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Complexation in the copper(II)—L-histidine—D-ornithine system as studied by ESR spectroscopy

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Equilibria in the copper (II)—L-histidine—D-ornithine system were investigated by ESR spectroscopy in an aqueous solution in the pH range 2—11. Analysis of the spectrum lineshape at different pH and ligand to metal ratios showed that the mixed-ligand complexes $Cu(OrnH)(HisH_2)^{4+}$, $Cu(OrnH_2)(HisH)^{3+}$, and Cu(Orn)(His) occur in the system along with the binary complexes. The stability constants, *g*-factors, HFC constants, and relaxation parameters of the complexes were determined, and the structures of the complexes were suggested.

Key words: copper(II), D-ornithine, L-histidine, mixed-ligand complexes, ESR spectroscopy, full lineshape analysis in ESR spectra.

Compounds of biometals with amino acids model the protein—metal complexes in the human and animal bodies. A majority of processes in the living organism include the interaction of the metal ions with several ligands. Mixed-ligand complexes with two amino acids are known[1] to play an important role in the metal transport.

The mixed-ligand complexation in solution are difficult to study by ESR because of a poor sensitivity of the spectrum parameters, *viz*., the line width and position, to changes in the coordination environment resulting in superimposing of the signals from different complexes.

The copper(II) mixed-ligand complexes with amino acids have been studied by polarography,[2] UV and CD spectroscopies,[3] and potentiometry;[4] and their stability constants have been found. The use of ESR allows one to reveal the structural characteristics and thermodynamic parameters of the complexes.

Earlier,[5—9] aimed at the estimation of parameters of the complexes, we used a computer program for modeling the ESR spectra of the binary systems in liquid solutions containing Cu^{II} and an organic ligand at different pH and the metal to ligand ratios. Processing of the experimental data resulted in a good agreement between the theoretical and experimental spectra, enabling us to determine the relative concentrations and stability constants of the complexes formed in the solution.

The purpose of this work was to develop an algorithm for calculation of the characterisctics of complexes in solution from the ESR spectra of the mixed-ligand system containing copper(II) and two amino acids, L-histidine and D-ornithine. The calculation procedure imposes no restrictions on the number and composition of the species involved in various equilibria.

Experimental

The salt $Cu(NO_3)_2$ was prepared by the dissolution of a specimen of metallic copper (analytical grade) in HNO_3 (analytical grade). Hydrochlorides of D-ornithine and L-histidine (both analytical grade) were used. Solutions of amino acids were prepared by the weighing method. The constant ionic strength was maintned by a 1 *M* solution of KNO_3 (reagent grade), and pH was measured by an EV-74 ionomer calibrated with two buffer solutions.

The ESR spectra of solutions were recorded on a Radiopan SE/X 2543 spectrometer with a built-in JTM NMR magnetometer (accuracy of estimation of magnetic induction was $2 \cdot 10^{-5}$ T). The exact frequency value was measured by the spectrum of diphenylpicrylhydrazyl. The experimental spectra were loaded into the computer memory through a special interface attached to the spectrometer.

The ESR spectra of solutions containing copper(II) (C_{Cu} = 0.05 mol L^{-1}) and amino acids in the $C_{Cu} : C_{His} : C_{Orn} = 1 : 1 : 1$, 1 : 1 : 2, 1 : 2 : 2, and 1 : 1 : 4 ratios were recorded at pH from 2 to 11.

Practically all spectra of the solutions under study comprised a sum of the spectra of independent species. In this case, the most reliable method for estimation of the spectral characteristics is a comparison of the theoretical and experimental spectra by their computer modeling. The spectra were interpreted with a specially developed program,[10] which provides for the automatic estimation of the ESR parameters and stability constants from the composite spectrum containing contributions from several complexes.

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The main program units are the module for calculating the molar fractions of the system components by the standard procedure for solving the set of material balance equations;[11] the module for calculating the lineshape of the ESR spectrum; and the module for optimization of the parameters by the scanning and coordinate descent metods.

The experimental ESR spectrum is usually described as a convolution of the Gaussian and Lorentz lines. The theoretical spectrum was constructed by a superposition of the Lorentz curves, and this method gave a satisfactory agreement in all the cases. The correction for nonuniform broadening of the experimental signal was made by introducing the unresolved HFS. The effect of anisotropy of the HFC of an unpaired electron with the metal nucleus on the line width was calculated by the Wilson—Kivelson equation[12,13]

$$\Delta H_i = \alpha + \beta m_i + \gamma m_i^2,$$

where m_i is the projection of the nuclear spin and ΔH_i is the width of the corresponding HFS component.

The relaxation parameters α, β, and γ, the *g*-factor of the species, the HFC constants, and stability constants of the complexes were calculated by their optimization until the minimum of the function *F* was reached

$$F = \frac{1}{N}\sum_{i=1}^{N}\left(Y_i^{\text{exp}} - Y_i^{\text{theor}}\right)^2, \qquad (1)$$

where N is the number of the experimental points and Y_i is the intensity of the corresponding signal in the ESR spectrum.

Simultaneous calculation of many independent parameters is a complicated task. Therefore, we solved the problem by several steps. To enhance an accuracy of the parameters under estimation, we processed simultaneously up to four spectra recorded at different pH and the component ratios.

At the first step, four experimental spectra recorded at the same $C_{\text{Cu}} : C_{\text{His}} : C_{\text{Orn}}$ ratio and close pH values were optimized and the parameters of at most three compounds prevailing in the equilibrium system were calculated. The scanning method, which allows one to avoid falling into the local minimum of the function described by Eq. (1), was used as the optimization procedure at this step.

At the next step, the obtained values were refined by processing the spectra recorded at different pH and compositions of solutions, which can contain greater number of species. This calculation procedure imposes no restrictions on the number of complexes, whose parameters can be estimated simultaneously.

The minimization process was stopped when the further variation of the parameters did not decrease the function *F* and the theoretical and experimental spectra coincided visually. Based on the values obtained in the final steps of variation of the parameters, the errors of their estimation were calculated.

The reliability of the results was provided by the great number of the experimental points (80 spectra containing 800—1000 points were processed for the system with mixed-ligand complexation) and by the low value of *F* (0.003—0.005 for spectra normalized to unity). Figure 1 presents the experimental and calculated spectra for one of the solutions.

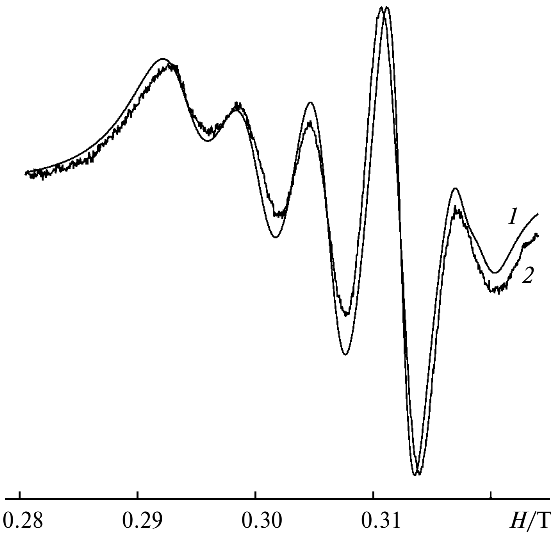


Fig. 1. Theoretical (*1*) and experimental (*2*) ESR spectra of a solution with the $C_{\text{Cu}} : C_{\text{His}} : C_{\text{Orn}} = 1 : 2 : 2$ ratio at pH 2.81.

Results and Discussion

The found parameters of the binary copper(II) complexes with D-ornithine and L-histidine are presented in Table 1. The stability constants are comparable to those determined by other methods (see Refs. 14, 15). These findings were further used for processing of the ESR spectra of complexes containing copper(II) and both amino acids.

The data processing for several series of solutions with different copper(II) to amino acid ratios showed that the best convergence was reached for the occurrence in the system of three mixed-ligand complexes, whose param-

Table 1. Stability constants (*K*) and the structural (*g*, *A*) and relaxation (α, β) parameters of the complexes under study in a 1 *M* the solution of KNO_3 at 25 °C

Complex	log*K*	*g*	*A*	α	−β
			mT		
$Cu(H_2His)$	13.9(2)	2.1579(2)	5.01(2)	3.6	1.1
$Cu(H_2His)_2$	20.0(4)	2.1339(2)	5.53(3)	2.9	0.8
CuHis	10.7(2)	2.1201(2)	6.19(3)	3.0	1.3
Cu(HHis)(His)	10.6(2)	2.1166(2)	7.60(2)	3.2	1.1
$Cu(His)_2$	17.1(1)	2.1191(1)	8.22(1)	3.5	1.0
$Cu(H_2Orn)$	10.4(2)	2.1474(2)	6.01(2)	5.2	0.7
$Cu(H_2Orn)_2$	20.4(2)	2.1263(2)	5.55(2)	3.0	0.8
Cu(HOrn)	7.6(2)	2.1333(2)	5.83(3)	3.9	1.4
$Cu(HOrn)_2$	14.4(1)	2.1286(1)	7.19(2)	3.7	2.6
$Cu(H_2Orn)(HOrn)$	17.5(2)	2.1160(2)	6.22(2)	2.9	1.7

Note. Accuracy of determination of the latter figures is shown in parentheses. Here and in Table 2 the charges of complexes are not shown. $K = ([Cu_pL_qH_r])/([Cu]^p[L]^q[H]^r)$ (L is His or Orn).

Table 2. Stability constants (K) and structural (g, A) and relaxation (α, β) parameters of the copper(II)—L-histidine—D-ornithine system in 1 M solution of KNO_3 at 25 °C

Complex	log$K$*	g	A	α	$-\beta$
			mT		
$Cu(H_2Orn)(H_2His)$	37.4(1)	2.1560(2)	6.02(2)	3.5	0.8
$Cu(H_2Orn)(HHis)$	33.9(1)	2.1141(2)	5.02(2)	2.0	0.7
Cu(Orn)(His)	16.3(1)	2.1197(1)	7.26(1)	4.1	1.1

* $K = ([Cu_pHis_qOrn_sH_r])/([Cu]^p[His]^q[Orn]^s[H]^r)$.

eters are shown in Table 2. The distribution diagrams for the complexes in the series with the $C_{Cu} : C_{His} : C_{Orn}$ = 1 : 2 : 4 ratio are shown in Fig. 2.

Different acid-basic properties of the ligands give rise to different modes of coordination depending on the pH value. The complex in which both ligands are coordinated through the carboxylic group is formed in the pH range 1.5—3.5. The Hamiltonian parameters corresponding to this mode of coordination are presented in Table 2, being in agreement with our data for the binary complexes and the earlier reported data.[16]

The complex $[Cu(H_2Orn)(HHis)]^{3+}$ with the anomalously low HFS constant forms at pH 2—5. According to the reported data,[17,18] this fact can indicate a distortion of the square-planar coordination polyhedron. In our opinion, this is due to the involvement in coordination of the carboxylic group and the N atom of the imidazole ring of histidine to form the seven-membered chelate cycle. According to the literature data,[19] such binary complexes were not found in aqueous solutions but were observed by the IR-spectra in the solid state.[20]

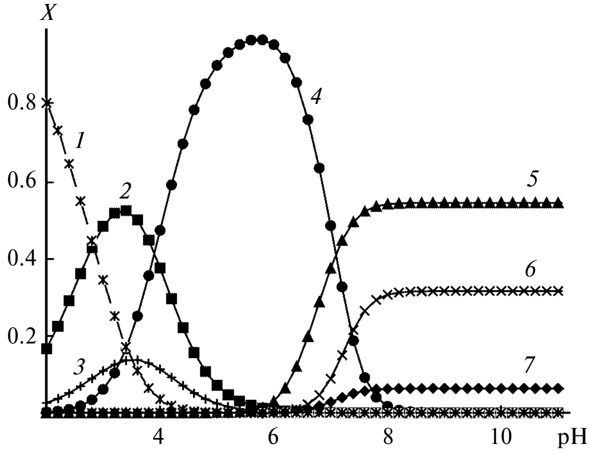
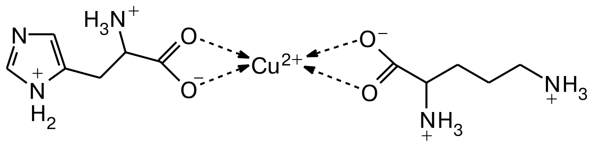
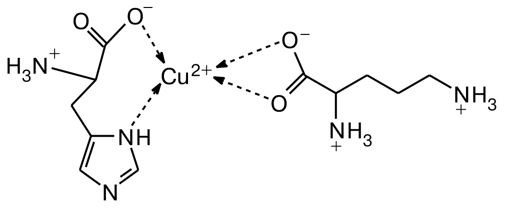


Fig. 2. Molar fractions (X) of the complexes as a function of pH of the solution with the concentration ratio $C_{Cu} : C_{Orn} : C_{His}$ = 1 : 4 : 2: *1*, $Cu(H_2Orn)(H_2His)$; *2*, $Cu(H_2Orn)(HOrn)$; *3*, $Cu(H_2Orn)(HHis)$; *4*, $Cu(HOrn)_2$; *5*, $Cu(His)_2$; *6*, $Cu(Orn)_2$; *7*, Cu(Orn)(His).

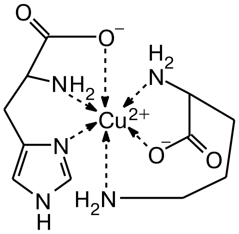
The complex Cu(Orn)(His) occurs in an alkaline medium, and its Hamiltonian parameters correspond to the presence of three nitrogen atoms in the equatorial plane[19] and likely to additional bonding in the axial plane through the carboxylic group of histidine and the ω-amino group of ornithine. At pH > 11 the spectra did not change. This indicates that the H_2O molecule coordinated in the axial position is not deprotonated. Therefore, this indirectly confirms the occupancy of the axial positions by the third donor group of the ligand. The suggested structures of the mixed-ligand copper(II) complexes with L-histidine and D-ornithine are presented below.



$Cu(H_2Orn)(H_2His)$



$Cu(H_2Orn)(HHis)$



Cu(Orn)(His)

Hence, we showed a possibility of studying complicated equilibria by the processing of the data on the full lineshape in a number of poorly-resolved ESR spectra of liquid solutions.

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