Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 136 (2015) 761–770

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

New water-soluble copper (II) complexes including 4,7-dimethyl-1,10-phenanthroline and L-tyrosine: Synthesis, characterization, DNA interactions and cytotoxicities ☆

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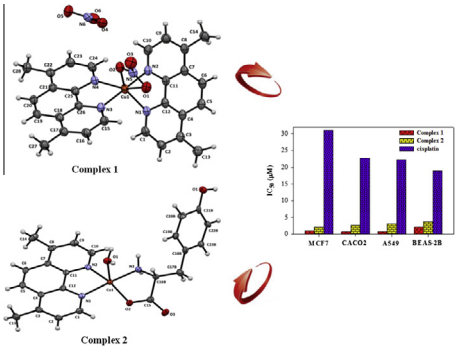
HIGHLIGHTS

- Water soluble new copper(II) complexes and the diquarternary salt of dmphen have been synthesized and characterized.
- Single crystals X-ray study confirms the structure of the copper(II) complexes.
- DNA binding studies reveal that these compounds bind to CT-DNA via intercalation.
- The copper(II) complexes efficiently cleavage the super-coiled pBR322 plasmid DNA.
- XTT assay of the copper(II) complexes show prominent cytotoxic activity against the selected human tumor cell lines.

GRAPHICAL ABSTRACT

Binary and ternary Cu(II) complexes (**1**–**2**) containing 4,7-dimethyl-1,10-phenanthroline (dmphen) and L-tyrosine (tyr) and the diquarternary salt of dmphen (dq-dmphen) have been synthesized and characterized by elemental analysis, ^{1}H NMR, ^{13}C NMR and IR spectroscopy, thermal analysis and single crystal X-ray diffraction techniques. They have been tested for their *in vitro* DNA binding activities by the spectroscopic methods. Binding studies of CT DNA with these complexes and dq-dmphen show 49–62% of hypochromicity and a minor red-shift (~2 nm) in the charge transfer band. The apparent binding constant ($\log K_{app}$) and Stern–Volmer quenching constant ($\log K_{sv}$) values of these complexes and dq-dmphen were obtained from fluorescence measurements. The results showed that the magnitudes of the calculated $\log K_{app}$ and $\log K_{sv}$ values of all of the compounds were in the order complex **1** > complex **2** > dq-dmphen. The DNA denaturation experiment shows a minor shift in the melting temperature (T_m). The cleavage activity of super-coiled pBR322 plasmid DNA under aerobic condition reveals moderate activity with complex **1** and complex **2** while dq-dmphen shows no activity. Complexes **1** and **2** exhibit high cytotoxicity with low IC_{50} values against selected human tumor cell lines (Caco-2, A549 and MCF-7) and healthy cells (BEAS-2B). Also, the dq-dmphen and Cu^{2+} solutions exhibit much less activity against these cancer cells than does complexes **1** and **2** and cisplatin.



☆ This study is a part of a MSc thesis of the first author accepted on 16.01.2013 by the Graduate School of Natural and Applied Sciences of Uludag University.

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http://dx.doi.org/10.1016/j.saa.2014.09.093
1386-1425/

ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 18 July 2014
Accepted 22 September 2014
Available online 30 September 2014

Keywords:
Copper(II)
4,7-Dimethyl-1,10-phenanthroline
L-tyrosine
Single crystal X-ray diffraction
DNA binding and cleavage
Cytotoxicity

ABSTRACT

Two new water-soluble copper(II) complexes, $[Cu(dmphen)_2(NO_3)]NO_3$ (**1**), $[Cu(dmphen)(tyr)(H_2O)]NO_3{\cdot}H_2O$ (**2**) and the diquarternary salt of dmphen (dmphen = 4,7-dimethyl-1,10-phenanthroline and tyr = L-tyrosine), have been synthesized and characterized by elemental analysis, ^1H NMR, ^{13}C NMR and IR spectroscopy, thermal analysis and single crystal X-ray diffraction techniques. The CT-DNA binding properties of these compounds have been investigated by absorption, emission spectroscopy and thermal denaturation measurements. The supercoiled pBR322 plasmid DNA cleavage activity of these compounds has been explored by agarose gel electrophoresis. The cytotoxicity of these compounds against MCF-7, Caco-2, A549 cancer cells and BEAS-2B healthy cells was also studied by the XTT method. Complexes **1** and **2** exhibit significant cytotoxicity, with lower IC_{50} values than those of cisplatin.

Introduction

DNA plays an important role in the life process because it contains all genetic information required for cellular function [1]. The binding of metal complexes with DNA base pairs has been a major focus in the study of bioinorganic chemistry [2]. These metal complexes are known to bind to DNA via both covalent and non-covalent interactions. In covalent binding, the labile ligand of the complexes is replaced by a nitrogenous base of DNA such as guanine N7. However, non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of cationic metal complexes along the outside of the DNA helix, along the major or minor groove [3–5].

Copper(II) is known as to have a significant role in biological systems and pharmacological agents. Because of its biological relevance, a large number of copper(II) complexes have been synthesized and explored for their biological activities [6–8]. Among these copper complexes, attention has been mainly focused on the copper(II) complexes of 1,10-phenanthroline (phen) due to their high nucleolytic efficiency, anti-tumor, anticandidal, and antimicrobial activities [9–11]. Many metal complexes have been synthesized using various modified phen ligands with the purpose of enhancing their interaction with DNA [12,13], and their DNA cleavage activity has also been investigated [14–19]. However, most studies have focused primarily on metal complexes containing fully planar ligands, while metal complexes containing substituted ligands such as methyl groups have rarely been reported [20]. In fact, some of these complexes also exhibit very interesting properties upon binding to DNA [21–23]. A variation in the nature and position of the substituents at the binding site of the ligand can create some interesting differences in the space configuration and electron density distribution of the metal complexes, resulting in differences in spectral profiles, DNA binding properties, enantioselectivities, and even DNA cleavage activities [15]. Studies of such differences can be very useful to thoroughly understand the binding mechanism of metal complexes to DNA [20].

Amino acids have been widely used in the production of agrochemicals, racemic drugs, fragrances and pharmaceuticals [24]. Furthermore, cancer chemotherapy has also involved transition metal complexes with amino acids as subsidiary ligands [25]. L-tyrosine (Fig. 1) has an important role in the synthesis of neurotransmitters, dopamine and noradrenaline, as well as the thyroxin thyroid hormone [26]. L-tyrosine forms coordination compounds with metal ions using carboxylate, amino and/or hydroxyl groups. However, the most popular coordination mode is a chelating one involving $-COO^-$ and $-NH_2$ groups [27]. Copper complexes containing amino acids have been studied as models for the behavior of copper enzymes [28], and some copper complexes with amino acid ligands have been reported to exhibit potent antitumor and artificial nuclease activities [29–31].

Although interaction of copper complexes of some 1,10-phenanthroline derivatives as primary ligands and some amino acids as a secondary ligands with DNA has been studied [12–20], interaction of copper complexes of 4,7-dimethyl-1,10-phenanthroline and L-tyrosine with DNA has not been shown yet. We previously investigated the stability constants of copper(II) complexes with substituted 1,10-phenanthroline groups and L-amino acids in aqueous solution [32]. Therefore, in this study, we synthesized and characterized two water-soluble Cu(II) complexes that include dmphen (Fig. 1) and tyr, $[Cu(dmphen)_2(NO_3)]NO_3$ (**1**) and $[Cu(dmphen)(tyr)(H_2O)]NO_3{\cdot}H_2O$ (**2**), and the diquarternary salt of dmphen (dq-dmphen). The CT-DNA binding properties of these compounds were investigated by UV–vis, fluorescence and thermal denaturation experiments. Their cleavage behavior toward pBR322 plasmid DNA and the cytotoxicities of these compounds against MCF-7 (breast adenocarcinoma), Caco-2 (colon adenocarcinoma), A549 (lung adenocarcinoma) and BEAS-2B (bronchial epithelium) cell lines were obtained.

Experimental

Materials

All reagents were obtained from commercial sources and used as received. Copper(II) nitrate trihydrate and 4,7-dimethyl-1,10-phenanthroline were purchased from Merck and Alfa-Aesar, respectively. L-tyrosine, methanol, KOH, NaCl, tris-(hydroxymethyl)aminomethane-HCl, CT-DNA, agarose (molecular biology grade), ethidium bromide, bromophenol blue, xylene cyanol,

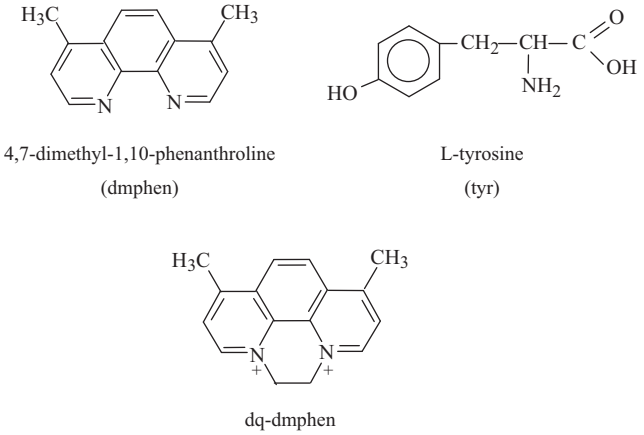

Fig. 1. Structures of the ligands used in this study and the diquarternary salt of dmphen (dq-dmphen).