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Spectroscopic and Magnetic Resonance Studies on Ni(II), Cu(II) and Pd(II) Complexes with Gly-Leu-Tyr and Tyr-Gly-Gly Tripeptides

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The structure of tetragonal Ni(II), Cu(II) and Pd-(II) complexes with Tyr-Gly-Gly and Gly-Leu-Tyr has been established by absorption spectroscopy, NMR and EPR methods.

The aromatic ring interaction with the metal ion has been proposed and it was found to be most effective for Ni(II). In Cu(II) Gly-Leu-Tyr solution the dimer formation occurs at pH 8–10 and the dimer suffers decomposition at pH above 12.

In the complexes with all three metal ions the ligands act as tetradentate at pH up to 11 and as tridentate in higher pH regions.

Introduction

Nickel(II), copper(II) and palladium(II) ions are capable of forming similar tetragonal complexes with polypeptides. They have been the subject of extensive studies for several years [1–22]. In the presence of the peptide containing three or more amino acid residues nickel(II) undergoes a stereochemical change from octahedral to planar with peptide linkage deprotonation [12–18, 21, 22]. The Cu(II) is the only ion among those under consideration, whose coordination number could exceed four upon the coordination of tri- and tetra-peptides. The predominant influence on the tetragonal Cu(II) complex properties, however, could be expected to derive from the nitrogen donors coordinated in the complex plane.

The palladium(II) ion is the most effective in induction of the amide nitrogen ionization [7] and its complexes are usually planar.

This paper presents the absorption spectra, NMR and EPR results for Ni(II), Cu(II) and Pd(II) complexes with two tripeptides containing tyrosine residue at the N or C peptide terminal.

The tyrosine residue side-chain plays the important role of inducing the peptide conformation in Pd(II) and Cu(II) complexes with dipeptides [24, 25]. The use of tripeptides would also allow to involve the Ni(II) planar complexes into this studies and to compare their structure with that of the Cu(II) and Pd(II) complexes.

Experimental

K_2PdCl_4, $Ni(ClO_4)_2 \cdot 6H_2O$, $Cu(ClO_4)_2 \cdot 6H_2O$ and both tripeptides were obtained from Fluka AG.

The absorption spectra were recorded on a UNICAM SP-700 spectrophotometer. The NMR and EPR spectra were recorded on JEOL 100 MHz JNM PS-100 and JES-ME-3X spectrometers, respectively. All NMR spectra were recorded at 26 ± 1 °C using t-butyl alcohol as an internal standard. The EPR spectra were recorded at 120 K. The pH was measured on a Mera-Elmat N-512 pH-meter. Analyses of the NMR and EPR spectra were carried out on a JEC-6 computer.

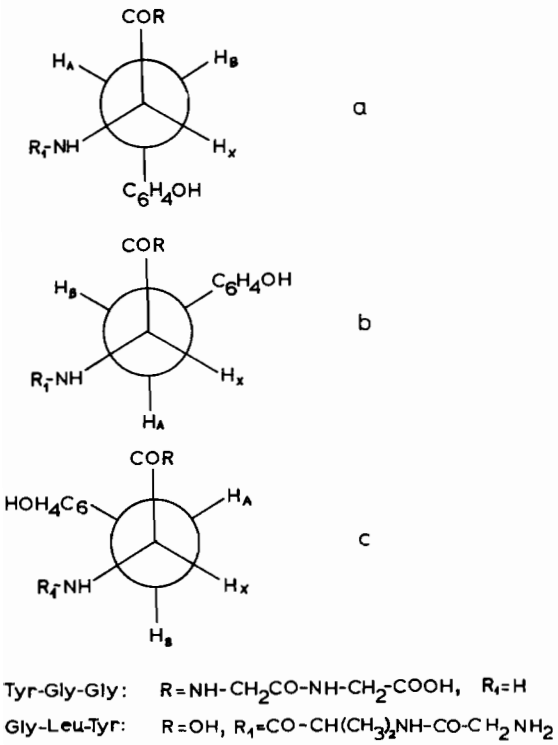
Results

Metal-free Tripeptides

The NMR spectrum of the tyrosine αCH-βCH_2 protons is of ABX type in glycyl-L-leucyl-L-tyrosine (Gly-Leu-Tyr) and of A_2X or degenerated ABX type in L-tyrosyl-glycyl-glycine (Tyr-Gly-Gly). In the latter tripeptide the spectra of the above mentioned protons are of ABX type only in the narrow pH range from 9.0 to 9.5.

The conformation of tyrosine residue in both tripeptides was found by the Feeney approximation [23] for the rotamer notation given in Fig. 1. The populations of these rotamers are presented in Table I. The dihedral angles in the rotamers presented in Fig. 1 may be different from 60 or 180°. In some cases deviations of about 5° from the ideal angles were considered to allow for non-bonded repulsions between bulky groups and electrostatic interactions between charged substituents. These allowances, however, have a slight influence on the fractional population data (see references [23, 37]).

In the case of A_2X spectra of tyrosine residue in Tyr-Gly-Gly the $(J_{AX} + J_{BX})$ values and the total population of "a" and "b" rotamers (Fig. 1) may be considered. It seems reasonable, however, that p_a is larger than p_b as was found at pH = 9.13 (Table I). In both tripeptides the "a" rotamer should predominate in agreement with the data for the other peptides containing the tyrosine residue [25, 26].



Tyr-Gly-Gly: $R = NH\text{-}CH_2CO\text{-}NH\text{-}CH_2\text{-}COOH$, $R_1 = H$

Gly-Leu-Tyr: $R = OH$, $R_1 = CO\text{-}CH(CH_3)_2NH\text{-}CO\text{-}CH_2NH_2$

Figure 1. The rotamer notation of tyrosine residue in Tyr-Gly-Gly and Gly-Leu-Tyr molecules. The dihedral angles are assumed to be 60° and 180° for simplicity. For more detailed discussion see text and ref. [23, 37].

In Gly-Leu-Tyr peptide solutions the "c" rotamer population was found to increase with increasing pH. At pH above 13 the amount of "c" rotamer was almost the same as that of the "a" one. The population of rotamer "b" was constant within the experimental error. In Tyr-Gly-Gly solutions the rotamer "c" contributed only slightly to the tyrosine residue conformation.

The results reported by Dale and Jones [26] indicate that the influence of other amino acid residues in the polipeptide on the tyrosine conformation is negligible. Thus the interactions within the tyrosine residue are responsible for the rotamer stability. Molecular models of both tripeptides with a planar *trans*-peptide bond suggest that the steric interactions between the aromatic ring and the nearest carbonyl or carboxyl group could be most important in the rotamer population distribution. It is likely that the rotamer "a" population in Gly-Leu-Tyr is larger especially at lower pH values up to 9 because the carboxyl group of the tyrosine residue seems to be more effective in the stabilization of this rotamer than the carbonyl one in Tyr-Gly-Gly.

Nickel(II) Complexes

The absorption spectra of 1:1 Ni(II):Tyr-Gly-Gly molar ratio solutions with metal ion concentration 0.02*M* show an intense band at 23100 cm^{-1} at pH above 7 with a molar absorbance of 255 M^{-1} cm^{-1}. The spectrum is typical of planar diamagnetic Ni(II) complexes with tripeptides [4]. The concurrent octahedral Ni(II) species spectrum is also observed up to pH = 9 with d–d transitions at 9600, 16400 and 25400 cm^{-1}. The donor groups coordinated in octahedral species present in solution are suggested on the bases of the average environment rule using the data given by Bair and Larsen [27].

The first transition energy value, equal to crystal field Δ, is sensitive to the number of nitrogen and oxygen donors coordinated to the nickel(II) ion. The value of 9600 cm^{-1} probably corresponds to two nitrogen donors which could be NH_2 and N^-. PMR selective line broadening at pH above 7 suggests that Ni(II) coordination begins at the tripeptide N-terminal.

The PMR spectrum of 1:1 Ni(II) : Tyr-Gly-Gly solution at pH = 9.5 is characteristic of diamagnetic systems and shows considerable changes of the chemical shifts in comparison with the metal free ligand (Table I). There are upfield chemical shifts for αCHtyr (0.265 ppm) and for both glycine residue methylene protons (0.65 and 0.43 ppm). The glycine protons are of A_2 type in the spectrum of the coordinated ligand whereas $\alpha CH\text{-}\beta CH_2$ tyrosine protons are of the A_2X type (deceptively simple ABX) with $J_{AX} + J_{BX} = 9.0$Hz. Thus the rotamer population of tyrosine residue is quite different in the formed complex from that of the metal-free ligand. The coordination of the metal ion to the peptide molecule may change the dihedral angles in the rotamers. The considered tyrosine side-chain in both tripeptides is not directly involved in the metal ion coordination, therefore any angle variation cannot be important. Thus, although this approximation will lead to some errors in the calculated fractional populations, it is possible to follow the trends in fractional populations for the tyrosine residue both in the coordinated and metal-free tripeptides.

The obtained PMR data of Ni(II) Tyr-Gly-Gly diamagnetic complex indicate that the "c" rotamer population increases upon coordination up to 0.79 and the sum of p_a and p_b decreases down to 0.21.

Ni(II) Gly-Leu-Tyr solutions behave almost in the same way as those mentioned above. The absorption spectra at pH 7 indicate the presence of a square planar Ni(II) complex with (characteristic for these kind of complexes) transition at 23000 cm^{-1} with molar absorbance equal to 225 M^{-1} cm^{-1}. There is also an octahedral species in solutions at pH from 7 to 9 indicated by the transitions at 9800, 16400 and 26800 cm^{-1}. The octahedral complex spectra are identical with those for the above mentioned nickel(II) Tyr-Gly-Gly case. Also in this case the coordination of two nitrogens of peptide molecule to the metal ion in the octahedral species is most probably to be a case.

TABLE I. NMR Data for Tyrosine Residue in Metal-free Tripeptides and Their Complexes.

pH	$\bar{\nu}$A	$\bar{\nu}$B	$\bar{\nu}$X	J_{AX}	J_{BX}	p_a[a]	p_b[a]	p_c[a]
Gly-Leu-Tyr								
0.96	170.2	193.2	338.6	9.6	5.3	0.74	0.16	0.10
2.85	168.0	191.6	334.2	9.5	5.2	0.72	0.16	0.12
4.64	159.8	186.4	315.7	8.6	4.7	0.61	0.13	0.26
9.34	159.1	185.5	314.3	8.7	4.7	0.60	0.11	0.29
10.40	159.2	181.1	312.0	7.8	4.7	0.48	0.13	0.26
13.17	158.4	172.5	302.8	7.6	4.6	0.45	0.13	0.42
Tyr-Gly-Gly								
1.12	190.5		300.8	14.6		1.00		0
4.18	190.5		300.9	14.7		1.00		0
9.13	159.6	165.1	242.2	8.9	5.1	0.64	0.16	0.20
12.88	153.9		236.5	13.6		0.88		0.12
Ni(II) Tyr-Gly-Gly								
10.0	167.0		213.0	10.0		0.20		0.80
Pd(II) Gly-Leu-Tyr								
9.60	179.0	203.5	307.4	8.9	3.6	0.58	0	0.42
10.71	172.8	195.3	306.5	7.7	5.2	0.49	0.19	0.32
Pd(II) Tyr-Gly-Gly								
2.85	181.2	188.3	245.8	9.0	3.3	0.54	0	0.46
9.71	157.8	180.3	241.7	9.6	2.4	0.50	0	0.50
13.35	157.0	178.1	239.8	9.7	3.3	0.65	0	0.35

[a] p_a, p_b, p_c, rotamer populations.

The ^{1}H NMR spectrum of the planar diamagnetic species at pH = 10 is not well resolved. Upfield chemical shifts are observed for CH_2gly (0.437 ppm), αCHleu (0.693 ppm) and αCHtyr (0.523 ppm) protons. The βCH_2tyr multiplet is overlapped with the A_2 singlet of CH_2gly, but the (J_{AX} + J_{BX}) value can be calculated from the αCHtyr multiplet, and is equal to 8.0Hz. Thus the population of "c" rotamer in tyrosine residue in planar Ni(II) Gly-Leu-Tyr complex is even higher than that for the Ni(II) Tyr-Gly-Gly one and is equal to 0.92.

Copper(II) Complexes

The absorption spectra of the solutions of both Cu(II) tripeptides with 1:1 molar ratio show d–d transition at 17900 cm^{-1} in pH region 8–10.5 (Table II). The molar absorbances are equal to 184 and 137 M^{-1} cm^{-1} for Cu(II) Gly-Leu-Tyr and Cu(II) Tyr-Gly-Gly solutions, respectively. This transition energy as well as the molar absorbances are characteristic for Cu(II) complexes with polypeptides with three nitrogen donors bound to the metal ion [5, 13]. At pH over 12 it is expected that hydrolysis of the metal carboxyl bond will occur and it may explain the slight d–d transition energy decrease [24, 28, 29].

TABLE II. EPR Parameters for Cu(II) Tyr-Gly-Gly and Cu(II) Gly-Leu-Tyr Solutions.

Complex	$g_\parallel$	$g_\perp$	A [Gs]	α^2	β^2	$\bar{\nu}$ cm^{-1}
Cu(II) Tyr-Gly-Gly						
pH = 10.2	2.169	2.054	207	0.81	0.63	17800
pH = 12.37	2.216	2.056	165	0.75	0.83	17500
Cu(II) Gly-Leu-Tyr						
pH = 6.46	2.262	2.072	172	0.83	0.86	16900
pH = 10.2	2.205	2.058	190	0.80	0.77	17900
pH = 12.51	2.200	2.052	191	0.79	0.74	17700

The g and A tensors of the EPR spectra for the solutions of both systems studied are of axial symmetry at pH above 8. The spin Hamiltonian parameters are given in Table II. These parameters are similar to those previously obtained for other Cu(II) polypeptide complexes with three nitrogen donors [13, 16, 33]. The EPR spectrum of Cu(II) Gly-Leu-Tyr solution at pH = 10.2 shows a transition with Δm = 2 and the line at g ≅ 2 is relatively broad. As the pH increases up to 12.5 the latter line becomes sharper

and the intensity of the half-field spectrum decreases considerably. A dimer species for Cu(II) Gly-Leu-Tyr system has already been found in the solid state [30]. The absorption spectra ($\bar{\nu}$ = 17900 cm^{-1}) and the EPR parameters suggest that in solution there are three nitrogens coordinated to Cu(II) rather than two as was found for the solid state [30]. At pH above 12 the breaking of the metal carboxyl bond occurs which is probably involved in the formation of the dimeric species.

In the Cu(II) Tyr-Gly-Gly solutions no distinct dimer-like EPR spectrum was observed. A quite large variation of the $g_{\|}$ and $A_{\|}$ values occurs as the pH increases from 10.2 to 12.37 (Table II). According to the absorption spectra, three nitrogens are coordinated to the metal ion also at pH above 12 (Table II). The selective PMR line broadening (for cautions see [34]) has shown that in the case of Cu(II) solutions at pH above 6 the coordination to the metal ion begins at the N-terminal of the tripeptide molecule.

Palladium(II) Complexes

The absorption spectra of a solution of Pd(II) Gly-Leu-Tyr with 1:1 molar ratio at pH 2.54 show a broad absorption, typical of d–d transition, centered at about 30000–32000 cm^{-1} with ϵ = 900 M^{-1} cm^{-1}. At higher pH values (up to 10) the energy of this absorption increases to above 32300 cm^{-1}, overlapping the band corresponding to the transition within the phenol ring [31]. For Pd(II) ion Tsuchida's rule is usually fulfilled quite satisfactorily [32] and the transition energy above 32000 cm^{-1} is reasonably believed to correspond to three nitrogens coordinated to the metal ion [6]. At pH above 13 the d–d transition energy decreases to 31250 cm^{-1} as a result of hydrolysis of the metal carboxyl bond [6].

^{1}H NMR spectra of Pd(II) Gly-Leu-Tyr solutions at pH 9.60 and 10.7 are well resolved and their analysis can be meaningfully performed (Table I). Tyrosine αCH-βCH_2 proton spectrum is of the ABX type and that of CH_2gly is of the A_2 type.

At pH = 9.6 the chemical shifts of CH_2gly (0.442 ppm), CHleu (0.20 ppm) and CHtyr (0.08 ppm) protons are upfield relative to the metal-free ligand in its zwitterionic form, while both βCH_2tyr protons have the downfield chemical shifts of 0.20 (A) and of 0.18 ppm (B) (Table I).

The population of "a" and "c" rotamers at pH = 9.6 is equal to 0.58 and to 0.42, respectively. The rotamer "b" does not exist, at least with Feeney approximation. At pH of 10.71 there is a slight drop of p_a and p_c values to 0.49 and 0.32, respectively, and the rotamer "b" population, p_b, increases to 0.19. At higher pH region the PMR spectra are not well resolved, but it was found that the CHleu proton is shifted upfield by about 0.20 ppm and CH_2gly protons downfield by 0.10 ppm.

This indicates that the hydrolysis of the Pd(II) carboxyl bond causes some deformation of the chelate ring and/or conformational changes of the leucine residue side-chain.

A downfield chemical shift of 0.12 ppm is also observed for the CHtyr proton when the metal carboxyl bond is broken. It may be caused by a conformation variation of the tyrosine residue similar to that found for the Pd(II) Gly-Tyr complex [25].

The absorption spectra of 1:1 Pd(II) : Tyr-Gly-Gly solutions are more resolved than those of the Pd(II) Gly-Leu-Tyr ones. At pH = 2.59 the d–d transition appears at 30900 cm^{-1}. This band may correspond to the complex with two nitrogens (NH_2, N^-) coordinated with the metal ion as was suggested for the Pd(II) dipeptide systems [8]. At pH 6–10 the d–d transition is observed at 33000 cm^{-1} with molar absorbance equal to 1200 M^{-1} cm^{-1}. This transition corresponds to the species with all tripeptide nitrogens bound to the metal ion. At pH above 12 the transition energy decreases to 31400 cm^{-1}, due to the hydrolysis of the metal carboxyl bond.

The ^{1}H NMR spectra of the Pd(II) Tyr-Gly-Gly solutions support these conclusions. At pH = 2.85 there are upfield shifts of CHtyr proton (0.55 ppm) and of CH_2 protons of a central glycine residue (0.197 ppm), and there are no changes of the terminal glycine metylene protons in comparison with the metal-free ligand. It means that at this pH there are only two consecutive nitrogens coordinated to the metal ion, *i.e.* NH_2tyr and N^-. At pH between 6 and 10 the upfield chemical shift of central glycine CH_2 protons increases to 0.30 ppm, and the terminal glycine metylene protons are shifted downfield by 0.08 ppm.

The conformational analysis of tyrosine residue in Pd(II) Tyr-Gly-Gly complexes gives results analogous to those obtained for the Pd(II) Gly-Leu-Tyr system. At pH = 2.85 the population of "a" and "c" rotamers is almost the same, *i.e.* p_a = 0.54 and p_b = 0.46. By increasing the pH up to about 10 the population of the "c" and "a" rotamers changes only slightly and $p_a = p_b = 0.50$.

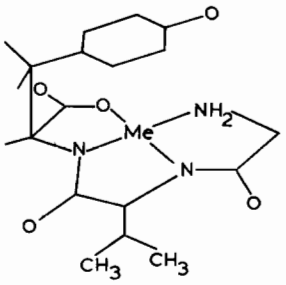
The hydrolysis of metal carboxyl bond occuring at pH above 12 causes an increase of the "a" rotamer population to 0.65. In the whole pH range the "b" rotamer is either absent or its amount is below the experimental error.

Discussion

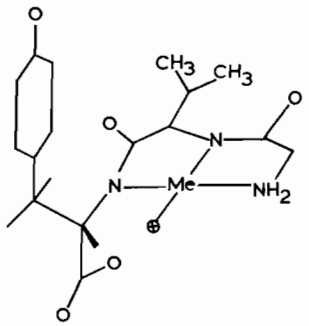
In all three metal ion complexes at relatively high pH, the tripeptides act as a tetradentate ligand coordinating through NH_2, two deprotonated nitrogens of the peptide linkages, and the carboxyl group. The metal–carboxyl bond is hydrolyzed at pH above 12 both for Pd(II) and Cu(II) complexes whereas precipi-

tation in the nickel solutions did not allow us to find the metal–carboxyl group breaking for its complexes.

The conformation analysis of the tyrosine residue suggests rather unusual interactions between the aromatic ring and the metal ion. These interactions stabilize the rotamer "c" as its population is considerably higher in the complex than in the metal-free ligand. When the ligand acts as tetradentate these interactions are observed for the tyrosine residue on C and N terminals of tripeptide molecule (Fig. 2A, 3).



A



B

Figure 2. The conformation of the metal Gly-Leu-Tyr complexes with metal aromatic ring interaction (A) and the most stable rotamer at high pH region (B).

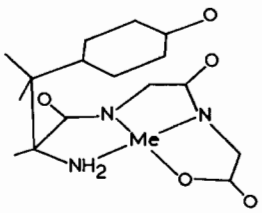


Figure 3. The conformation of the metal Tyr-Gly-Gly complex with metal aromatic ring interaction.

The interaction between the metal ion and the aromatic ring has been already found by X-ray study for Cu(Gly-Leu-H_1Tyr)·$4H_2O$·$1/2Et_2O$ complex [30] and has been suggested through NMR spectroscopy for Pd(II) Gly-Tyr 1:1 complex in water solution [25].

By comparing the population of rotamer "c" for Ni(II) and Pd(II) complexes it may be seen that the former metal ion interacts more effectively with the aromatic ring than the latter one.

The similar ligand conformation might be proposed for the cupric complexes. The d–d transition energy values and EPR parameters are consistent with the coordination of three nitrogen in the basal plane whereas only two where found to coordinate in the solid state [30]. By assuming the same structure in solution and in the solid state, the hydrolysis of metal–carboxyl bond should not destroy the dimeric species [30] and the d–d transition energy should be less than 17000 cm^{-1} as for Cu(II) dipeptide systems [24, 27–29, 33] with molar absorbance equal to about 100. The tetrahedral distortion in the basic plane of the square pyramid found in the solid state [30] should additionally diminish the d–d transition energy and should affect the EPR parameters more considerably [24, 38]. The absorption spectra ($\bar{\nu}$ = 16900 cm^{-1}) and EPR parameters found for the species formed in solution at pH = 6.5 are in good agreement with the assumption of two coordinated nitrogens to the cupric ion (Table II).

Thus the data reported here are consistent with the assumption that in the pH region from 8 to 10 the Cu(II) ion coordinates all three tripeptide nitrogens.

The hydrolysis of the metal–carboxyl bond of the Cu(II) Tyr-Gly-Gly complex changes the α^2 and β^2 molecular orbital coefficients (Table II). Both parameters are only of qualitative meaning but their distinct variation would suggest some more drastic changes of the chelate ring conformation which could decrease the $A_{\parallel}$ value rather considerably [24, 25]. For the Cu(II) Gly-Leu-Tyr complex the changes of EPR parameters are within the experimental error.

More information about the effect of hydrolysis on the complex structure was obtained for Pd(II) complexes. The decrease of the rotamer "c" population in the Pd(II) Tyr-Gly-Gly complex with unbound carboxyl group may be caused by the repulsive interaction of this negatively charged group with the aromatic ring. In the case of Pd(II) Gly-Leu-Tyr complex a downfield chemical shift of αCHtyr proton at pH above 13 should also suggest that the "c" rotamer population decreases [25]. The breaking of the metal–carboxyl bond would lead to similar changes as those found in the Pd(II) Gly-Tyr complex, *i.e.* the "a" rotamer population will increase and the aromatic ring will be placed in the pseudoaxial position in relation to the complex plane (Fig. 2B) [25].

Consideration of the glycine methylene proton spectra in Ni(II) and Pd(II) complexes (A_2 type in all cases) allows to suggest that the chelate rings formed by this amino acid residue are almost planar in agreement with the results achieved for the other similar systems [2, 4, 25, 36]. It is also possible that the

exchange rate between different chelate ring conformers is fast enough to average the spectra to A_2 type.

Thus the most important conformational changes are to be expected for the tyrosine residue because of its bulky aromatic ring and its possible interactions with the metal ion.

These unique properties of the tyrosine residue may be responsible for the mechanism of the enzymatic activities of the tyrosinase [35] or of other enzyme systems where this amino acid residue is adjacent to an active center containing a metal ion.

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