z $[M + H]^+$ Calc.: 211.0502, Found: 211.0533; $[M + Na]^+$ Calc.: 233.0321, Found: 233.0365.

2.2.2. Hydrazone-functionalized phenanthrolines

The ligands *N*'-(6-oxo-1,10-phenanthroline-5(6*H*)-ylidene)acetohydrazide (phenAH) and -benzohydrazide (phenBH) were prepared according to the following general procedure by condensing phenD with the appropriate hydrazide [2].

The respective hydrazide was dissolved in ethanol, phenD and a catalytic amount of *p*-toluenesulfonyl chloride was added dropwise. The mixture was stirred under reflux for 6 h. After storing overnight at −24 °C the formed precipitate was filtered off. The crude product was recrystallized in ethanol to obtain the respective hydrazone-functionalized phenanthroline.

2.2.2.1. N'-(6-oxo-1,10-phenanthroline-5(6H)-ylidene)acetohydrazide (phenAH). Acetohydrazide (0.194 g, 2.515 mmol), ethanol (100 mL), phenD (0.505 g, 2.403 mmol) and *p*-toluenesulfonyl chloride (0.015 g, 0.077 mmol) were used for the synthesis of the ligand phenAH, a yellow solid. Yield: 51%. *Anal.* Elemental analysis: Calc.: $C_{14}H_{10}N_4O_2$ (%): C, 63.15; H, 3.79; N, 21.04. Found: C, 63.15; H, 3.95; N, 21.03. ^{1}H NMR (400 MHz, DMSO d_6): δ = 2.49 (s, 3H^{acetyl}); 7.73 (m, 2H^{phen}); 8.59 (m, 2H^{phen}); 8.87–9.11 (dd, 2H^{phen}, J = 4.40 Hz, J = 90.06 Hz); 13.96 (s, 1H^{H}) ppm. HR ESI-MS: m/z $[M + H]^+$ Calc.: 267.0877, Found: 267.0892; $[M + Na]^+$ Calc.: 289.0696, Found: 289.0741.

2.2.2.2. N'-(6-oxo-1,10-phenanthroline-5(6H)-ylidene)benzohydrazide (phenBH). Benzhydrazide (0.324 g, 2.380 mmol), ethanol (100 mL), phenD (0.502 g, 2.390 mmol) and *p*-toluenesulfonyl chloride (0.013 g, 0.070 mmol) were utilized for the synthesis of the ligand phenBH, a yellow-orange solid. Yield: 78% (literature: 83% [2]). *Anal.* Elemental analysis: Calc.: $C_{19}H_{12}N_4O_2$ (%): C, 69.51; H, 3.68; N, 17.06. Found: C, 69.59; H, 3.76; N, 17.12. ^{1}H NMR (400 MHz, DMSO d_6): δ = 7.70 (m, 5H^{benzoyl}); 8.01 (d, 2H^{phen}, J = 7.87 Hz); 8.64 (dd, 2H^{phen}, J = 8.03 Hz, J = 16.99 Hz); 8.90–9.11 (dd, 2H^{phen}, J = 2.77 Hz, J = 86.39 Hz) ppm. HR ESI-MS: m/z $[M + H]^+$ Calc.: 329.1033, Found: 329.1060; $[M + Na]^+$ Calc.: 351.0852, Found: 351.0893.

2.3. Synthesis of the Cu(II) complexes

The Cu(II) complexes were prepared according to the following general procedure based on literature reports for the synthesis of the compounds $[Cu(phen)_2](NO_3)_2$ (**1**$(NO_3)_2$) and $[Cu(phenD)_2](NO_3)_2$ (**2**$(NO_3)_2$) [24,25].

$Cu(NO_3)_2·3H_2O$ was dissolved in a small amount of ethanol. The ligand was suspended in ethanol and the suspension was heated up to 60 °C before the $Cu(NO_3)_2·3H_2O$ solution was slowly added. A 1:2 ratio of Cu(II):ligand was ensured, when both components were combined. The occurring blue (**1**), green (**2**), red-brownish (**3**) or green-brownish (**4**) solution was heated to reflux for 1 h. Upon storage of the solution at −24 °C overnight the turquoise **1**$(NO_3)_2$, mint green **2**$(NO_3)_2$, brown **3**$(NO_3)_2$ and lime green **4**$(NO_3)_2$ precipitates were filtered off and dried *in vacuo*.

2.3.1. $[Cu(phen)_2](NO_3)_2$ (**1**$(NO_3)_2$)

$Cu(NO_3)_2·3H_2O$ (0.135 g, 0.560 mmol), phen (0.200 g, 0.110 mmol) and 30 mL ethanol were used for the synthesis of **1**$(NO_3)_2$, a turquoise solid. Yield: 73%. *Anal.* Elemental analysis: Calc.: $C_{24}H_{16}CuN_6O_6$ (%): C, 52.61; H, 2.94; N, 15.34. Found: C, 52.63; H, 3.06; N, 15.41. HR ESI-MS: m/z $[\mathbf{1}]^{2+}$ Calc.: 211.5330, Found: 211.5376; $[\mathbf{1} + NO_3]^+$ Calc.: 485.0544, Found: 485.0612; $[\mathbf{1} + Cl]^+$ Calc.: 458.0354, Found: 458.0424.

2.3.2. $[Cu(phenD)_2](NO_3)_2$ (**2**$(NO_3)_2$)

$Cu(NO_3)_2·3H_2O$ (0.059 g, 0.244 mmol), phenD (0.103 g, 0.488 mmol) and 15 mL ethanol were used for the synthesis of **2**$(NO_3)_2$, a mint green solid. Yield: 71%. *Anal.* Elemental analysis: Calc.: $C_{24}H_{12}CuN_6O_{10}$ (%): C, 47.42; H, 1.99; N, 13.82. Found: C, 47.58; H, 2.04; N, 13.91. HR ESI-MS: m/z $[\mathbf{2}]^{2+}$ Calc.: 241.5072, Found: 241.5118; $[\mathbf{2} + NO_3]^+$ Calc.: 545.0027, Found: 545.0122; $[\mathbf{2} + Cl]^+$ Calc.: 517.9838, Found: 517.9951.

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