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Solution and solid state study of copper(II) ternary complexes containing amino acids of interest for brain biochemistry – 2: Homocysteine with aspartate, glutamate or methionine

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ABSTRACT

Alzheimer's disease is characterized by the loss of synapses and by the presence of extra-cellular amyloid β-peptide (Aβ) plaques, and it has been associated to copper metabolism. The plasma of patients with Alzheimer's disease presents higher levels of the amino acid homocysteine (hCys).

In this study, three new ternary complexes of copper(II) with homocysteine and three amino acids of Aβ (aspartate, glutamate or methionine) were studied both in solution (pH-potentiometry and UV–Vis) and in the solid state (CHNS, AAS, TGA, EPR, and FT-IR). Molar conductance and electrochemical (CV) measurements were carried out as well. The binary complex $[Cu(hCys)_2]$ was also synthesized and characterized.

For the ternary complexes, the values of the stability constants (log β equal to 25.62 for CuAsphCys, 23.53 for CuGluhCys, and 18.61 for CuMethCys) are similar to those of the cysteine-containing ternary complexes CuAspCys and CuGluCys, recently reported by us, indicating a related N_2OS coordination.

The results found in the solid state are in agreement with the data found in solution studies whereby methionine, aspartic, and glutamic acids coordinate to copper(II) ion through the nitrogen atom of the amino group and one oxygen atom of the α-carboxylate group, and homocysteine coordinates through nitrogen and sulfur atoms. EPR data suggest that, in solution, the complexes are in a distorted tetrahedral geometry.

1. Introduction

Due to the advances in medicine, human life expectancy has increased in the last decades and, therefore, concerns with neurodegenerative diseases became greater than ever. Alzheimer's disease (AD) accounts for more than 70% of all cases of dementia [1]. AD is characterized by the presence of amyloid β-peptide (Aβ) plaques [2,3] and loss of synapses. The Aβ1–42 peptide is the predominant species found in the deposits of neuritic (senile) plaques [4]. In the last few years, various studies have been providing evidence that metal ions are critically involved in the pathogenesis of major neurological diseases. Zn(II), Cu(II), and Fe(III) are present along with Aβ in the senile plaques in Alzheimer's disease, where Al(III) is also detected [5,6]. The toxicity of Aβ plaques is related to the production of hydrogen peroxide [7], and studies show that the presence of copper ions potentiates the toxicity of the plaques [7–9].

In the plasma of patients with Alzheimer's disease, augmented levels of homocysteine (2-amino-4-mercaptobutyric acid, hCys) were found [10,11]. Homocysteine is an endogenous sulfur-containing amino acid produced metabolically by the demethylation of methionine. Homocysteine is involved in several biochemical processes and is a constituent of many proteins, which are good complexing agents. However, in spite of its beneficial functions, a plasma hCys concentration of more than 15 μM is described as hyperhomocysteinemia, a condition associated to inherited disorders in the metabolism of the main enzymes of hCys or to nutritional deficiencies of the vitamin cofactors B_6 and B_{12} [12].

The determination of hCys in biological fluids (especially plasma) is very important because hyperhomocysteinemia has been associated with several conditions, such as coronary artery and cerebrovascular diseases, neural tube defects, mental disorders [12], and is a risk factor for the development of AD [13]. Studies reported that a major risk factor for vascular disease is related to elevated levels of hCys in plasma [13,14], and that people with cardiovascular risk factors have an increased risk of developing AD [13–17].

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In this context, the main objective of the present work was to study, in the solid state, ternary complexes of copper(II) containing homocysteine and, as co-ligand, methionine (Met), aspartic acid (Asp) or glutamic acid (Glu), the three latter amino acids being part of the structure of Aβ. Studies in solution have also been performed, as they contribute to the determination of the structures of complexes in the solid state and allow for a better understanding of the processes occurring in biological systems because they represent simplified models of the chemistry of body fluids.

This work is part of a broader study on copper(II) complexes containing, as ligands, amino acids of interest for brain biochemistry. The aim of this study is to contribute to an improvement of our knowledge about several anomalies which lead, ultimately, to the accumulation of neurotoxic substances in the brain, oxidative stress, neurodegeneration, and cellular death.

2. Experimental

2.1. Materials

DL-Aspartic acid (Sigma) and L-homocysteine (Sigma), L-glutamic acid (Vetec), and L-methionine (Reagen) were used without further purification. Copper(II) chloride, potassium nitrate, potassium hydrogen phthalate, hydrochloric acid, 0.1 mol L^{-1} potassium hydroxide, 0.1 mol L^{-1} EDTA, and the pH 4.0 and 7.0 buffer solutions were purchased from Merck.

2.2. Potentiometric studies

2.2.1. Reagents

A 0.1 mol L^{-1} carbonate-free potassium hydroxide solution was prepared from Titrisol ampoules. A 0.01 mol L^{-1} copper(II) chloride solution was prepared by dissolving the salt in deionized and bi-distilled water, and was standardized against a standard solution of 0.1 mol L^{-1} EDTA (Titriplex). All solutions were prepared with bi-distilled, deionized, and carbonate-free water.

2.2.2. Apparatus

The titrimetric data were obtained using a Metrohm 809 automatic titrator coupled with a Metrohm combined glass electrode (Ag/AgCl). The glass electrode was calibrated before each titration with two Merck standard buffer solutions in nitrate medium: first with the pH 7.0 solution (the same as in the bulb), and then with a pH 4.0 solution. All titrations were performed at 25.0 ± 0.1 °C by coupling the titration cell to a thermostatic bath. N_2 was bubbled in the solution during all the procedure.

2.2.3. Procedure

For the determination of the acid dissociation constants of the ligands (hCys, Asp, Glu, and Met), a 1×10^{-3} mol L^{-1} aqueous solution of the protonated ligands was titrated with 0.1 mol L^{-1} KOH at 25 °C under ionic strength of 0.1 mol L^{-1}, using a 1.2 mol L^{-1} KNO_3 solution in order to obtain I = 0.1 mol L^{-1}. For the determination of the binary systems [one ligand and Cu(II)], solutions containing the ligands and Cu(II) ions were titrated at a 1:1 Cu(II):ligand ratio for Asp, Glu, and Met, and at a 1:2 ratio for hCys. For the ternary systems, the ratio used was 1:1:1 Cu(II):hCys:L2 (Asp or Glu or Met). In the ternary systems, the concentration of hCys, Asp, Glu or Met solutions in the titrated samples was always the same and equal to 1×10^{-3} mol L^{-1}. All binary and ternary systems were titrated with 0.1 mol L^{-1} KOH under the same conditions mentioned above. Each set of titrations was performed at least in triplicate in order to verify the repeatability of the data. All the dissociation and the complex formation constants were determined using the HYPERQUAD program [18], and the speciation as a function of pH was determined using Hyss [19].

2.3. Synthesis

2.3.1. General

One binary and three ternary complexes were obtained and characterized in the solid state by elemental analysis (CHN), atomic absorption spectrometry (Cu), electron paramagnetic resonance (EPR), conductimetry, thermogravimetry, and infrared and UV–Vis spectroscopies.

2.3.2. Synthesis of $[Cu(hCys)_2]\cdot H_2O$ (1), $[Cu(Asp)(hCys)]\cdot H_2O$ (2), $[Cu(Glu)(hCys)]\cdot H_2O$ (3), and [Cu(Met)(hCys)] (4)

These complexes were prepared by dissolving 3 mmol of hCys in 20 mL of distilled water, than adding this solution to an equal volume of an aqueous solution containing 3 mmol of Asp or Glu or Met, for the ternary complexes 2–4. For the binary complex 1, 6 mmol of hCys were used. Each mixture was then stirred for 1 h at room temperature for homogenization. After that, a $CuCl_2\cdot 2H_2O$ solution (3 mmol, 0.511 g) was added and the system was left to react for 2 h, when the pH of the solution was adjusted to 6.5 (complex 2) or 6.0 (complexes 1, 3, and 4) by adding a 1 mol L^{-1} KOH solution dropwise. The resulting systems, after being additionally stirred for 48 h at 25 °C, were concentrated to half of their initial volumes. The precipitates obtained were filtered off and washed with absolute ethanol, acetone, and ether, and then dried.

2.4. Elemental analysis

Carbon, hydrogen, nitrogen, and sulfur (CHNS) determinations were performed in an elemental analyzer EA 1110 by CE Instruments. The copper content was determined by atomic absorption spectrometry in a Varian AA5 instrument.

2.5. Thermogravimetric analysis

Thermogravimetric analyses (TGA) were performed with a Perkin-Elmer TGA 7. The temperature range used was from 20 to 900 °C, with a scanning rate of 10.0 °C min^{-1} in a nitrogen atmosphere.

2.6. Spectral analysis

Cu(II) complexes were studied by UV–Vis spectroscopy both in aqueous solutions prepared *in situ* from the ligands and copper(II) and in solutions directly obtained by dissolving the solid complexes in water. A double-beam Perkin-Elmer spectrophotometer model Lambda 35, operating in the range of 190–1000 nm with a 1 cm path length quartz cell, was used. The analyses were performed at 25 °C. The solutions were prepared in the same way as previously described for potentiometry. The same metal:ligand ratio was used. The solutions were analyzed in a wide range of pH values, adjusted by adding small increments of 0.1 mol L^{-1} KOH solution in order to obtain the maximum absorbance of each predominant species, as was previously determined in the species distribution diagrams for all binary and ternary systems. The ionic strength was 0.1 mol L^{-1} (1.2 mol L^{-1} KNO_3 as stock solution). Bi-distilled and deionized water was used as the reference sample for all measurements. The spectra of the complexes were obtained after dissolving them in bi-distilled and deionized water.

EPR spectra were measured at 298 K (powder and dissolved solid) and 77 K (dissolved solid) in a Bruker ESP 300E spectrometer. The values of the EPR parameters were obtained by treating and simulating the experimental spectra using the Windows software programs Win-EPR and SimFonia.