Equilibrium and Spectral Studies of Putrescine Complexes with Copper(II)

Lechoslaw Lomozik and Anna Gąsowska

Faculty of Chemistry, Adam Mickiewicz University, 60-780 Poznań, Poland

Summary. It has been found that in the putrescine-copper system several types of complex compounds (MHL, ML, ML₂, ML₂OH) are formed. In the pH range of 7–9, despite a multiple excess of ligand, a precipitation occurs. When adenosine is introduced to the system, the ability to observe the complexation reaction in solution is largely increased, because the additional ligand prevents precipitation. On the basis of computer analysis of potentiometric titration data the stability constants of the compounds have been determined. The coordination mode of the complexes is discussed.

Keywords. Complexes; Copper; Putrescine; Stability constants.

Gleichgewichte und spektroskopische Untersuchungen an Putrescin-Komplexen mit Kupfer(II)


Introduction

Inorganic cations such as Na⁺, K⁺, Mg²⁺, and Ca²⁺ or cations of transition metals participate in numerous processes occurring in the cells of living organisms [1–5]. These processes involve very often a formation of coordination compounds with bioligands. The group of compounds which plays an important role in the processes of genetic information transfer comprises the following polyamines: putrescine (Put), spermidine (Spd), and spermine (Spm). These compounds occur at high concentrations in cells which tend to grow very quickly, including cancerous cells [6–7]. Results of recent studies undoubtedly prove the great importance of biogenic amines [8–16]; however, no convincing evidence how this group of compounds behaves in vivo or in vitro has been provided as yet. Amines occur in the physiological environment almost exclusively in the protonated form, as cations, and as such show a considerable affinity to some of the anion fragments of biomolecules. Besides, in the case of spermidine and spermine it was found that these ligands tend to form complex compounds with metals [17–25]. Recently, we have also proved, contrary
to the opinions expressed by some authors [17–18], that putrescine also forms complexes with copper (II) in solution [25–26].

The present work is the next step in our studies of coordination compounds of this bioligand with metals.

Experimental Part

The following compounds were used in this study: putrescine (Put·2HCl), adenosine (Ado·HCl), cytidine (Cyd·HCl), deoxyctydine (dCyd·HCl) (Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Poznań), and deoxyadenosine (dAdo) (Sigma Chemical Company). All these compounds were subjected to elemental analysis, the results of which in each case (%N, %C, %H) were in agreement with the results obtained from theoretical calculations (±0.5%). Additionally, for Ado and dAdo the melting points were determined (235 °C and 187 °C, respectively). Cu²⁺ was used in the form of its perchlorate (for the preparation see [27]). The concentration was determined by a complexometric method [27]. Potentiometric measurements were carried out using a Radiometer PHM 26C pH-meter equipped with a TTT11 titrator and ABU 1-c autoburette. A previously calibrated GK 2401c electrode was employed in the measurements [28]. The concentrations of Put, Ado, dAdo, Cyd, and dCyd in the titrated samples ranged from 1.0·10⁻³ to 1.75·10⁻³ at metal:ligands ratios from 1:3.5:3.5 to 1:4.5:4.5, respectively. All titrations were performed under an argon atmosphere at an ionic strength of \( \mu = 0.1 \) (NaClO₄), \( T = 293 \pm 1 \) K, NaOH free of CO₂ being used as the titrant (0.0464 M). Calculations were carried out using SCOGS [29], MINIQUAD [30], and HALTAFALL [31] programs. Samples for \(^1\)HNMR and \(^{13}\)C NMR were prepared by dissolving Put and copper perchlorate in D₂O. Measurements of \( pD \) were corrected according to the formula \( pD = pH + 0.4 \) [32]. The concentration of Put in samples was 0.02 mol/dm³, and the ratio of metal to ligand ranged from 1:50 to 1:100. \(^1\)HNMR spectra were recorded on a TESLA 587A spectrometer with t-butanol as the internal standard, the \(^{13}\)C NMR spectra on a NMR JEOL FX 90Q spectrometer with dioxane as the internal standard.

Results and Discussion

The complexing ability of putrescine is determined by the presence of two nitrogen atoms separated by four methylene groups. The reactions of this ligand with copper cations depend on the basicity of donor atoms and spatial arrangements. Thus, a high stability of the nitrogen–hydrogen bond (appropriately high \( pK \) values) as well as a formation of a seven-membered ring, which is thermodynamically unfavorable, account for the poor complexing property of this bioligand. Previously, we claimed that in the copper-putrescine system in water two complex species are formed. A compound of the \( ML_2OH \) type (\( M \)-metal, \( L \)-putrescine) appears at \( pH > 9 \), whereas the \( MHL \) species occurs at \( pH < 7 \) [26]. However, for \( MHL \) species the values of the stability constant was not determined because of a too narrow \( pH \) range in which no precipitation proceeds and in which the potentiometric titrations data can be used to calculate equilibrium constants. Yet, spectral data clearly indicate the formation of a protonated compound. Under the conditions of the present study, even at a multiple excess of ligand, at a \( pH \) range from 7 to 9 precipitation occurs, which is undoubtedly related to the formation of compounds of the \( M(OH)_x \) and \( ML(OH)_x \) type (for the sake of simplicity, in all formulae ion charges were omitted). Any attempts for a precise determination of the composition of the precipitates failed, however, the results obtained indicate the formation of hydroxo complexes. At a \( pH \) of about 9.0 the precipitate dissolves.