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

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Summary. It has been found that in the putrescine-copper system several types of complex compounds (MHL, ML, ML_2, ML_2OH) 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)

Zusammenfassung. Es wurde festgestellt, daß sich im Putrescin-Kupfer System einige Typen von Komplexen bilden (MHL, ML, ML_2 and ML_2OH). Im *pH*-Bereich von 7–9 tritt trotz eines mehrfachen Ligandenüberschusses ein Niederschlag auf. Bei Einführung von Adenosin in das System wird die Beobachtbarkeit der Komplexreaktion verbessert, da der zusätzliche Ligand die Niederschlagsbildung verhindert. Mittels Computeranalyse der potentiometrischen Titrationsdaten wurden die Stabilitätskonstanten der Verbindungen ermittelt. Die Art der Komplexierung wird ebenfalls diskutiert.

Introduction

Inorganic cations such as Na^+, K^+, Mg^{2+}, and Ca^{2+} 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), deoxycytidine (*dCyd*·HCl) (Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Poznań), and deoxyadenosine (d*Ado*) (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 ($\pm 0.5\%$). Additionally, for *Ado* and *dAdo* the melting points were determined (235 °C and 187 °C, respectively). Cu^{2+} 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 $1.0 \cdot 10^{-3}$ to $1.75 \cdot 10^{-3}$ 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 $\mu = 0.1$ ($NaClO_4$), $T = 293 \pm 1$K, NaOH free of CO_2 being used as the titrant (0.0464 *M*). Calculations were carried out using SCOGS [29], MINIQUAD [30], and HALTAFALL [31] programs. Samples for ^{1}H NMR and ^{13}C NMR were prepared by dissolving *Put* and copper perchlorate in D_2O. Measurements of *pH* were corrected according to the formula $pD = pH + 0.4$ [32]. The concentration of *Put* in samples was 0.02 mol/dm^3, and the ratio of metal to ligand ranged from 1:50 to 1:100. ^{1}H NMR 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 hydroxocomplexes. At a *pH* of about 9.0 the precipitate dissolves.

Table 1. Stability constants [$\log\beta$] of putrescine complexes with H^+ and Cu(II); ($Cu_pH_qL_r$)

p	q	r	$\log\beta_{pqr}$
0	2	1	20.51(1)
0	1	1	10.83(1)
1	1	1	15.83(17)
1	0	1	8.62(13)
1	0	2	13.40(18)
1	−1	2	0.065(6)

In order to give a more comprehensive explanation of the complexing reaction, further studies of putrescine complexes with copper were performed (preliminary results in [25]). In the course of the studies of ternary systems, comprising besides Cu(II) and *Put* also adenosine (*Ado*), it was found that the presence of the additional ligand increases the ability to observe the reaction between Cu and putrescine. Already the initial titrations at *pH* ranging from 3 to 12 indicated that within the whole *pH* range studied no precipitation occurred, however, a significant excess of ligand was still applied (as for the Cu/*Put* system, in the binary Cu/*Ado* system precipitation was observed in the basic medium).

Table 1 shows stability constants for particular complex species. These constants were obtained from computer analysis of the potentiometric titration curves. The data confirm the presence of the ML_2OH form (they are in perfect agreement with the results obtained from the analysis of binary systems) and allow to determine the values of stability constants of MHL, ML, and ML_2 species. The ranges where the two simple complexes, i.e. ML and ML_2 predominate, practically overlap the precipitation ranges in binary systems.

On the basis of the obtained values of stability constants, a diagram of complex species distribution in the system under study was constructed (Fig. 1). For the sake of clarity, all the compounds containing adenosine were omitted in the diagram because in this part of the study, the adenosine ligand was considered only a factor preventing a solid phase formation. However, in further considerations it should be remembered that this distribution diagram concerns a ternary system. Therefore, as a result of competitive metal-amine-adenosine reactions, the concentrations of the analyzed species are relatively low. The MHL complex can be formed at a *pH* of about 6 and tends to dominate at a *pH* ca. 7. It binds, at maximum, about 10% of metal (in systems without adenosine the value does not exceed 20%). The fact that measurements can be carried out only in a narrow *pH* range accounts for the difficulties in determining equilibrium constants in binary systems. ML_2 dominates at *pH* ca. 8.5 and then binds ca. 21% of metal. ML_2 and ML_2OH dominate at a *pH* of 9.5 and 10.5, respectively. A hydroxocomplex is formed beginning from *pH* about 9.5 as a result of the reaction $ML_2 + OH^- \rightleftharpoons ML_2OH$, and unlike the hydroxo-complexes formed at *pH* range 7–9, it is water-soluble.

As we have already stated, the ML_2OH complex has a coordination of the 3N or 4N type (at higher *pH*). In the last case a planar-square coordination, in which

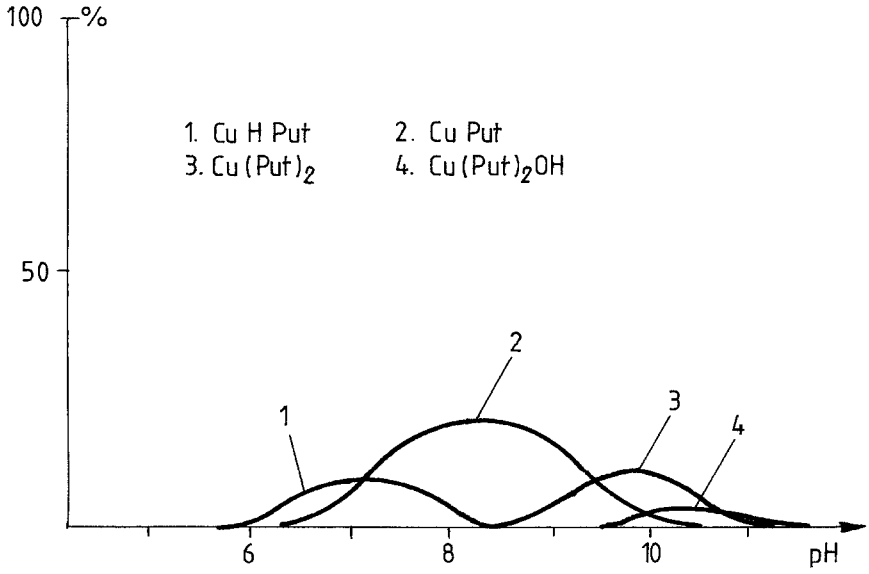

Fig. 1. Distribution diagram for the *Put*-Cu system; percentages of the species refer to total metal; $c_{Put} = 1 \cdot 10^{-3} M$, $c_{Cu} = 4 \cdot 10^{-4} M$

all nitrogen atoms from both putrescine molecules are involved, is favored due to a total ligand deprotonation (H^+ does not compete with Cu(II) at high *pH*). As follows from the distribution diagram, the concentration of ML_2OH in the ternary system is very low. In the binary system, on the other hand, due to the absence of an additional complexing agent (adenosine) and higher concentration of the compound, it is possible to measure absorption spectra (VIS) and to determine the coordination mode [26]. A comparison of the values of $\log \beta$ of a simple *ML* putrescine complex with copper (II) (Table 1) and of corresponding values obtained for 1,3-diaminopropane (*Put*-3) [25, Table 1] clearly shows that in the Cu*Put* complex a seven-membered chelate ring is formed (the difference in stability constant values equals only to one unit of $\log \beta$). A lower stability of the *Put* complex with respect to *Put*-3 results from a formation of a six-membered ring in the case of the latter. Unfortunately, due to the presence of adenosine in the system, it is not possible to confirm this coordination mode by means of electron spectroscopy.

As the issue of formation of putrescine coordination compounds with metals in solution [17, 18] became controversial, we decided to confirm this reaction by using other ligands which might improve the ability to observe this reaction. When cytidine (*Cyd*) and deoxycytidine (*dCyd*) were used, precipitation took place beginning from a *pH* of about 7.8. Likewise, the results of computer analysis proved the presence of a protonated *MHL* compound. The following $\log \beta$ values were obtained for these compounds: 15.92(14) for the system with cytidine, and 15.97(17) for the one with deoxycytidine. When deoxyadenosine was added as the third component, precipitation occurred already at a *pH* ca. 7 (similarly, as in the case of the binary system). The observed differences in *Ado* and *dAdo* behavior are a subject of our further investigations.

The position of absorption bands of electron spectra (VIS) in a weakly acid medium ($\lambda_{max} = 750$ nm) is indicative of the presence of noncoordinated copper [26].

However, at *pH* ca. 7 a band shift to 705 nm is observed, which corresponds to a coordination of the 1N type [18, 21] and to a formation of the *MLH* complex without a chelate ring. Similar values of log β obtained for the *MHL*-type complexes of putrescine (15.83) and 1,3-diaminopropane (15.78) [25] prove the formation of analogous compounds. The differences in the length of the methylene chain are not of significant importance, as the metal is attached only to one nitrogen atom.

In the Cu*HPut* complex an increase in the lability of the hydrogen atom is observed; this is associated with a change in the electron charge density due to the presence of metal ion in the compound. The protonation constant, $K^{H}_{MHL} = \beta_{MHL}/\beta_{ML}$, of the reaction $ML + H^+ = MHL$ is equal to 7.21 (for the ligand $pK_1 = 9.68$, $pK_2 = 10.83$). When comparing the complex with the ligand itself, which in the physiological solution (*pH* ca. 7.4) occurs mostly in the protonated form, one can see that the possibility of the complex interaction with other bioligands changes drastically. Instead of a symmetrically distributed charge, as in *Put*, one of the nitrogen atoms is mostly deprotonated, while at the other end of the molecule a significant positive charge (Cu^{2+}) is accumulated.

Using the obtained values of stability constants and the HALTAFALL computer program (to calculate species concentration) the molar absorptivity value of the Cu*HPut* complex was determined as 197 $dm^3\,mol^{-1}\,cm^{-1}$ (at *pH* 6.37, $\lambda_{max} = 735$ nm, i.e. when *MHL* is the only compound in the solution).

The results of ^{1}H NMR and ^{13}C NMR measurements, which are presented in Fig. 2 and Table 2, confirm the conclusions drawn hitherto. The ^{1}H NMR spectrum of putrescine shows two signals (symmetric amine): one at 4.96 ppm corresponds to equivalent protons at C1 and C4, and the other at 3.66 ppm to protons at C2 and C3 (Fig. 2a). In the spectrum of the Cu/*Put* complex (*pH* = 6.37) the signal at

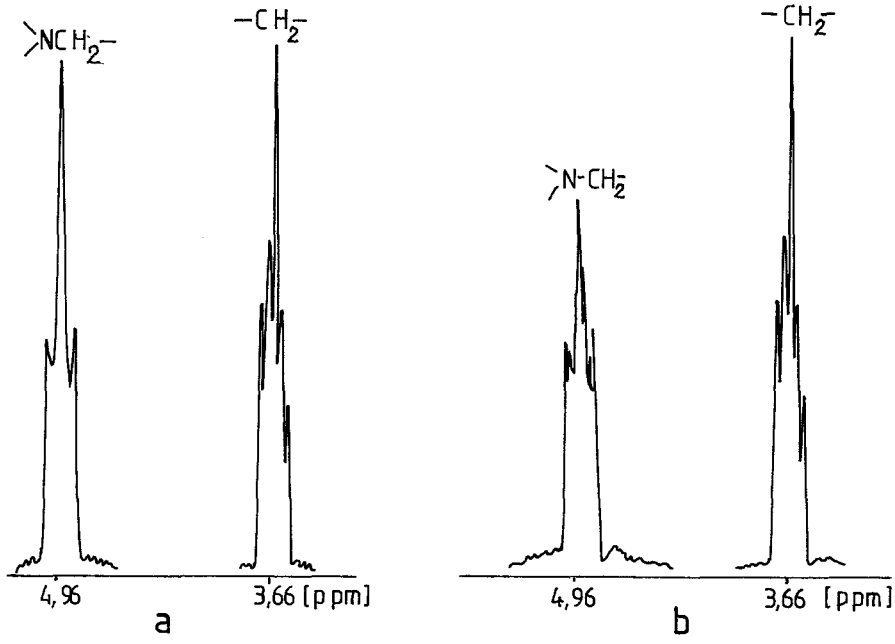

Fig. 2. ^{1}H NMR spectra: a – *Put*, b – Cu/*Put*; $c_{Put} = 0.02\,M$, $c_{Cu} = 0.0004\,M$, *pH* = 6.37

Table 2. ^{13}C NMR signals positions of putrescine, NH_2–C(1)H_2–C(2)H_2–C(3)H_2–C(4)H_2–NH_2, and its complexes with Cu(II), [ppm]

		[C(1), C(4)]	[C(2), C(3)]
Put	($pH = 6.37$)	39.71	24.70
Put/Cu(II)	($pH = 6.37$)	38.76	23.81
$\Delta\delta$		0.95	0.89

lower field undergoes splitting as the result of a bond formation at the neighboring nitrogen atom (Fig. 2b). Because of a fast exchange (on the NMR scale) it is not possible to distinguish protons at C1 and C4 in the protonated Cu*Put* complex. In the ^{13}C NMR spectrum also two signals are observed (Table 2). When the complex is formed both of them are shifted, yet the change in signal from C1 is greater. The differences in chemical shift are slight (but reproducible), which results from the low concentration of copper ions in the system (which is inevitable, as Cu^{2+} is paramagnetic). The signals yielded are a resultant of averaging of the position of ligand and complex signals. The tendency observed in the changes of chemical shifts is in agreement with the results of electron spectra analysis as well as with equilibrium studies and confirm the above formulated conclusions on the reactions studied.

When analyzing the importance of biogenic amines, in particular in the processes of genetic information transfer, one should take into account the formation of metal-bioligand compounds. This may affect both the molecule structure and charge distribution during formation of ion pairs (polyamine-nucleic acid fragment). Moreover, it may also change the interactions between bioligands as the metal may block active sites during the interaction between heteropolar fragments of biomolecules.

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