Rotational dynamics of copper(II) amino acid complexes by EPR and NMR relaxation methods

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Abstract. Rotational dynamics of the copper(II) bis-complexes with glycine and L-aspartic acid has been studied by EPR and NMR relaxation methods in aqueous solutions at several temperatures. Dynamical parameters obtained by EPR were compared with nuclear magnetic relaxation dispersion (NMRD) results and were found to be in a good agreement. From EPR data dominating trans isomer for Cu(Gly)$_2$ and cis isomer for Cu(L-Asp)$_2^2-$ was found. On the basis of distance of closest approach of protons to central ion inferred from NMRD and crystal structure data the average slope angles of axial water molecule to equatorial plane were calculated and axial coordination of only one water molecule in the Cu(L-Asp)$_2^2-$ complex was established.

1. Introduction
Investigation of dynamical behaviour of molecules in solutions is very important fundamental problem of physics and chemistry of condensed matter. The reason of interest is lack of complete understanding of molecular dynamics depending on solvent nature, structure, and properties of molecules. Also investigations of rotational dynamics in solutions draw big attention because rotation of molecules and their fragments often determine the ways and effectiveness of chemical reactions.

In this work dynamical behaviour of the copper(II) amino acid bis-complexes with glycine and L-aspartic acid in aqueous medium was studied by EPR and NMR relaxation methods. Joint application of these two methods to study dynamics of particles in solution is already known for VO(II) and Gd(III) complexes [1, 2]. Copper(II) complexes with amino acids model many proteins and metal enzymes and serve as transport forms of copper in living cells so their investigation induces additional significant theoretical and practical interest in terms of understanding of living systems.

2. Materials and methods
Copper nitrate, glycine (GlyH) and L-aspartic acid (L-AspH$_2$) from Reanal were used to prepare solutions. The pH was adjusted to 6.90 and 7.29 respectively for copper(II) - glycine and copper(II) - L-aspartic acid systems by adding HNO$_3$ and KOH with an accuracy of 0.01 pH unit. Concentrations of copper(II) and both amino acids were 4.87 mM and 14.84 mM respectively. The salt background 1.0 M was created by addition of potassium nitrate recrystallized from water solution.
Measurements of the pH values were carried out with a Basic Titrino 794 automatic titrator from Metrohm. X-band EPR spectra were recorded on a Bruker ESP 300 spectrometer. Magnetic field was checked by an ER 035M magnetometer and microwave frequencies were measured with a Hewlett-Packard 5335A counter. NMRD measurements (0.1 – 20 MHz) in temperature range (278 – 300K) were carried out on a Stelar Spinnmaster FFC2000 NMR relaxometer located in Polymerphysik laboratory of Institute of Physics at Technische Universität Ilmenau.

3. Results and discussion

3.1. EPR

EPR spectra of the studied compounds were registered in the temperature range 278-323 K. Simulations of the EPR spectra were performed with the modified computer program \cite{3} that allows to determine the spin Hamiltonian parameters ($g_o$, $A_o$, $A_o(N)$), rotation correlation time ($\tau_R$), and percentages of the complexes ($P$). EPR line positions were described by the following isotropic spin-Hamiltonian:

$$\hat{H} = g_o \beta S_i H_z + A_o \sum_i T_i S_i + \sum_i A_i \sum_j T_j S_j$$  \hspace{1cm} (1)

where $g_0$ is the isotropic $g$-factor, $A_o$ and $A_i$ are the hyperfine (hf) and superhyperfine (shf) constants, and other symbols have their usual meaning.

Line positions of single isotope molecule were calculated within the second-order perturbation approach. EPR spectra were fitted by assuming Lorentzian line shape with line width expressed by Wilson and Kivelson theory \cite{4}:

$$\Delta H_{m_i} = \alpha^\prime + \alpha^\prime + \beta m_i + \gamma m_i^2 + \delta m_i^3$$  \hspace{1cm} (2)

where coefficients $\alpha^\prime$, $\beta$, $\gamma$, $\delta$ depend on anisotropy spin-Hamiltonian parameters, microwave frequency, rotational correlation time ($\tau_R$) and $\alpha^\prime$ is the residual line width.

Examples of experimental and simulated EPR spectra of Cu(L-Asp)$_2^{2-}$ complex at different temperatures are presented on Fig.1. Simulations were performed taking into account two isomers with cis and trans arrangement of amino groups. Parameters of spectra simulated for 295K can be seen in Table 1.

**Table 1.** EPR spectra parameters of the Cu(Gly)$_2$ and Cu(L-Asp)$_2^{2-}$ complexes at 295K.

| Complex     | $g_0 \pm 0.0005$ | $A_o$, G | $\tau_R$, $10^{-11}$ s | $A_N$, G | $P \pm 0.05$ |
|-------------|------------------|----------|------------------------|----------|--------------|
| Cu(Gly)$_2$ |                  |          |                        |          |              |
| trans isomer| 2.1288           | 64.4 ± 0.4 | 2.9 ± 0.1 | 9.3, 9.3 | 0.67         |
| cis isomer  | 2.1280           | 75.8 ± 0.5 | 2.9 ± 0.1 | 10.7, 10.7 | 0.33        |
| Cu(L-Asp)$_2^{2-}$ |       |          |                        |          |              |
| trans isomer| 2.1286           | 59.6 ± 0.3 | 6.2 ± 0.2 | 9.1, 9.1 | 0.39         |
| cis isomer  | 2.1274           | 72.5 ± 0.4 | 6.2 ± 0.2 | 10.1, 10.1 | 0.61        |

\(^a\) Rotational correlation times for two isomers are supposed to be the same. 
\(^b\) Errors in $A_N$ values are not more than 1G.

As can be seen from Table 1 trans isomer dominates over cis isomer in the Cu(Gly)$_2$ complex and opposite is true for the Cu(L-Asp)$_2^{2-}$ complex. In our opinion the reason of this is axial coordination of a carboxyl group of ligand in the Cu(L-Asp)$_2^{2-}$ complex. In the case of trans isomer two carboxyl groups locate at one site of coordinated plane and compete with each other for the axial position because of their electrostatic repulsion. In cis isomer such repulsion doesn’t take place (see Scheme 1). So cis isomer is more stable than trans isomer in the Cu(L-Asp)$_2^{2-}$ complex.
Figure 1. Experimental and simulated EPR spectra of copper(II) bis complex with aspartic acid (Cu(L-Asp)$_2^{2-}$) at different temperatures.

Assumed that, temperature dependences of rotational correlation times fulfill Arrhenius law ($\tau_R = \tau_0 \exp(E_a/RT)$) the activation energy of rotational motion of the complexes and $\tau_0$ coefficient were found (see Table 2).

3.2. NMRD
In the Cu(Gly)$_2$ and Cu(L-Asp)$_2^{2-}$ complexes water molecules occupy axial positions and exchange rapidly with other molecules in solution [5]. In the case of Cu(L-Asp)$_2^{2-}$ complex one axial position can also be occupied by carboxyl group of the ligand (Scheme 1).

![Scheme 1](image)

trans isomer  cis isomer  trans Cu(L-Asp)$_2^{2-}$ (1 ax. H$_2$O)  cis Cu(L-Asp)$_2^{2-}$ (1 ax. H$_2$O)

R = H for Gly$^-$
R = CH$_3$COO$^-$ for Asp$^{2-}$

Proton relaxation of axial water molecules results in so called inner-sphere paramagnetic contribution in the observed relaxation times. The modified Solomon-Bloembergen-Morgan equation [6] taken into account $g$-tensor anisotropy of complexes was used to describe this contribution:

$$ (T_{1p}^{-1})_{dd} = \frac{2}{15} \hbar^2 \gamma_I^2 \gamma_S^2 S(S+1)r^{-6} \left( A' \tau_c + B \frac{\tau_c}{1 + \omega_I^2 \tau_c^2} + A'' \frac{\tau_c}{1 + \omega_S^2 \tau_c^2} \right) $$

where $\gamma_I$ and $\gamma_S$ are nuclear and electron gyromagnetic ratios respectively, $S$ is electron spin, $\omega_I$ and $\omega_S$ are the corresponding nuclear and electron Larmor frequencies, $r$ is distance between protons and paramagnetic ion, coefficients $A'$, $A''$, and $B$ depend on $g$-tensor anisotropy and angle between electron-nuclear spin dipole and symmetry axis [6]. Correlation time $\tau_c$ equals $\tau_R$ for studied compounds [7].
To get acceptable fitting it was also necessary to account outer-sphere relaxation due to translational motion of water molecules near the complex coordination sphere. This contribution is described by the following equations [8]:

\[
(T_{1p})_{\omega} = \frac{32}{405} \frac{N_A[M] \gamma_0^2 S(S+1)}{d(D_M + D_L)} \{7J(\omega_D) + 3J(\omega)\},
\]

where

\[
J(\omega) = \frac{1 + 5z/8 + z^2/8}{1 + z + z^2/2 + z^3/6 + 4z^4/81 + z^5/81 + z^6/648},
\]

\[
z = \sqrt{2} \left( \omega \tau_D + \frac{\tau_D}{\tau_S} \right)^{1/2}; \quad \tau_D = \frac{d^2}{D_M + D_L}.
\]

In these equations \(d\) is the distance of closest approach of outer sphere water molecule protons to paramagnetic ion, \(\tau_D\) is the diffusion correlation time, \(D_M\) and \(D_L\) are the diffusion coefficients for the solvent protons and the complex.

On Fig. 2 experimental (points) and simulated (lines) \(T_{1p}^{-1}\) proton relaxation profiles are presented for two solutions of copper(II) bis-complexes with glycine and L-aspartic acid. On Fig. 3 one can see comparison of inner-sphere and outer-sphere contributions for both systems at 284 K.

**Figure 2.** Experimental (dots) and simulated (lines) paramagnetic contributions in \(T_1p^{-1}\) relaxation rates of solvent protons in Cu(Gly)\(_2\) (a) and Cu(L-Asp)\(_2\)\(^{2-}\) (b) water solutions at different temperatures.

**Figure 3.** Inner-sphere and outer-sphere contributions for Cu(Gly)\(_2\) (a) and Cu(L-Asp)\(_2\)\(^{2-}\) (b) at 284K.
The parameters used to model NMR relaxation data are presented on the Table 2. In general there are six parameters for simulation. But it is not correct to vary all of them because they are correlated to each other and several solutions are possible. So in the case of Cu(Gly)$_2$ system we fixed rotational correlation times (namely $\tau_0$ coefficient and activation energy of rotational motion) at the values found by EPR. Then by fitting relaxation data we could obtain distance $r$ and parameters of outer sphere contribution.

**Table 2.** NMRD fitting parameters and parameters found by EPR.

| Complex        | Inner-sphere component | Outer-sphere component |
|----------------|------------------------|------------------------|
|                | $\tau_0$, $10^{-14}$ s | $E_a$, kJ/mol | $r$, Å | $\tau_{0(os)}$, $10^{-14}$ s | $E_{a(os)}$, kJ/mol | $d$, Å |
| Cu(Gly)$_2$    |                        |                       |       |                        |                       |       |
| EPR            | 3.0±0.9                | 16.8±0.7              | 3.50±0.05 | 1.6±0.4                | 18.8±0.5              | 4.01±0.05 |
| NMRD           | 3.0$^a$                | 16.8$^b$              | 3.50±0.05 | 1.6±0.4                | 18.8$^b$              | 4.29±0.05 |
| Cu(L-Asp)$_2$$^{2-}$ |                    |                       |       |                        |                       |       |
| EPR            | 3.2±0.2                | 18.6±0.2              | 3.51 (1 ax. H$_2$O) | 1.6$^b$    | 18.8$^b$              | 4.29±0.05 |
| NMRD           | 3.2±0.4                | 17.5±0.6              | 3.51 (1 ax. H$_2$O) | 1.6$^b$    | 18.8$^b$              | 4.29±0.05 |

$^a$ Values were fixed at those found from EPR.

$^b$ Taken as for Cu(Gly)$_2$ complex.

Using a such scheme in the case of Cu(L-Asp)$_2$$^{2-}$ complex results in non acceptable fitting of relaxation data. So to model experimental data of this system we fixed outer sphere relaxation parameters $\tau_{0(os)}$ and $E_{a(os)}$ at the values found for Cu(Gly)$_2$ system and obtained parameters for inner sphere component and distance $d$.

As can be seen from the Table 2 $\tau_0$ coefficient found from NMR relaxation equals to one obtained from EPR. At the same time the value of activation energy is a little lower than in EPR. The distance of closest approach of protons to paramagnetic ion for outer sphere relaxation a little bigger for the Cu(L-Asp)$_2$$^{2-}$ complex then for Cu(Gly)$_2$ that is in agreement with bigger size of L-aspartic acid and presence of carboxylic groups screened paramagnetic ion from water molecules.

Comparison of rotational correlation times obtained by two methods is presented on Fig. 4. The difference of these values for the Cu(L-Asp)$_2$$^{2-}$ complex due to difference in activation energy is not understood yet and is the subject of the next studying.

**Figure 4.** Temperature dependences of rotational correlation times for Cu(Gly)$_2$ (a) and Cu(L-Asp)$_2$$^{2-}$ complexes (b) obtained by EPR and NMR relaxation methods.
Let us take average length of Cu-O$_{ax}$ bond equalled approximately 2.85 Å for both complexes according to literature data [9,10]. Then using distance of closest approach $r$ of axial water molecule protons found from NMR relaxation data one can calculate the average slope angle of water molecule to equatorial plane of complexes (Cu-O$_{ax}$-H angle). For the Cu(Gly)$_2$ complex this angle equals 127°. It means that water molecule doesn’t slope to equatorial plane of the complex. In the Cu(L-Asp)$_2^{2-}$ complex there are two possible ways of coordination: with two and one axial water molecule. In the first case we again obtain 127° and in the second case only 95°. As this complex has negative charge the axial water molecule should indeed bend to equatorial plane by their hydrogen atoms. So we can conclude that in the Cu(L-Asp)$_2^{2-}$ complex only one axial position is occupied by water molecule while second one is taken up by carboxyl group of the ligand. This result is in agreement with above mentioned EPR data and higher stability constants of the Cu(L-Asp)$_2^{2-}$ ($\log\beta = 16.2$ [11]) relative to the Cu(Gly)$_2$ complex ($\log\beta = 15.2$ [11]).

4. Conclusions
Rather good agreement was found in rotational dynamics parameters of Cu(Gly)$_2$ and Cu(L-Asp)$_2^{2-}$ bis-complexes obtained by EPR and NMR relaxation methods. It was shown that combination of these two methods can be used to obtain reliable fitting parameters of proton relaxation profiles.

As opposed to Cu(Gly)$_2$ the cis-isomer of Cu(L-Asp)$_2^{2-}$ dominates in solution over trans isomer due to electrostatic repulsion of additional carboxyl groups of two L-Asp$^{2-}$ anions in the latter.

On the basis of $T_1^{-1}$ proton relaxation rates simulation and crystal structure data average slope angles of axial water molecules were estimated for the Cu(Gly)$_2$ and Cu(L-Asp)$_2^{2-}$ complexes.

Axial coordination of only one water molecule in the Cu(L-Asp)$_2^{2-}$ complex was confirmed by EPR and NMR relaxation methods.

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