Ph.D. Thesis Abstract

An ESR study of some copper(II) - bioligand equilibrium systems in aqueous solution by the two-dimensional evaluation method: microspeciation and coordination modes

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1. Introduction

In biological systems, copper(II) is mainly bound to proteins, and it catalyses various bioredox processes; in most cases, it is in the active centres of these metalloenzymes. In order to clarify the role of copper(II) in detail, the complexes of smaller ligands as models are frequently investigated instead of the complexes of large biomolecules. Various oligopeptide complexes, e.g., can be good models for the active centres of metalloenzymes. The histidine side-chains of various metalloproteines frequently take part in the copper(II) coordination. Accordingly, I studied the coordination of copper(II) to some oligoglycines, and to the ligands L-histidylglycine and glycyl-L-histidine in aqueous solution in order to model the ligation of the backbone and side-chain donor groups, important in metalloenzymes and other biological systems. The above and related small ligands and their copper(II) complexes themselves also show biological activity.

Copper(II) complexes of bis(aminomethyl)phosphinic acid and of its N-substituted derivatives were also investigated because of the great biological activity of the ligands. Multidentate ligands containing in-chain phosphinate groups are applied as inhibitors of a number of bacterial enzymes, and symmetric phosphinic acids have been found to be powerful inhibitors of HIV protease; bis(L-prolino-N-methyl)phosphinic acid and bis(iminodicarboxymethyl-N-methyl)phosphinic acid, ligands studied in my Thesis, have been shown to have plant growth-regulating properties.

2. Experimental methods

The donor groups of the ligands are in competition for the binding sites of the metal ion, and a number of complexes of different composition and structure can be formed, depending on the metal and ligand concentrations and pH. Frequently, the alternative structures coexist. If we want to elucidate the natures and positions of the donor groups around the central ion, which determine the biological effects of the complexes, we have to apply spectrum decomposition techniques in order to obtain the individual spectra of various species from the experimental curves. For paramagnetic complexes, the electron spin resonance (ESR) spectra recorded in fluid solution offer an excellent possibility for spectrum decomposition. A great advantage of this method is, in contrast to other methods, that we can

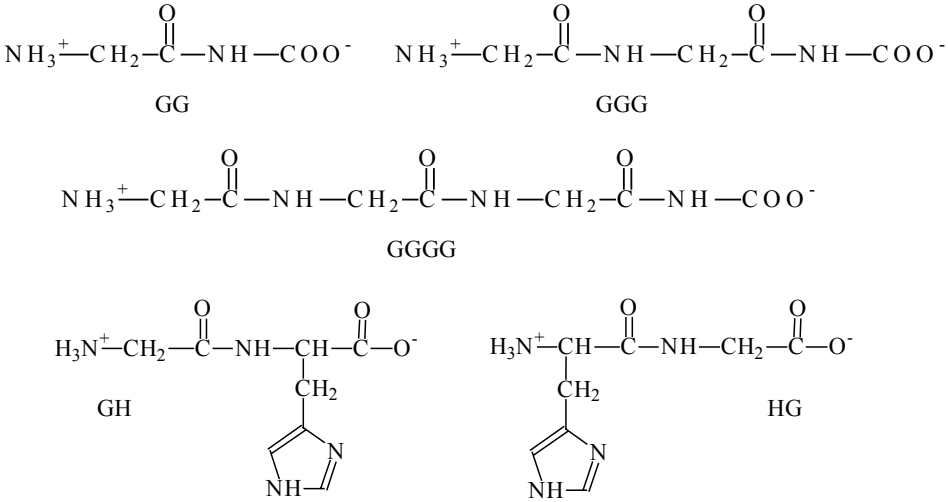
easily distinguish the microspecies or isomers (metal complexes of identical compositions but different coordination modes), and oligomerization processes can be detected easily, too.

I applied a new spectrum decomposition method, the so-called "two-dimensional" ESR simulation which involves the simultaneous analysis of series of ESR spectra recorded at various concentrations and pH's. It gives the formation constants of the metal complexes including isomers; furthermore, it furnishes the magnetic parameters of the ESR-active species, which inform about the nature of the coordinating groups and additional details concerning the coordination modes.

As the number of parameters to be fitted is large, and therefore the unique decomposition of spectra requires good initial values for the parameters, we have to know the main features of the equilibrium model beforehand, as well as possible. Accordingly, I followed the procedure outlined below. a) On the basis of preliminary pH potentiometric data, I chose the optimum conditions (concentrations and pH's) for data acquisition. b) I recorded the spectra in a circulating system at identical settings of the ESR spectrometer in order to ensure the linear relationship between the signal intensity and concentration. c) After some numerical preparation of spectra, I carried out matrix rank analysis (by the method of residual intensities) on the series of spectra. By this model-free, purely mathematical method which does not use any chemical assumption, I obtained the minimum number of independent, ESR-active species. In this way, I could check the pH potentiometric model, and complete it by considering new independent species if necessary. d) Then I carried out the two-dimensional analysis on the spectrum package. When the spectral fit was poor for a smaller group of spectra, I further refined the equilibrium model by taking into account an isomeric (micro) or an oligomerization equilibrium for the complex represented in relatively high concentration in the respective spectra. After the spectral fit could not be further improved, I checked whether each minor complex causes a significant improvement of spectral fit. e) On the basis of the magnetic parameters, I drew conclusions on the natures of coordinating donor groups and other features of coordination modes for the various species.

3. Ligands

The ligands diglycine (GG), triglycine (GGG), tetraglycine (GGGG), glycyl-L-histidine (GH), and L-histidylglycine (HG) were from Sigma. The secunder phosphinic acids (bis(aminomethyl)phosphinic acid (L1), bis(N-glycino-N-methyl)phosphinic acid (L2), bis(N-benzylglycino-N-methyl)phosphinic acid (L3), bis(L-prolino-N-methyl)phosphinic acid (L4) and bis(iminodicarboxymethyl-N-methyl)phosphinic acid (L5)) were prepared in the Department of Inorganic and Analytical Chemistry of the University of Debrecen.



GG GGG

GGGG

GH HG

L1 L2

L3 L4

L5

4. Results

The sensitivity of the two-dimensional ESR evaluation method allowed to identify and characterize a number of new independent species in the systems studied. The majority of them are protonated mono and bis complexes formed in acidic solutions, where one or two ligand(s) is/are bound to the metal ion by weak donor(s) (mostly by carboxylate group(s)), without any change in protonation state. In alkaline media, new deprotonated species were detected in some cases. Equilibria of monomeric and oligomeric complexes could be shown in several systems as well. Furthermore, I have concluded that isomeric equilibria occur very frequently for both mono and, particularly, for bis complexes.

1. The two-dimensional ESR evaluation method allowed an insight in the great structural variety of the copper(II) - oligoglycine complexes, offering data on the microspeciation and coordination modes for 14 metal complexes in each of the copper(II) - diglycine, copper(II) - triglycine and copper(II) - tetraglycine systems.

a) For the mono complexes formed by the stepwise deprotonation of the subsequent peptide groups, structures involving coupled chelate rings are stabilized by particularly strong copper(II) - N in-plane σ-bonds.

b) For the bis complexes, the formation of coupled chelate rings is also favored: i) The tridentate equatorial ligation of the ligand LH_{-1} makes the complex $[CuL_2H_{-1}]^-$ of diglycine predominant in a wide pH range, inhibiting the microprocesses leading to the formation of $[CuL_2H_{-2}]^{2-}$. ii) In the major isomer of $[CuL_2H_{-1}]^-$ of tetraglycine, $(LH_{-2}+LH)$ coordination occurs. iii) The predominant mode of coordination in both isomers of $[CuL_2H_{-2}]^{2-}$ of triglycine and tetraglycine is $(LH_{-2}+L)$.

2. For the copper(II) - glycyl-l-histidine system, the side-chain imidazole group in C-terminal position plays different role in acidic and nearly neutral solutions than in alkaline media.

a) Below pH 10, the presence of the side-chain imidazole group in the glycyl-l-histidine complexes only slightly affects the mode of coordination compared to the simple dipeptides: it is bound to the metal ion only in the $[CuLH_{-1}]$ complex, and in the 'LH_{-1}' ligand of the bis complexes; in these species it displaces the carboxylate group from the third equatorial site. The 'LH' and 'L' ligands in the $[CuL_2]$ and $[CuL_2H_{-1}]^-$ complexes, respectively, are ligated through the amino nitrogen and the peptide oxygen donors, either with the first in equatorial and the second in axial position, or inversely.

b) Over pH 10, the deprotonation of the pirrolic nitrogen causes dramatic changes in the coordination. In equimolar solution, up to pH 12, an equilibrium of three species occurs: the minor monomer $[CuLH_{-2}]^-$ with a deprotonated and equatorially-bound imidazole nitrogen, the corresponding ESR-inactive cyclic tetramer $[Cu_4L_4H_{-8}]^{4-}$ as major species, and the minor mixed hydroxo complex $[CuLH_{-1}OH]^-$ are formed.

3. For the copper(II) - L-histidylglycine system, it has been shown that the competition between the histamine-like N-terminal part and the dipeptide backbone of the ligand results in a variety of coordination modes. The great stability of structures with coupled chelate rings can be observed for this system, too: the dipeptide-like tridentate coordination by the backbone donors is present almost under any conditions (except for strongly acidic media and nearly neutral solutions at ligand excess).

a) In strongly acidic media, the side-chain plays a decisive role by participating in monodentate imidazole binding, and then histamine-like equatorial ligation through the amino and imidazole donors ($[CuLH_2]^{3+}$ and $[CuLH]^{2+}$, respectively).

b) Over pH 3, deprotonation of the peptide group also occurs, with somewhat higher probability than that for histamine-like coordination (in the isomers of $[CuL]^+$). At a ligand excess, in the complex $[CuL_2H]^+$, both the dipeptid-like tridentate coordination and the monodentate imidazole ligation occur in the equatorial sites.

c) At equal metal and ligand concentrations, in the neutral and alkaline region up to pH 12, peptide-like tridentate equatorial coordination by the backbone donors takes over the leading role from the histamine-like binding mode. Here, the side-chain is only a bridging group, though a strong one (active dimer $[Cu_2L_2H_{-2}]$), and even this role ceases in strongly alkaline solution (hydroxo complexes $[Cu_2L_2H_{-3}]^-$ and $[CuLH_{-2}]^-$).

d) At a ligand excess, in nearly neutral media, the N-terminal part and histamine-like coordination achieves an advantage over the backbone: the diequatorial coordination of the first ligand L is accompanied by diequatorial or equatorial-axial ligation of the second ligand (isomers of $[CuL_2]$).

e) At a ligand excess above pH 9, the backbone again predominates in the deprotonated ligands of the above-mentioned mono complex and binuclear species, and $[CuL_2H_{-1}]^-$). In the latter case, histamine-like binding of the second ligand also takes place.

4. The coordination modes and stabilities of the copper(II) complexes of N-substituted bis(aminomethyl)phosphinates can be interpreted as the resultants of a number of various effects such as strong coordination ability of one, two or three donor groups on both ends of

the ligands, tension and rigidity of structures with coupled chelate rings influenced differently by the non-coordinating substituents, different basicities of the N donors, competition between the phosphinate and carboxylate groups for the copper binding sites.

a) In the event of nonsubstituted terminal amino groups (ligand L1), various mono and bis complexes are formed, where the deprotonated ligand is bound in a diamin-like way, while aminophosphinate-type coordination occurs for the ligand with one amino group protonated.
b) When each amino group bears a carboxymethyl substituent, mono complexes and the aminocarboxylate binding mode are favored (ligands L2, L3 and L4). Simultaneous coordination of all terminal donors appreciably enhances the stability of the complex CuL, particularly for L2. At the same time, the steric effect of the noncoordinating substituents on the N atoms (L3), or the rigidity of the ligand with the amino group built into a ring (L4), in part counterbalance the former effect: at a ligand excess dimerization occurs, while at a metal excess the binuclear complex Cu_2L is formed in significant amounts.
c) Two carboxymethyl substituents on each N atom (L5) make tridentate coordination of the terminal parts favoured. This binding mode is observed mainly in the binuclear and dimer species, and is accompanied by considerable distortion of the complexes.

Publications connected to the Thesis

Articles:

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4. T. Szabó-Plánka, A. Rockenbauer, **N. V. Nagy**, G. Peintler, I. Nagypál, L. Korecz: Use of the two-dimensional EPR evaluation method in the study of equilibria of paramagnetic metal complexes, *Progress in Coordination and Bioinorganic Chemistry,* **6**, 175-180 (2003), Eds. M. Melník and A. Sirota, Slovak Technical University Press, Bratislava, Slovakia. IF=0

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2. **Nagy N.**, Plánkáné Szabó T., Rockenbauer A. és Korecz L.: A képződési állandók és a koordinációs módok ESR-spektroszkópiai meghatározása a réz(II) - L-hisztidil-glicin egyensúlyi rendszerben (lecture in Hungarian: ESR spectroscopic determination of formation constants and coordination modes in the copper(II) - L-histidyl-glycine equilibrium system), *XXXV. Komplexkémiai Kollokvium (XXXVth Colloquium on Coordination Chemistry),* Kecskemét, 2000

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Articles:

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2. Zs. Árkosi, T. Szabó-Plánka, A. Rockenbauer, **N. V. Nagy**, L. Lázár, F. Fülöp: A two-dimensional EPR study of copper(II)-β-substituted β-amino acid systems. Steric effects of the substituents on equilibria of metal complexes. *28th International Conference on Solution Chemistry,* Debrecen, Hungary, 2003 p. A-P2