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Thermodynamic and spectroscopic study of the interaction of Cu(II), Ni(II), Zn(II) and Ca(II) ions with 2-amino-*N*-(2-oxo-2-(2-(pyridin-2-yl)ethyl amino)ethyl)acetamide, a pseudo-mimic of human serum albumin

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Abstract

The protonation equilibria of 2-amino-*N*-(2-oxo-2-(2-(pyridin-2-yl)ethyl amino)ethyl)acetamide ([H_2(556)–N]) and the complexation of this ligand with Cu(II) Ca(II), Zn(II) and Ni(II) have been studied by glass electrode potentiometry and UV–visible spectrophotometry. From pH ~2.00–11.00, five models for Cu(II) with the following complexes; MLH, ML, MLH_{-1}, MLH_{-2} and MLH_{-3} were generated and observed to describe the experimental data equally well as far as the statistical criteria were concerned. The MLH_{-2} complex predominates at physiological pH in all five models, while the MLH_{-1} complex species exists only at low concentration in two models. The coordination in the MLH_{-2} complex suggested the involvement of one amino, two deprotonated peptides and one pyridyl nitrogen atoms. Molecular mechanics (MM) calculations confirmed the MLH_{-2} complex as the most stable species. Speciation calculations, using a blood plasma model, predicted that the Cu(II)–[H_2(556)–N] complex is able to mobilize Cu(II). Octanol/water partition of $CuLH_{-2}$ showed that 30% of the complex went into the octanol phase, hence promoting percutaneous absorption of copper. The complex is a poor mimic of native copper–zinc superoxide dismutase.

Keywords: Thermodynamic; Spectroscopic; Potentiometry; Complexation; Speciation; Protonation

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic and destructive inflammatory polyarticular joint disease that chiefly affects the synovial membranes of multiple joints in the body [1,2]. It is characterized by massive synovial proliferation and subintimal infiltration of inflammatory cells, which along with angiogenesis leads to the formation of a very aggressive tissue, the pannus [3,4]. Expansion of the pannus induces bone erosion and cartilage thinning, leading to the loss of joint function. The rheumatoid pannus can be considered as a local tumor.

Although the disease is currently controlled by immunosuppressive drugs and symptoms treated with anti-inflammatory drugs [5,6], the use of metal chelating agents has grown. Within the last decade there has been an upsurge

Abbreviations: RA, rheumatoid arthritis; HSA, Human serum albumin; ([H_2(556)–N]), 2-amino-*N*-(2-oxo-2-(2-(pyridin-2-yl)ethyl amino)ethyl)acetamide; [H(555)–N], *N*-(2-(2-aminoethylamino)ethyl)picolinamide; β_{pqr}, overall formation constant; ESTA, equilibrium simulation for titration analysis; esff, extensible systematic force field; ECCLES, evaluation of constituent concentrations in large equilibrium systems; p.m.i., plasma mobilizing index; l.m.w., low molecular weight; SOD, superoxide dismutase; Gly-Gly-Ha, glycylglycylhistamine; Ala-Gly-Ha, alanylglycylhistamine; pK_a, protonation constant; Z_H-bar, protonation curves; Z_M-bar, complex formation function; *Q*-bar, deprotonation function; σ_{pqr}, standard deviation in log β_{pqr}; R_f^H, Hamilton *R*-factor; R_{lim}^H, Hamilton limit; n_T, number of titrations; n_P, titration points; MM, molecular mechanics; ε, extinction coefficient; trien, 1,4,7,10-tetraazadecane; LMCT, ligand to metal charge transfer; $P_{oct/aq}$, octanol/water partition coefficient; NBT, nitroblue tetrazolium; edta, ethylenediaminetetraacetic acid.

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of interest in metal ion therapeutics for both diagnosis and treatment [7,8]. For example Cu(II)-L-histidine [9] has been used in the treatment of Menkes disease [10,11]. Such interest has been due to the biochemical and pharmacological properties of the metal–ligand system, with extensive research carried out to determine the role of the ligand in copper uptake into cells. On the other hand many articles [12,13], indicating the effective role of various copper chelating agents in the alleviation of inflammation associated with RA have also appeared in literature, indicating the physiological importance of these agents as well as their therapeutic applications. Important in this regard is the ability of biologically essential metal ions such as Cu(II), Ca(II), Zn(II) and Ni(II) to interact with various ligand systems, particularly those containing a pseudo-peptide-mimic of the N-terminus of serum albumin [14–18].

Serum albumin (SA) is a major metal binding protein in the body, with about 40 μg of copper able to bind to the albumin contained in 1 ml of human plasma [19]. The Cu(II) transport site of serum albumin is one of the most extensively studied binding site of any protein [20]. The proposed structure (Fig. 1) of the major Cu(II) binding site in HSA involves the α-NH_2 nitrogen, two peptide nitrogens, and the imidazole nitrogen of the N-terminal Asp-Ala-His residues [12]. Recently, we have shown, using animal experiments, that Cu(II)-*N*-(2-(2-aminoethylamino)ethyl)picolinamide ([H(555)–N]), a system related to Cu(II)–HSA, is capable of surviving *in vivo* [21] with a relatively long biological half life. Encouraged by these results, we have extended our efforts to the thermodynamic and spectroscopic study of the solution equilibria of Cu(II), Ca(II), Zn(II) and Ni(II) with 2-amino-*N*-(2-oxo-2-(2-(pyridin-2-yl)ethyl amino)ethyl)acetamide ([H_2(556)–N]) (Fig. 2). The major difference between [H_2(556)–N] and HSA is the replacement of the imidazole group by a pyridyl system. Speciation modelling studies [22] have shown that ligands containing the pyridyl group have a higher Cu(II) mobilizing ability than analogous ligands with the imidazole group. Presumably this is related to metal ion specificity and hence *in vivo* competition with

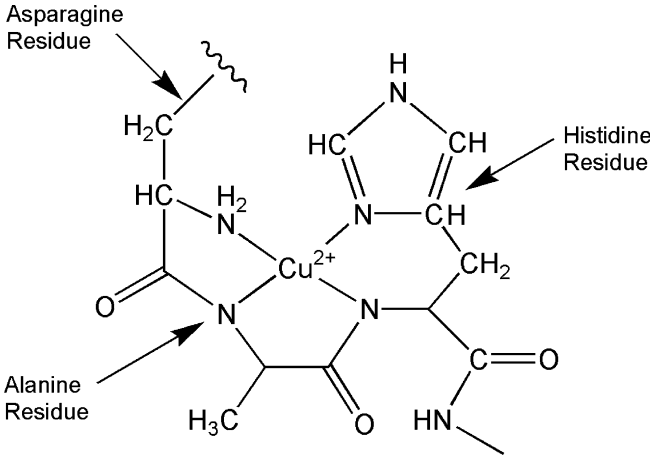
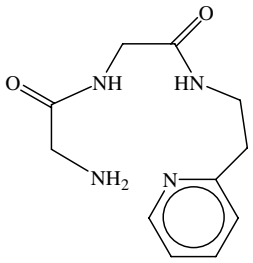


Fig. 1. Copper binding site in Human Serum Albumin (HSA).



[H_2(556)-N]

Fig. 2. Schematic structure of chelating agent.

Ca(II) and Zn(II) which are present in much higher concentration than Cu(II) in blood plasma [20,23].

2. Experimental

Formation constants were measured at 298 K and an ionic strength of 0.15 M (NaCl) using a procedure described previously [24]. The reagents used were all of analytical grade and the synthesis of the metal chelating agent was based on a method described previously [17]. The overall formation constant, β_{pqr}, refers to the equilibrium below, where p, q and r are the stoichiometric coefficients of the components in the complex

$$pM + qL + rH \rightleftharpoons M_pL_qH_r \quad (1)$$

$$\beta_{pqr} = \frac{[M_pL_qH_r]}{[M]^p[L]^q[H]^r} \quad (2)$$

The species formed in the investigated systems can be characterized by this general equilibrium process (1) where charges are omitted. The formation constants (β_{pqr}) given by (2) for the generalized reaction (1) were evaluated from the pH-potentiometric titration data using the Equilibrium Simulation for Titration Analysis (ESTA) computer program [25].

Molecular mechanics calculations were performed on a SyncMaster 1100p workstation using the Discover module of InsightII software [26]. The extensible systematic force field (esff) [27] was used for all energy calculations. All calculations were done in vacuum.

Electronic spectra in aqueous solution were recorded in 1.00-cm quarts cells using a Varian UV-Cary 100 spectrophotometer equipped with a temperature-controlled cell holder.

Water/octanol partition coefficients were measured as a function of pH using the shake flask method [28–30]. The aqueous solutions in the vials at different pH were spiked with $^{64}CuCl_2$ solution of activity 7.50–9.00 mCi, before shaking with an equal volume of water saturated octan-1-ol. An equal amount (1 ml) of each phase was counted in a Minaxi Auto gamma counter (5000 Series-Packard) using a window set at 340–540 keV [31–34].

Blood plasma modelling was carried out by incorporation of the formation constants determined in this study into the blood plasma model consisting of data for 10