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Kinetic study of formation/dissociation of Cu(II) and Zn(II) complexes of *cyclen* macrocyclic ligand with pendant thiol group

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ABSTRACT

The kinetic properties of macrocyclic ligand 2-[1,4,7,10–tetraazacyclododecane-1-yl]-ethanethiol (**L1**, DOSH) were studied with Cu(II) and Zn(II) metal ions, using conventional molecular absorption spectroscopy in pH regions 2.5–4.7 and 4.3–6.0 for Cu(II) and Zn(II), respectively. The obtained partial rate constants, measured at 25 °C and ionic strength $I = 0.1$ M (KCl), are slightly lower than for parent *cyclen* (1,4,7,10-tetraazacyclododecane) macrocyclic ligand supporting the hypothesis that the thiol pendant arm is not directly involved in the formation of these metal complexes. The study of the dissociation of the copper(II)-L1 complex in the presence of perchloric acid and at higher temperatures (75–95 °C) showed that this complex is more kinetically inert than the copper(II) complex with *cyclen*, indicating that the presence of the thiol pendant arm plays a key role as protecting group of copper(II) ion. On contrary, the dissociation of zinc(II) complex in presence of copper(II) ion measured at laboratory temperature (25 °C) and in 0.1 M-solution of hydrochloric acid is going via the metal-exchange reaction mechanism. Altogether, these findings show that L1 ligand presents favorable kinetic properties for further conjugation with biomolecules, aiming at their use in medicinal chemistry.

1. Introduction

The majority of radiopharmaceuticals currently available in nuclear medicine make use of metallic radioisotopes. Copper is one of the most interesting elements for various biomedical applications and its radioisotopes due to their unique properties are applied in imaging ($^{60-62,64}$Cu) and for targeted radionuclide therapy ($^{64/67}$Cu) [1–5]. The most utilized is ^{64}Cu, which is suitable both for positron emission tomography (PET) imaging (β^+ emission) and for targeted radiotherapy (β^- emission) [1–9]. Several approaches have been made for tethering these radionuclides to biologically active molecules, *e.g.* monoclonal antibodies, small peptides, hormones, sugars, *etc.*, which targets to the cells in the body [1–8]. The use of Bifunctional Chelating Agents (BFC's) is one of the most relevant approaches to firmly attach the radiometal to the carrier moiety of the radiopharmaceutical [1,10–11]. A critical prerequisite for *in vivo* applications of a metalloradiopharmaceutical is the high *in vivo* stability of the metal chelate complex, as if the radiometal is released *in vivo* it will almost certainly accumulates in bone, giving a radiation dose to the radiosensitive bone marrow. Thus, it is important to choose BFC's that allow the formation of radiometal complexes of high thermodynamic stability. However, high thermodynamic stability is not the sole requirement of a metal chelate complex because it only reflects the direction, not the rate of the reaction. As a matter of fact, the solution stability of a metalloradiopharmaceutical in the blood stream is predominantly determined by the kinetic inertness of the metal chelate complex [2,4,12–15]. In this respect, it is important to know the influence of linker on thermodynamic and kinetic properties (formation and dissociation rate constants) of radiometal-chelate complexes in order to optimize the structural design of the corresponding BFC's [4,16–17].

Nowadays it is well known that, in general, fast dissociation kinetics are characteristic of metal complexes of acyclic chelators, while metal complexes containing macrocyclic chelators are much more kinetically inert [2–4,17–18]. In addition, according to the Irwing-Williams order for metal complexes, the more stable copper(II) complexes are formed with ligands with nitrogen and sulphur donor atoms [19]. In fact, a variety of N- and S-donor acyclic chelators have been used in several instances to obtain Cu(II) complexes with relevance for biomedical applications. Several tetraazamacrocyclic ligands with nitrogen and/or oxygen pendant arms have been used for labeling peptides or antibodies with $^{64/67}$Cu [1–5,14,20–22] but, on contrary, there are only a few examples in literature dealing with the synthesis of new

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tetraazamacrocyclic ligands bearing thiol and/or thioether groups as pendant arms [23–26].

As part of our ongoing research work on copper(II) complexes with biological interest [27–29], we have synthesized and characterized a mono-N-substituted *cyclen* derivative with a 2-ethanethiol pendant arm, 2-[1,4,7,10-tetraazacyclododecan-1-yl]-ethanethiol (**L1**, DOSH) [27]. In a previous paper, we have described the thermodynamic properties of protonation and complexation of **L1** ligand with chosen metal ions, *e.g.* Cu(II), Zn(II), Cd(II) [27]. Following our previous work on searching to have a better insight into the kinetic properties of L1 ligand, herein we report the study dealing with formation and dissociation of its Cu(II) and Zn(II) complexes.

2. Experimental

The studied ligand, **L1**, was synthesized according to a published procedure [27]. Other analytical grade chemicals were purchased from Lachema (Czech Republic), Fluka or Merck and used as received. The $CuCl_2$ and $ZnCl_2$ stock solutions were standardized by chelatometric titration according to the recommended procedure [30,31].

The formation kinetic study was carried out at 25 °C in a solution of ionic strength 0.1 M which was adjusted by addition of potassium chloride. The chloracetate/acetate buffers were used for pH region 2.5 to 4.7 for Cu(II) complexation and acetate/MES (morpholine-ethansulfonic acid) buffers for pH region 4.3–6.0 for Zn(II) complexation (all buffers 0.025 M). The concentrations of ligand and metal ions were set to fulfill the pseudofirst-order conditions while concentration of ligand was kept constant at 0.1 mM and Cu(II) ion concentration was varied in range 1.0–2.5 mM. In case of Zn(II) ion complexation (c_M = 0.01 mM, c_L = 0.01 mM), the 1,10-phenanthroline was used as a competing ligand and the ratio phen/Zn(II) was varied in ratio 0.3–1.

The dissociation kinetic study was carried out with metal complexes prepared mixing aqueous solutions of ligand and metal ion in the molar ratio 1.2 and the pH was adjusted about 5. The solutions were let to stand at least 24 h prior to the dissociation study. The dissociation of the copper(II) complex (1×10^{-4} M) was measured in perchloric acid solution (0.05–5 M), in the temperature range 75–95 °C and the ionic strength was adjusted to 5 M adding sodium perchlorate. The dissociation of zinc(II) complex ($\sim 1 \times 10^{-4}$ M) was measured at room temperature (25 °C) in weakly acidic conditions adjusted by hydrochloric acid (pH 1) in the presence of Cu(II) ions.

The kinetic measurements were performed on Hewlett Packard 8453A (Agilent, USA) and Varian Cary 50 Bio (Varian, Australia) spectrophotometers. Some measurements were carried out on AMINCO BOWMAN 2 fluorimeter (Thermo, USA). The rate constants were calculated with Pro-K II software (Applied Photophysics, UK).

3. Results and discussion

3.1. Formation kinetic study

As already postulated in a former publication [27], the complex formation was slow enough for copper(II) ion to follow the process by conventional molecular spectroscopy. The rate of the copper complex formation of the title ligand can be described as:

$$v = k_{2,f}[Cu]_{tot}[L]_{tot} \quad (1)$$

In the presence of excess of copper(II) ion relatively to the ligand, the previous equation can be simplified as

$$v = k_{f,\,obs}[L]_{tot} \quad (2)$$

Being the pseudo-first rate constant is defined as

$$k_{f,\,obs} = k_{2,f}[Cu]_{tot} \quad (3A)$$

and/or in logarithmic form

$$\log k_{f,\,obs} = \log k_{2,f} + \log [Cu]_{tot} \quad (3B)$$

This relationship allowed us to assess the stoichiometry of the intermediate species of the reaction (see Fig. 2), as the slope of Eq. (3B) was about one (within uncertainty of measurement) meaning that the species formed is CuL, *i.e.* the M:L stochiometry is 1:1. This hypothesis was also verified by fitting the pseudo-first rate constants, $k_{f,obs}$, measured in the pH region 2.5–4.3, as a function of the concentration of copper(II) ion, which gave the second-order rate constants, $k_{2,f}$, as slopes of the Eq. (3A) (Figure is not

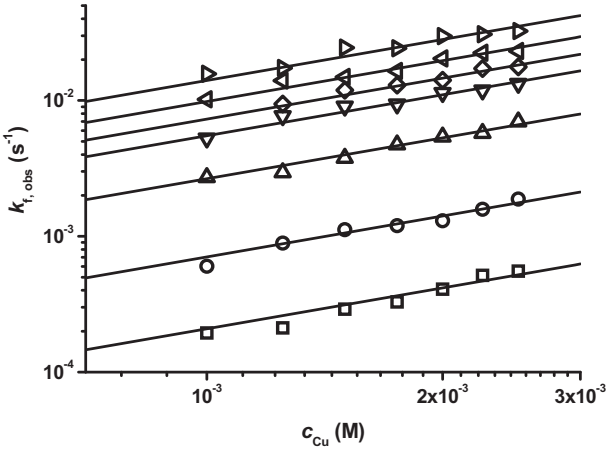

Fig. 2. The dependence of pseudo-first rate constant on copper(II) ion concentration for metal complex formation (pH is increased gradually as 2.56, 2.93, 3.39, 3.73, 3.93, 4.06 and 4.26, t = 25 °C).

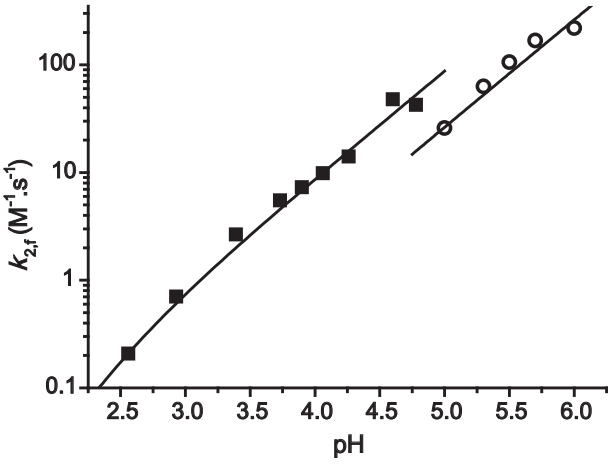

Fig. 3. The pH dependence of the second-order rate constants for the formation of studied ligand with divalent transition metal ions (Cu(II) – closed rectangles, Zn(II) – opened circles, I = 0.1 M (KCl), t = 25 °C).

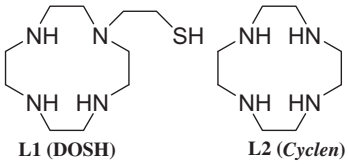

Fig. 1. The structural formulas of discussed ligands.