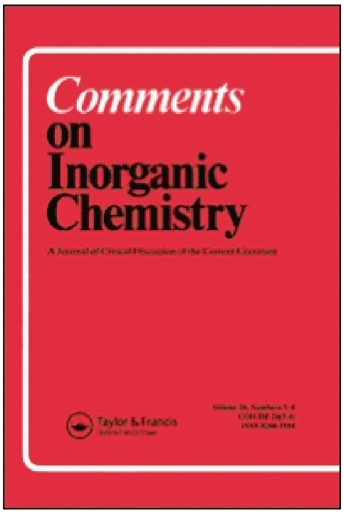

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Metal complexes of peptides containing monodentate or chelating imidazole nitrogen donors: Factors influencing the coordination of amide groups and imidazole side chains

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Metal Complexes of Peptides Containing Monodentate or Chelating Imidazole Nitrogen Donors: Factors Influencing the Coordination of Amide Groups and Imidazole Side Chains

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Transition metal complexes of peptides containing monodentate or chelating imidazole side chains have been studied by the combined application of potentiometric and spectroscopic techniques. The results obtained on the complexes of peptides containing C-terminal histidyl residues (Gly_3His, Gly_4His and Gly_5His) provided clear evidence that both amino and imidazole functions are effective metal binding sites. The formation of various macrochelates were described *via* the coordination of both termini, but the major species were characterised by 4N-coordination starting from the N-termini. The coordination chemistry of a series of peptide molecules containing bis(imidazolyl) agents revealed that the donor functions of the peptide backbone cannot compete with chelation of the bis(imidazolyl) residue. However, the presence of terminal amino group promotes amide coordination, while imidazole residues act as additional donor sites or bridging ligands.

Keywords: *peptides; histidyl residues; bis(imidazolyl) agents; amide group; copper(II); nickel(II) and zinc(II) complexes*

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INTRODUCTION

Peptide molecules are selective and very effective ligands for a wide range of metal ions, because they offer a big variety of potential metal binding sites. The investigation of the interactions between metal ions and peptides was stimulated by its biological relevance and the most important results have already been reviewed by several authors.[1–7] It is obvious from these studies that the metal binding ability of peptide molecules is affected by many external and internal factors including the properties of the metal ions, the presence of primary ligating groups or anchors and especially the presence of other additional donor functions in the side chain residues. It has already been well documented that among the labile metal ions palladium(II) and copper(II) are the most effective to induce deprotonation and coordination of the amide functions of peptides. In the case of palladium(II) complex formation takes place under strongly acidic conditions (pH ~ 1–3) and the stoichiometry of the complexes is largely affected even by the non-coordinating side chains.[8] Copper(II) complexes of peptides are generally formed in slightly acidic solutions (pH ~ 4–5) and the coordination takes place *via* the terminal amino, deprotonated amide and carboxylate donor groups with both metal ions.[1,2] The coordination chemistry of the longer peptides is described by the metal binding of the subsequent nitrogen donors starting from the terminal amino group. The terminal amino group is, therefore, considered as a primary ligating group or anchor for metal binding and the N-protected peptides generally form much less stable complexes.

The metal binding pattern of the peptide backbone is, however, very much affected by the number and locations of other donor functions present in the side chain residues. In this paper we aim to summarise the role of imidazole nitrogen donor atoms in peptide molecules and raise the most important issues connected to the metal binding of histidyl residues. It is well known that imidazole nitrogen donor atoms are among the most common metal binding sites in metalloenzymes. As a consequence, the di- and tripeptides containing histidyl residues have already been thoroughly studied both in solution and in the solid state.[7] It turns out from these studies that stable histamine-like coordination of N-terminal histidyl residues generally suppresses deprotonation and coordination of the peptide amide groups. The insertion of histidine into the second or third position of a peptide chain, however, results in the enhanced metal binding ability of the ligands and the deprotonation of the peptide amide groups takes place at physiological pH even in the zinc(II) or cobalt(II) complexes.[9] The outstanding metal binding ability of these ligands was explained by the equatorial coordination of 3N (for GlyHis) and 4N (for GlyGlyHis) donors, including the terminal amino, one or two deprotonated amide and imidazole nitrogen atoms instead of the terminal carboxylate residues.[7]

However, in the case of tetra- or longer peptides the overall number of possible nitrogen donors is higher than the number of equatorial coordination sites, which may result in a competition between the N- and C-termini for metal binding. Therefore, in the last few years we have performed systematic studies on the copper(II) and nickel(II) complexes of tetra-, penta- and hexapeptides containing histidyl residues at the C-termini (Gly_3His, Gly_4His and Gly_5His).[10] In another group of our studies the amino acid or peptide ligands were linked to the chelating agent bis(imidazol-2-yl)methane, which is a very effective 6-membered chelator for a wide variety of metal ions. The results obtained on the transition metal complexes of these peptide derivatives make it possible to compare the metal binding abilities of peptide backbones and the chelating agents.[11–14]

COPPER(II) AND NICKEL(II) COMPLEXES OF OLIGOPEPTIDES CONTAINING HISTIDYL RESIDUES AT THE C-TERMINI

It has already been noted that the di- and tripeptides containing C-terminal histidyl residues form very stable metal complexes *via* the coordination of all possible nitrogen donors. The structures of the $[MH_{-1}L]$ complexes of GlyHis were determined by X-ray crystallography and the $[NH_2,N^-,N(Im)]$-coordination was suggested both for copper(II)[15] and palladium(II).[16] The outstanding metal binding ability of GlyGlyHis and related tripeptides with C-terminal histidyl residues were explained by the cooperative deprotonation of the amide groups resulting in 4N-coordination. The $[MH_{-2}L]^-$ complexes having the $[NH_2,N^-,N^-,N(Im)]$-coordinations were identified as the major species with a series of metal ions including copper(II)[17], nickel(II)[18], palladium(II) and gold(III).[19] Another important feature of the metal complexes of these peptides that the N(3)-coordination of the imidazole ring increases the acidity of the pyrrole-type N(1)H group. In the case of

GlyHis it results in the formation of tetranuclear complexes,[20,21] while in the gold(III) complex of GlyGlyHis even the coordinated amino group can deprotonate under mildly alkaline conditions.[19]

Metal complexes of tetra- or longer peptides with histidyl residues are much less studied[22–28] and the results are partly contradictory. The authors generally agree that imidazole-N donor atoms are the primary metal binding sites and the major species are described by the coordination of 4N donor atoms. However, these binding sites may include either the imidazole and amide nitrogens with the free amino group,[24] or the amino and amide nitrogens with uncoordinated imidazole.[28]

For the reliable identification of the metal binding sites of these types of ligands we performed systematic potentiometric and spectroscopic studies on the copper(II) and nickel(II) complexes of Gly_3His, Gly_4His and Gly_5His.[10] The stability constants of the copper(II) and nickel(II) complexes are collected in Tables I and II, respectively, while the species distribution in the various systems is demonstrated by Figs. 1 and 2.

TABLE I Stability constants (log β_{pqr}) of the proton and copper(II) complexes of Gly_nHis (T = 298 K, I = 0.2 mol/dm^3 KCl)

Species	*Gly_2His^a*	*Gly_3His[10]*	*Gly_4His[10]*	*Gly_5His[10]*
[HL]	8.06	8.03	8.05	8.00
$[H_2L]^+$	14.88	14.89	14.98	14.87
$[H_3L]^{2+}$	17.68	17.55	17.88	17.73
$[CuHL]^{2+}$	12.40	12.24	12.16	12.29
$[CuL]^+$	7.60	8.47	8.50	8.11
$[CuH_{-1}L]$	2.50	1.63	2.41	2.63
$[CuH_{-2}L]^-$	−1.55	−5.79	−5.39	−5.06
$[CuH_{-3}L]^{2-}$	–	−16.67	−15.71	−15.67
p*K*(His)	–	3.77	3.66	4.18
pK_1(amide)	–	6.84	6.09	5.48
pK_2(amide)	–	7.42	7.80	7.69
pK_3(amide)	–	10.88	10.32	10.61

a. Data from literature: R.W. Hay, M. M. Hassan and C. You-Quan, *J. Inorg. Biochem.*, **52**, 17 (1993)

TABLE II Stability constants (log β_{pqr}) of the nickel(II) complexes of Gly_nHis (T = 298 K, I = 0.2 mol/dm^3 KCl)

Species	*Gly$_2$His*	*Gly$_3$His*	*Gly$_4$His*	*Gly$_5$His*
$[NiHL]^{2+}$	11.35(1)	–	–	–
$[NiL]^{+}$	–	5.76(1)	5.82(2)	5.46(3)
$[NiL_2]$	–	9.65(2)	9.54(6)	9.06(7)
$[NiH_{-2}L]^{-}$	−6.83(2)	−11.51(1)	−11.35(3)	−10.75(3)
$[NiH_{-3}L]^{2-}$	–	−21.47(1)	−20.97(8)	−19.94(4)
p$K_{1,2}$(amide)	–	8.64	8.59	8.11
pK_3(amide)	–	9.96	9.62	9.19
log(K_1/K_2)	–	1.87	2.10	1.86

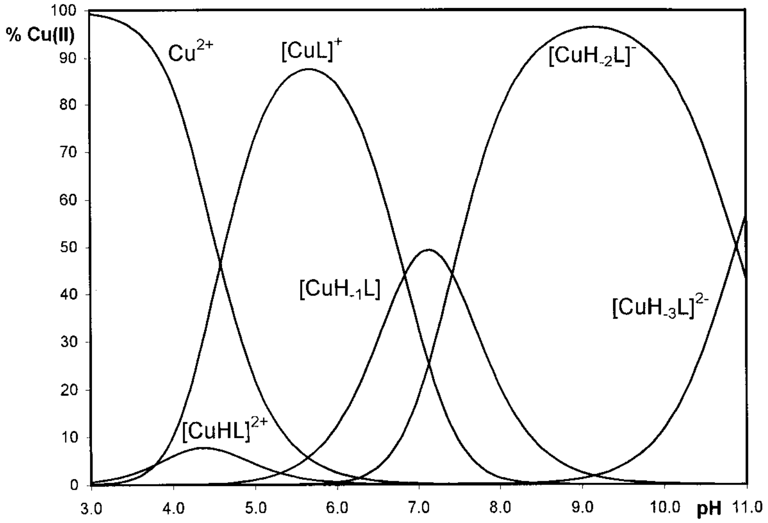


FIGURE 1 Species distribution of complexes formed in copper(II)-Gly_3His system $c_L = c_{Cu(II)} = 4 \cdot 10^{-3}$ mol dm^{-3}

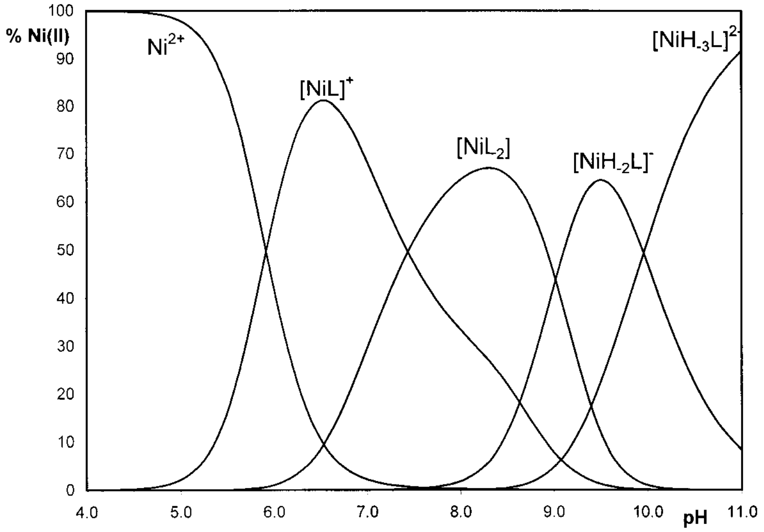
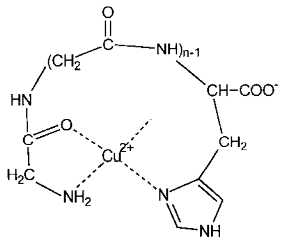


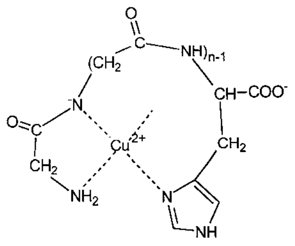
FIGURE 2 Species distribution of complexes formed in nickel(II)-Gly_3His system $c_L = 3 \cdot 10^{-3}$ mol dm^{-3}, $c_{Ni(II)} = 1.5 \cdot 10^{-3}$ mol dm^{-3}

The data in Table I and the speciation in Fig. 1 clearly demonstrate that only 1:1 complexes are formed in the copper(II)-Gly_nHis (n = 3–5) systems. The binding sites of the ligands were identified by UV-Vis and EPR spectroscopic measurements. It is clear from Fig. 1 that $[CuHL]^{3+}$ is a minor species, but the complex $[CuL]^{2+}$ is present in high concentration in all systems studied. The stability constants of the species $[CuL]^{2+}$ are generally significantly higher than those of (NH_2,CO)-coordinated peptide complexes.[29] The EPR spectral parameters ($g_\parallel = 2.299$ and $A_\parallel = 139 \cdot 10^{-4}$ cm^{-1}) strongly support the presence of two nitrogen donors in the equatorial plane of the metal ion, but in a strongly distorted environment. It can be explained by the formation of a macrochelate (**1**), which contains 14, 17 and 20 atoms in the case of Gly_3His, Gly_4His and Gly_5His, respectively. The binding sites of the species $[CuH_{-1}L]$, $[CuH_{-2}L]^-$ and $[CuH_{-3}L]^{2-}$ have been described by the successive deprotonation and coordination of amide nitrogens as they are shown by Structures (**2**) – (**5**).



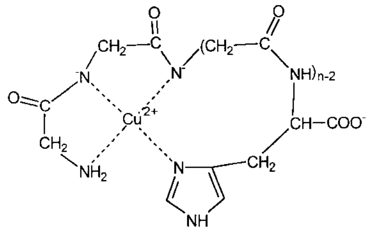
$[CuL]^{+}$ L=Gly$_n$His (n=3,4,5)

(1)



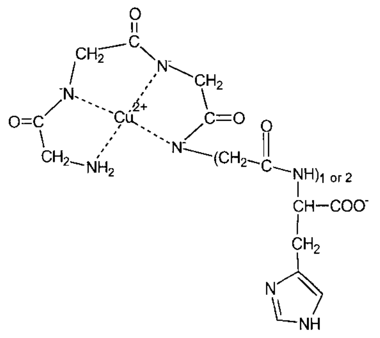
$[CuH_{-1}L]$ L=Gly$_n$His (n=3,4,5)

(2)



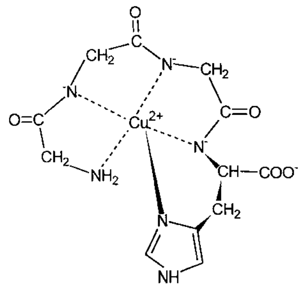
$[CuH_{-2}L]$ L=Gly$_n$His (n=3,4,5)

(3)



$[CuH_{-3}L]$ L=Gly$_n$His (n=4,5)

(4)



$[CuH_{-3}L]$ L=Gly$_3$His

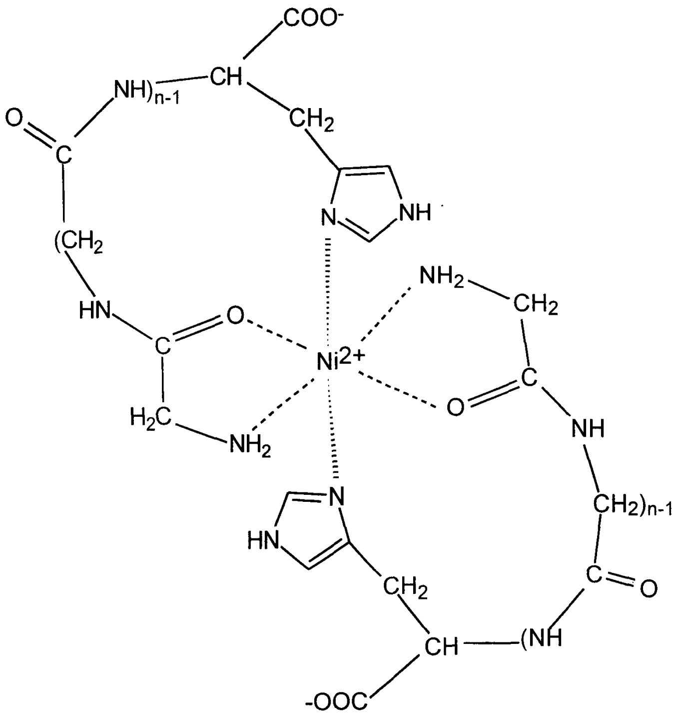
(5)

The species $[\mathrm{CuH_{-1}L}]$ is a 3N complex and the spectral parameters ($\lambda_{\mathrm{max}} = 620$ nm, $g_{\parallel} = 2.227$ and $A_{\parallel} = 156 \cdot 10^{-4}$ cm^{-1} for Gly$_4$His) also support a distorted geometry caused by the macrochelate (**2**). The binding sites of the species $[\mathrm{CuH_{-2}L}]^{-}$ are described in a similar way, but the metal ion is coordinated by 4N donor atoms in this case ($\lambda_{\mathrm{max}} = 560$ nm, $g_{\parallel} = 2.199$ and $A_{\parallel} = 200 \cdot 10^{-4}$ cm^{-1} for Gly$_4$His). In the case of the tripeptide GlyGlyHis the same complex $[\mathrm{CuH_{-2}L}]^{-}$ was identified as the major species both in solution and in the solid state. However, the 4N donor atoms create 3 fused chelate rings (5,5,6-membered) with GlyGlyHis, which results in a significant planarity of the coordination sphere of the metal ion ($\lambda_{\mathrm{max}} = 525$ nm, $g_{\parallel} = 2.178$ and $A_{\parallel} = 209 \cdot 10^{-4}$ cm^{-1} for Gly$_2$His). Another important difference in the complex formation processes of GlyGlyHis and the longer peptides is reflected in the cooperative deprotonation of the amide groups of GlyGlyHis. The coordination chemistry of Gly$_n$His peptides ($n = 3$–5) is, however, characterised by the successive deprotonation and coordination of the subsequent amide groups. Therefore, only the species $[\mathrm{CuL}]^{+}$ and $[\mathrm{CuH_{-2}L}]^{-}$ can be detected in the samples containing copper(II) and GlyGlyHis, while all species are present in measurable concentration with the other ligands. The relative stability of the various species can be evaluated by the comparison of the p*K*(amide) values in Table I. It is obvious from these data that the formation of stable macrochelates slightly suppresses coordination of amide functions. As a consequence, the formation of $[\mathrm{CuH_{-1}L}]$ is the most preferred in the case of Gly$_5$His, because the preceding macrochelate ($[\mathrm{CuL}]^{+}$) is too large (**1**). On the contrary, the formation of $[\mathrm{CuH_{-2}L}]^{-}$ is the most favoured with Gly$_3$His, because of the too small (12-membered) macrochelate of $[\mathrm{CuH_{-1}L}]$ (**2**).

The stoichiometry of the final species is $[\mathrm{CuH_{-3}L}]^{2-}$ in all cases and its spectral parameters correspond well to those of a 4N complex. The p*K* values for the deprotonation of the third amide groups are, however, much higher than those of tetra- or pentaglycine (9.18 and 7.89, respectively).[10] The increase can easily be explained by the effect of the 4N-macrochelate (**3**), which slightly suppresses the rearrangement of the coordination sphere of the metal ions. There are, however, two possibilities for the coordination of the four nitrogen atoms in $[\mathrm{CuH_{-3}L}]^{2-}$. Namely, it can be described from the C-termini with uncoordinated amino group[24], or from the N-termini with uncoordinated imidazole residue.[28] The spectroscopic parameters of the complexes and their comparison with the data obtained for oligoglycines, however, provide an unambiguous proof for the latter type of coordination. The spectroscopic data of the species $[CuH_{-3}L]^{2-}$ with Gly_4His and Gly_5His are almost the same as those of pentaglycine (λ_{max} = 512 nm, $g_{\|}$= 2.171 and $A_{\|}$ = $206 \cdot 10^{-4}$ cm^{-1}).[29] On the contrary, the species formed in the copper(II)-Z-GlyGlyHis system, which contains one imidazole and three amide nitrogen donors, has significantly different spectral parameters (λ_{max} = 540 nm, $g_{\|}$ = 2.183 and $A_{\|}$ = $202 \cdot 10^{-4}$ cm^{-1}).[30] These observations support that the species $[CuH_{-3}L]^{2-}$ of Gly_nHis ($n > 2$) peptides are always 4N complexes with (NH_2,N^-,N^-,N^-) binding sites. In the case of Gly_4His and Gly_5His the uncoordinated imidazole residue is too far from the metal centre **(4)**, while a weak axial interaction of the C-terminal imidazole nitrogen atom is expected in the copper(II) complex of Gly_3His **(5)**. It is reflected by the red shift of the characteristic absorption maxima (λ_{max} = 550 nm) and the appearence of a poorly resolved EPR spectra caused by the strong rhombic distortion.

The comparison of Tables I and II reveals that the stoichiometries of the nickel(II) complexes are very similar to those of copper(II). The major difference is the formation of the stable bis(ligand) $[NiL_2]$ complexes, which were not present in any copper(II) containing systems. The regular octahedral geometry of nickel(II), however, makes the tridentate coordination of the ligands possible **(6)** *via* the metal binding of both N- and C-termini in the form of a 5-membered chelate and a macrochelate, respectively. As a consequence, the stability constants of $[NiL_2]$ complexes of Gly_nHis peptides are much higher than those of oligoglycines.

Fig. 2 indicates that the bis(ligand) complex is one of the major species around the physiological pH and its formation suppresses the metal binding of amide nitrogens. Deprotonation of the amide nitrogens takes place only above pH 8, but, similarly to the nickel(II)-GlyGlyHis system, the formation of the 4N complex $[NiH_{-2}L]^-$ is a result of the cooperative deprotonation of two amide functions. This is another significant difference from the corresponding copper(II) complexes, where the species $[CuH_{-1}L]$ also were present in high concentration. On the other hand, the cooperative deprotonation in the nickel(II) complexes suggests a change in the coordination geometry of nickel(II). The absorption spectra (λ_{max} = 440 nm and ε = 150 M^{-1} cm^{-1}) and the magnetic behaviour of the species $[NiH_{-2}L]^-$ strongly support that it is a square planar, diamagnetic nickel(II) complex. Its structure corresponds to that of $[CuH_{-2}L]^-$ **(3)** containing a macrochelate. As a consequence, an addi-



$[NiL_2]$ L = Gli_3His

(6)

tional extra base consuming process can be observed above pH 9, which corresponds to the formation of $[NiH_{-3}L]^{2-}$. It is a square planar, diamagnetic species, again, but the absorption spectra are almost the same as those of tetra- or pentaglycine (λ_{max} = 410 nm). These data support that the metal binding sites in $[NiH_{-3}L]^{2-}$ can be best interpreted by the coordination of 4N donor atoms starting from the N-termini, similarly to the nickel(II) complexes of oligoglycines or to the corresponding copper(II) complexes (**4**).

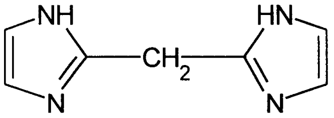
METAL COMPLEXES OF PEPTIDES CONTAINING CHELATING SIDE CHAINS

It is well known that the active sites of a series of copper(II) or zinc(II) enzymes contain two or more imidazole residues. Therefore, the ligands containing two or more imidazole rings linked *via* aliphatic carbon chains can potentially mimic the binding sites and catalytic activities of the enzymes. A series of poliimidazole ligands have been prepared for these purposes and their protonation equilibria and complex formation reactions has been thoroughly studied.[31–38] It is clear from these studies that the ligands containing the bis(imidazol-2-yl)methane chelating agent (abbreviated as "bis(imidazolyl)" ligands) form very stable mono- and bis(ligand) complexes *via* the coordination of 2 or 4 imidazole nitrogens in 6-membered chelate rings. The relative stability of the complexes is generally higher than those of the most common diamines owing to the π-acceptor properties of imidazole rings.

Another advantage and promising application of the bis(imidazolyl) ligands is that they may serve as general enzyme inhibitors. Moreover, specific enzyme inhibitors may be obtained by attaching the bis(imidazolyl) residues to peptides having the preferred amino acid sequence for the peptide cleavage. However, the metal complexes of amino acids or peptides containing chelating side chains have only been scarcely studied,[39] therefore we launched a systematic study on this field a few years ago.[11–14] These studies first involved the preparation and the investigation of the complexation reactions of bis(imidazol-2-yl)methane (BIM) **(7)** as the basic chelating agent. The amino (BIMA, **(8)**) and carboxylate (BIP, **(9)**) derivatives of BIM made it possible to attach the chelating agent to the C- or N-termini of amino acids and peptides *via* the formation of amide bonds. Three different groups of these ligands have been studied so far: (i) N-protected tripeptides of aliphatic amino acids with chelating side chains,[11] (ii) N-protected tripeptides containing histidyl residues and chelating side chains,[13] (iii) derivatives of amino acids with free amino groups and chelating side chains.[12,14]

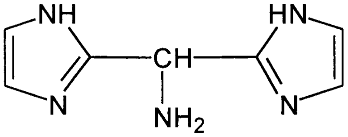
Metal complexes of BIM, BIP and BIMA

The protonation constants of BIM, BIP and BIMA and the stability constants of the corresponding copper(II), nickel(II) and zinc(II) complexes are collected in Table III.



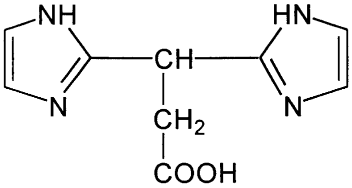
bis(imidazol-2-yl)methane (BIM)

(7)



bis(imidazol-2-yl)methanamine (BIMA)

(8)



3-[bis(imidazol-2-yl)]propionic acid (BIP)

(9)

TABLE III Stability constants (log β_{pqr}) of the proton,[11] copper(II),[11] nickel(II) and zinc(II)[11] complexes of BIM, BIP and BIMA (T = 298 K, I = 0.2 mol/dm^3 KCl)

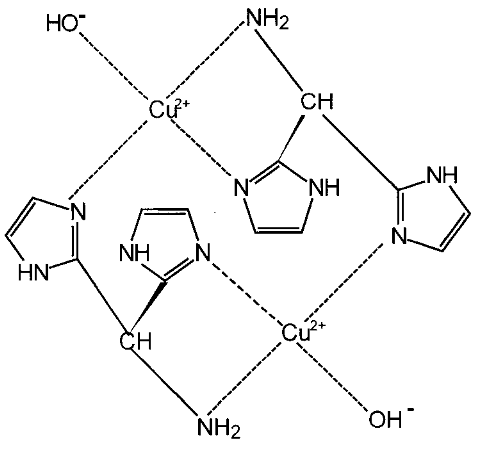
Species		*BIM*			*BIP*			*BIMA*	
[HL]		6.93			6.90			6.49	
[H_2L]		11.67			11.52			10.56	
[H_3L]		–			14.31				
	Cu(II)	*Ni(II)*	*Zn(II)*	*Cu(II)*	*Ni(II)*	*Zn(II)*	*Cu(II)*	*Ni(II)*	*Zn(II)*
[MH_2L_2]	–	–	–	–	–	–	25.21	21.76(6)	18.85
[MHL_2]	–	–	–	21.20	–	–	21.50	18.11(4)	14.25
[ML_2]	17.03	13.46(1)	10.22	17.29	13.64(5)	10.10	16.89	13.33(4)	9.92
[MHL]	–	–	–	13.36	10.84(3)	–	14.0	11.95(2)	9.66
[ML]	9.64	7.29(1)	5.53	10.13	7.76(1)	5.63	9.89	7.24(5)	5.38
[$MH_{-1}L$]	–	– -	–	–	–	–		–0.79(9)	–1.83
[$M_2H_{-1}L_2$]	–	–	–	–	–	–	18.43	–	–
[$M_2H_{-2}L_2$]	–	–	–	–	–	–	12.78	–	–
log(K_1/K_2)	2.25	1.12	0.84	2.97	1.88	1.16	2.89	1.15	0.84

It can be seen from Table III that the deprotonation of imidazole nitrogens of bis(imidazolyl) ligands occurs at lower pH values than that of free imidazole (p*K*(Im) = 6.95). The decrease in the basicity of the nitrogen atoms is especially significant in the case of BIMA because one of the nitrogen atoms cannot be protonated in this ligand in the measurable pH range. It can probably be explained by the electron withdrawing effect of the protonated ammonium group. The propionic acid side chain has much less influence on the p*K* values of nitrogen donors, but the acidity of the carboxylic function significantly increases as compared to that of propionic acid (p*K* = 4.67).

The complex formation processes of the three metal ions with BIM are very similar to each other. Namely, the species $[ML]^{2+}$ and $[ML_2]^{2+}$ are formed in all cases. The stability constants of both mono- and bis(ligand) complexes follow the Irving-Williams series and the formation of tris(ligand) complexes is negligible because of steric requirements. The spectral parameters obtained for the copper(II) complexes of BIM show the coordination of 2N and 4N donor atoms in the species $[CuL]^{2+}$ and $[CuL_2]^{2+}$, respectively. The absorption maxima observed at λ_{max} = 680 and 578 nm, while the EPR parameters are $A_{\parallel}$ = 181 and $199\cdot10^{-4}$ cm^{-1} and $g_{\parallel}$ = 2.307 and 2.237 for $[CuL]^{2+}$ and $[CuL_2]^{2+}$, respectively. It is also important to note that the metal ion coordination of the four equivalent nitrogen donor atoms in the bis(ligand) complexes can be seen very well in the 9-line superhyperfine splitting of the EPR spectra.

The coordination chemistry of BIMA and BIP is very similar to that of BIM and only the characteristic 2N and 4N binding sites can be observed under strongly acidic conditions. The presence of the extra functional groups, however, results in the formation of protonated complexes [MHL], $[MH_2L_2]$ and $[MHL_2]$. In the case of BIP the deprotonation of the uncoordinated carboxylic group is probably followed by a weak axial interaction of the oxygen donor atoms. This is reflected in the enhanced stability of the species $[CuL]^{+}$ and in the increased ratio of stepwise stability constants, but the equatorial coordination of 4N donor atoms remains intact. Nickel(II) and zinc(II) complexes of bis(imidazolyl) ligands have much lower thermodynamic stabilities, which shifts the complex formation reactions to higher pH values. As a consequence, the concentration of protonated complexes is much lower in the case of nickel(II) and they are not present at all with zinc(II).

Complex formation processes of BIMA are much more complicated than those of BIM or BIP. The imidazole-N donor atoms are the exclusive metal binding sites again up to pH 4, but deprotonation of the ammonium group results in the metal ion coordination of this donor function. In the case of the bis(ligand) complex the deprotonation is accompanied with a small red shift of the absorption maxima (λ_{max} = 597 nm for $[CuL_2]^{2+}$). Tridentate coordination of BIMA is even more evident in equimolar solutions. The potentiometric measurements indicate the titration of one more equivalent of base in the pH range 5 to 7, which should correspond to water soluble hydroxo complex formation. The EPR spectra strongly support the formation of a dimeric complex with the equatorial coordination of three nitrogen donors. It is clear from structure **(10)** that the formation of $[Cu_2H_{-2}L_2]^{2+}$ is accompanied with a structural rearrangement of the mononuclear complexes. The dimeric complex contains a 5-membered chelate formed by the amino and one of the imidazoles, while the remaining two coordination sites are occupied by the hydroxide ions and the bridging imidazole residues. The outstanding metal binding ability of BIMA results in the existence of protonated complexes in the nickel(II)- and zinc(II)-BIMA systems, too. The formation of soluble dimeric species, however, can be ruled out, but on the basis of the equilibrium data the tridentate coordination of the ligands can be suggested in the octahedral bis(ligand) complexes.

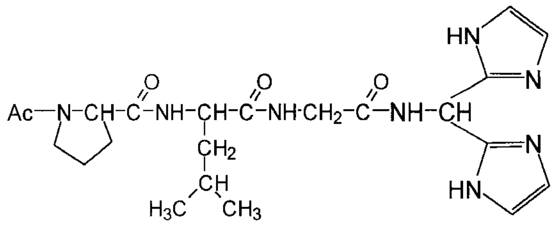


$[Cu_2H_{-2}L_2]^{2+}$ L = BIMA

(10)

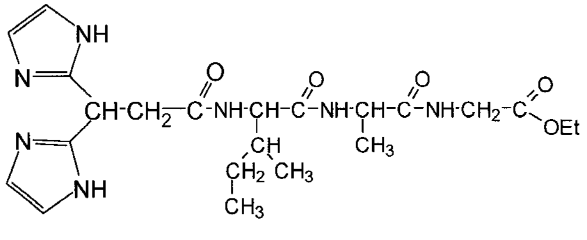
Copper(II) complexes of N-protected tripeptides containing chelating side chains

The peptides involved in this study, Ac-ProLeuGly-BIMA **(11)** and BIP-IleAlaGly-OEt **(12)**, were the fragments of the specific sequence of collagen – ProLeuGly-IleAlaGly – cleaved at the Gly-Ile bond by vertebrate collagenase a zinc(II) containing enzyme.[40] The use of two different bis(imidazolyl) agents (BIMA and BIP) made it possible to prepare derivatives of peptides containing the chelating agent either on the C- or their N-termini. The preparations of the peptides are described elsewhere[41] and they were aimed at inhibiting collagenase activity.



Ac-ProLeuGly-BIMA

(11)



BIP-IleAlaGly-OEt

(12)

Protonation constants of the ligands and the stability constants of the copper(II) complexes were determined by potentiometric measurements and the data are collected in Table IV. It is clear from these data that the basicities of the imidazole nitrogen atoms are lower than those of BIM because of the electron withdrawing effect of the amide bonds in the vicinity of the bis(imidazolyl) groups.

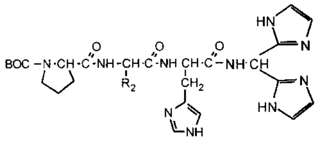
TABLE IV Stability constants (log β_{pqr}) of the proton and copper(II) complexes of Ac-ProLeuGly-BIMA[11] and BIP-IleAlaGly-OEt[11] (T = 298 K, I = 0.2 mol/dm^3 KCl)

Species	*$[HL]^+$*	*$[H_2L]^{2+}$*	*$[CuL]^{2+}$*	*$[CuL_2]^{2+}$*	*$\log(K_1/K_2)$*
Ac-ProLeuGly-BIMA	5.67	8.98	8.65	15.24	2.06
BIP-IleAlaGly-OEt	5.99	9.81	8.92	15.52	2.32

The complex formation processes of Ac-ProLeuGly-BIMA and BIP-IleAlaGly-OEt are, however, almost exactly the same as those of BIM and very stable mono- and bis(ligand) complexes, $[CuL]^{2+}$ and $[CuL_2]^{2+}$, were formed in both cases. The stability constants of copper(II) complexes are slightly lower than those of BIM, but it corresponds to the decreased basicity of the ligands. UV-Vis and EPR parameters of both $[CuL]^{2+}$ and $[CuL_2]^{2+}$ complexes show a close similarity to the corresponding BIM complexes supporting that the imidazole nitrogen atoms are the exclusive binding sites of the molecules **(11)** and **(12)**. Extra deprotonation processes were not observed even at elevated pH values providing further support that the peptide backbones of these ligands behave as non-coordinating side chain residues. In the case of BIP-IleAlaGly-OEt it can be easily understood because the imidazole and the neighbouring amide functions can only form a 7-membered chelate at the N-terminus. However, in the case of Ac-ProLeuGly-BIMA a 5-membered chelate would form at the C-terminus with the involvement of one amide and one of the imidazole nitrogen atoms. The trigonal carbonyl atom of the amide function is, however, out of the chelate ring of such a structure and its involvement is generally required for stable amide coordination.[42]

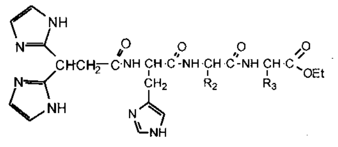
Copper(II) complexes of tripeptides containing both histidyl residues and chelating side chains

In the extension of the previous studies discussed in paragraph (b) six new compounds have been synthesized containing histidyl residues in all possible locations. These ligands involve BOC-ProLeuHis-BIMA (**13**), BOC-ProHisGly-BIMA (**14**), BOC-HisLeuGly-BIMA (**15**), BIP-HisAlaGly-OEt (**16**), BIP-IleHisGly-OEt (**17**) and BIP-IleAla-His-OMe (**18**).



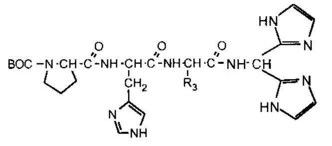
BOC-ProLeuHis-BIMA

(13)



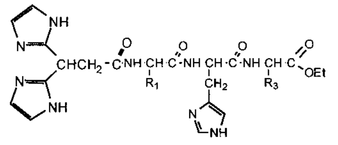
BIP-HisAlaGly-OEt

(16)



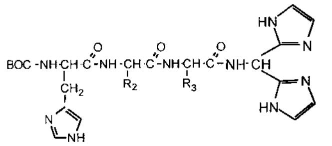
BOC-ProHisGly-BIMA

(14)



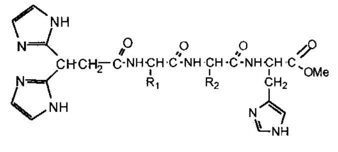
BIP-IleHisGly-OEt

(17)



BOC-HisLeuGly-BIMA

(15)



BIP-IleAlaHis-OMe

(18)

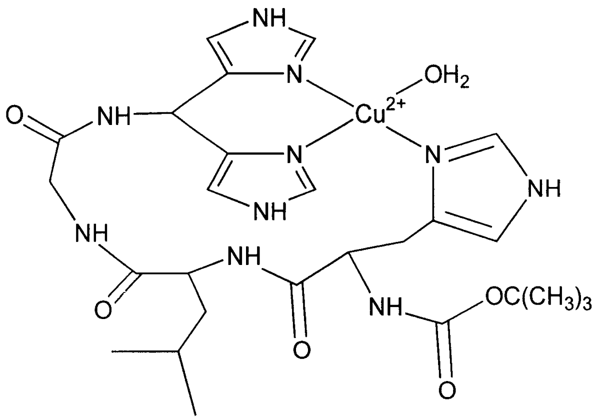
Previous literature studies have already indicated that imidazole-N donor atoms of histidyl residues can act as anchors for the metal induced deprotonation and coordination of amide nitrogens.[30,43] Therefore, these molecules can be considered as potentially ambidentate ligands capable of chelation at the bis(imidazolyl) residues and metal binding *via* the side chain imidazole and/or amide functions. Protonation constants of the ligands and stability constants of the copper(II) complexes were determined by potentiometric titrations and the data are collected in Table V and VI, respectively.

It can be seen from Table V that the deprotonation of the three imidazole nitrogen atoms takes place in the pH range 2 to 7 in partly overlapping processes. The lower basicities, however, are mainly attributed to the bis(imidazolyl) donor atoms and these values show a close similarity to those involved in Table IV. On the other hand, it is also clear that the decrease in the basicity of the bis(imidazolyl) residues is more pronounced in the case of C-terminal chelating agents (peptides of BIMA). The highest p*K* values correspond mainly to the ionization of the side chain histidyl residues and they are rather similar to those of other histidine containing peptides listed in Table I.

TABLE V Protonation constants (log β_{pqr}) of the histidine containing tripeptides[13] (T = 298 K, I = 0.2 mol/dm^3 KCl)

Species	$[HL]^+$	$[H_2L]^{2+}$	$[H_3L]^{3+}$
BOC-ProLeu**His**-BIMA	6.64	11.89	14.74
BOC-Pro**His**Gly-BIMA	6.38	11.80	14.91
BOC-**His**LeuGly-BIMA	6.65	11.89	14.67
BIP-**His**AlaGly-OEt	6.77	12.54	16.27
BIP-Ile**His**Gly-OEt	6.65	12.32	16.33
BIP-IleAla**His**-OMe	6.81	12.65	16.38

The data on the copper(II) complexes of the ligands in Table VI reveal a significant similarity in the complex formation reaction of all six tripeptides. Protonated mono- and bis(ligand) complexes are formed in the acidic pH range and the spectroscopic data suggest that the bis(imidazolyl) donor atoms are the exclusive metal binding sites in the species $[CuHL]^{3+}$ and $[CuH_2L_2]^{4+}$. Deprotonation of the non-coordinated histidyl residues, however, has a significant effect both on the thermodynamic stabilities and binding modes of the various species. Tridentate coordination of the ligands can be expected in the species $[CuL]^{2+}$, which enhances the stability of the corresponding complexes, but slightly suppresses bis(ligand) complex formation, as it is reflected in the increased ratios of stepwise stability constants. It is also clear from Table VI that tridentate coordination is more pronounced if the chelating agent is present at the N-termini (peptides of BIP). In the case of C-terminal peptides the ratio of stepwise stability constants increases with the increase of the distance between the chelating and monodentate side chains. As a consequence, the most stable I:1 complexes were obtained with BOC-HisLeuGly-BIMA and the outstanding stability of the species $[CuL]^{2+}$ was explained by the equatorial coordination of the histidyl residue in the form of a macrochelate **(19)**.



$[CuL]^{2+}$ L = BOC-HisLeuGly-BIMA

(19)

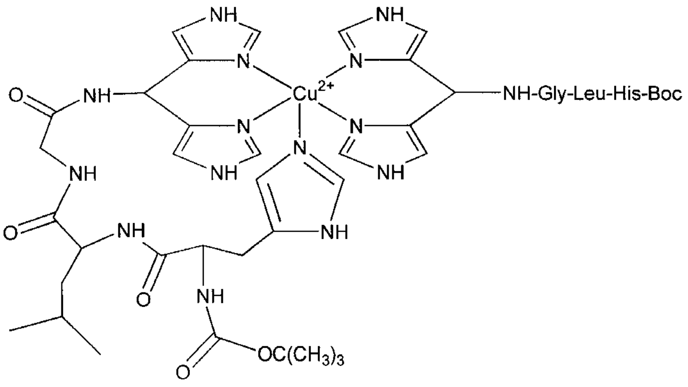
The deprotonation of the side chain imidazolyl residue is accompanied with a very significant blue shift of the absorption maxima (680 to 635 nm). The hyperfine coupling constants of the EPR spectra also suggested the coordination of 3N donor atoms in the equatorial plane, but in a slightly distorted environment. The values $A_\parallel = 172\cdot10^{-4}$ cm^{-1}, $g_\parallel = 2.30$ and $A_\parallel = 176\cdot10^{-4}$ cm^{-1}, $g_\parallel = 2.27$ were obtained for the species $[CuHL]^{3+}$ and $[CuL]^{2+}$, respectively.

TABLE VI Stability constants (log β_{pqr}) of the copper(II) complexes of the histidine containing tripeptides[13] (T = 298 K, I = 0.2 mol/dm^3 KCl)

Ligand	$[CuHL]^{3+}$	$[CuL]^{2+}$	$[CuH_2L_2]^{4+}$	$[CuHL_2]^{3+}$	$[CuL_2]^{2+}$	$log(K_1^H/K_2^H)$	$log(K_1/K_2)$
BOC-ProLeu**His**-BIMA	14.37	9.17	27.34	21.85	15.89	1.40	2.45
BOC-Pro**His**Gly-BIMA	14.70	9.51	27.44	21.88	15.69	1.96	3.33
BOC-**His**LeuGly-BIMA	14.67	11.42	27.45	23.01	16.67	1.89	6.17
BIP-**His**AlaGly-OEt	15.08	10.63	27.97	22.44	16.03	2.19	5.23
BIP-Ile**His**Gly-OEt	14.67	10.05	26.68	21.08	14.74	2.66	5.36
BIP-IleAla**His**-OMe	14.84	10.28	27.78	21.88	15.34	1.90	5.22

$\log(K_1^{H}/K_2^{H}) = 2\log\beta_{111} - \log\beta_{122}$

Similar, but generally smaller, spectral changes were observed with the other ligands in equimolar solutions supporting the equatorial coordination of the histidyl residues in the $[CuL]^{2+}$ complexes. On the contrary, deprotonation of the bis(ligand) complexes, namely the formation of $[CuL_2]^{2+}$ from $[CuH_2L_2]^{4+}$, is accompanied with the opposite change of absorption spectra (to higher vawelengths) indicating the axial coordination of the side chain histidyl residues. For example, in the case of the copper(II)-BOC-HisLeuGly-BIMA system the deprotonation of the histidyl residues resulted in a 41-nm red shift of the absorption maximum and a decrease in the hyperfine coupling constant ($\lambda_{max} = 595$ nm, $A_{\parallel} = 197 \cdot 10^{-4}$ cm^{-1} and $\lambda_{max} = 636$ nm, $A_{\parallel} = 191 \cdot 10^{-4}$ cm^{-1} were obtained for the species $[CuH_2L_2]^{4+}$ and $[CuL_2]^{2+}$, respectively). These parameters are in agreement with the axial coordination of at least one histidyl residues in the bis complexes resulting in a pentacoordinated copper(II) complex **(20)**.



$[CuL_2]^{2+}$ L = BOC-HisLeuGly-BIMA

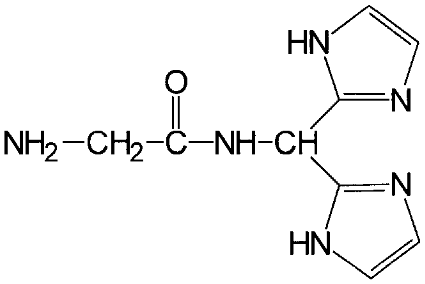
(20)

Similar spectral changes were observed in the copper(II) complexes of all other ligands and both thermodynamic and spectroscopic data suggest that the extent of axial coordination of histidyl residues is increasing with the distance between the chelating and the monodentate side chains. Finally it should be noted that extra deprotonation reactions were not observed in any of the systems, which rules out the deprotonation and coordination of both amide and pyrrole-N1(H) donor functions. As a consequence, the bis(imidazolyl) agents are the primary metal binding sites in all ligands **(13)** – **(18)**, while the side chain imidazole residues enhance the metal binding ability of the ligands *via* equatorial (for $[CuL]^{2+}$) or axial (for $[CuL_2]^{2+}$) coordinations.

Metal complexes of the ligands containing unprotected amino groups and chelating agents

The terminal amino groups, which are generally considered as the primary ligating groups or anchors for the metal binding of peptides were blocked in the previous ligands. Therefore, the reliable comparison of the metal binding ability of the peptide backbone and the chelating side chain made it necessary to include the ligands containing free amino groups.

The ligand Gly-BIMA **(21)** has been first synthesized[12,41] and it can be considered as an effective ambidentate ligand, which can form either a 6-membered chelate *via*the bis(imidazolyl) binding sites or two, fused 5-membered chelates with the tridentate coordination of the amino, amide and imidazole donor functions. Copper(II), nickel(II) and zinc(II) complexes of Gly-BIMA have been studied by potentiometric and various spectroscopic techniques and the equilibrium data are collected in Table VII.



Gly-BIMA

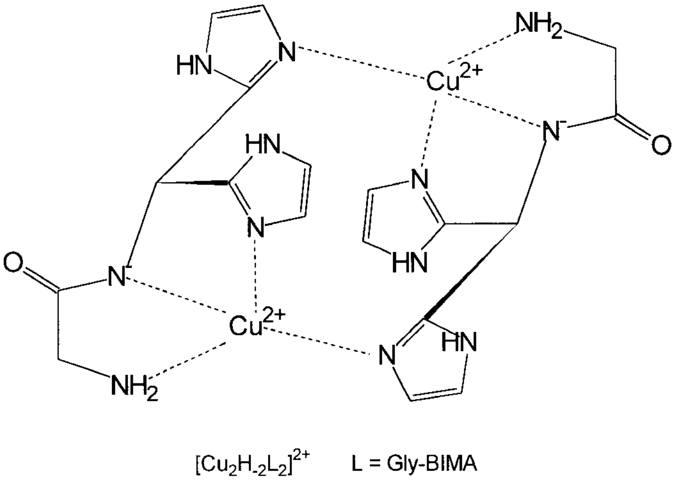
(21)

TABLE VII Stability constants (log β_{pqr}) of proton,[12] copper(II),[12] nickel(II) and zinc(II)[12] complexes of Gly-BIMA (T = 298 K, I = 0.2 mol/dm^3 KCl)

Species			
$[HL]^+$		7.95	
$[H_2L]^{2+}$		13.46	
$[H_3L]^{3+}$		16.68	
	Cu(II)	*Ni(II)*	*Zn(II)*
$[MH_2L_2]^{4+}$	31.64	27.82(3)	24.04
$[MHL_2]^{3+}$	–	21.51(7)	17.17
$[ML_2]$	18.97	13.47(8)	10.28
$[MH_{-1}L_2]^+$	11.12	4.7(1)	–
$[MHL]^{3+}$	17.11	14.21(4)	12.40
$[M_2H_{-2}L_2]^{2+}$	18.43	2.71(6)	0.40
$[MH_{-2}L]$	–0.92	–7.71(3)	–

It is clear from Table VII that the fully protonated form of Gly-BIMA contains 3 dissociable protons ($[H_3L]^{3+}$) and the p*K* values of the bis(imidazolyl) moiety are in good agreement with those of the peptide derivatives of BIMA. The highest p*K* value belongs to the deprotonation of the ammonium group and it shows a close similarity to those of common dipeptides.[29] The potentiometric titration curves of the copper(II)-Gly-BIMA system reveal that complex formation starts in a very acidic pH range. Fig. 3 demonstrates that the species $[CuHL]^{3+}$ and $[CuH_2L_2]^{4+}$ are the major species under these conditions and the spectroscopic parameters definitely support the exclusive coordination of bis(imidazolyl) donor atoms with protonated amino groups.

However, the deprotonation of the ammonium groups takes place above pH 4 and it is followed with another extra base consuming process in an equimolar solution of copper(II) and Gly-BIMA. This reaction is accompanied by a significant blue shift of the absorption spectra ($\lambda_{max} = 690$ and 590 nm are measured for the species $[CuHL]^{3+}$ and $[Cu_2H_{-2}L_2]^{2+}$, respectively), which suggests the coordination of one more nitrogen donor atom. The potentiometric titration curves can be fitted with the formation of either monomeric or dimeric species, but the EPR spectra provide an unambiguous proof for the existence of a dimer. Its binding mode is described by structure **(22)**, in which the Cu-Cu distance is around 390 pm as calculated from the EPR spectra.[12]



(22)

The ligand Phe-BIMA has also been synthesized containing the same metal binding sites as Gly-BIMA, but with a bulky aromatic side chain on the α-carbon atom. The complex formation processes of Phe-BIMA were almost the same as those of Gly-BIMA, the dimeric species dominating again under physiological conditions. The formation of dimeric complexes is almost exclusive in equimolar solutions, but Fig. 3 reveals that it overlaps with bis(ligand) complex formation in the presence of excess ligand. The spectroscopic measurements support that the bis(ligand) complexes $[CuL_2]^{2+}$ and $[CuH_{-1}L_2]^{+}$ contain both tridentate $[NH_2,N^-,N(Im)]$ and monodentate [N(Im)] coordinations of the ligands. It is also a significant observation that a further base consuming process starts above pH 9, which corresponds to hydrolysis, but the EPR spectra suggest the existence of polymeric complexes.

Nickel(II) and zinc(II) complexes of Gly-BIMA have also been studied and it is important to note that deprotonation and coordination of the amide nitrogens take place in these complexes, too. Similarly to copper(II), the bis(imidazolyl) residues were detected as the major metal binding site in acidic solutions, but the low ratio of the stepwise stability

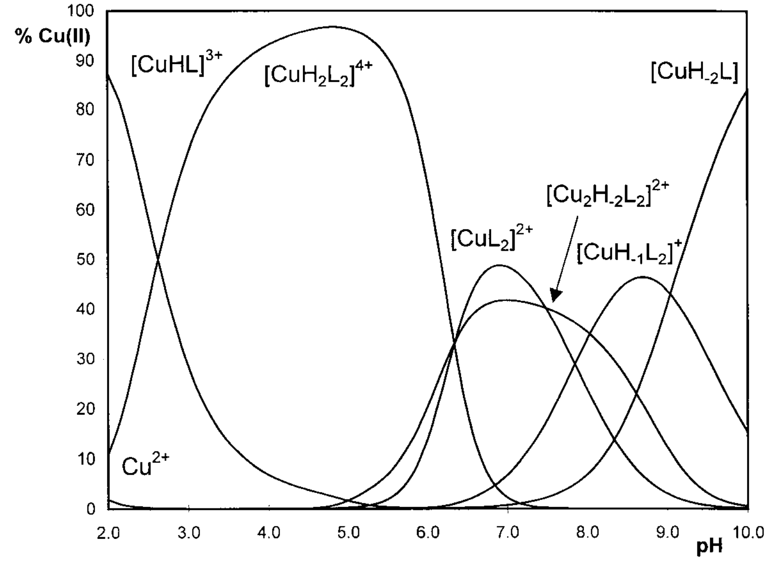


FIGURE 3 Species distribution of complexes formed in copper(II)-Gly-BIMA system $c_L = 4 \cdot 10^{-3}$ mol dm^{-3}, $c_{Cu(II)} = 2 \cdot 10^{-3}$ mol dm^{-3}

constants shows some differences in the binding modes of copper(II) and nickel(II) or zinc(II) complexes. Namely, protonated bis(ligand) complexes of copper(II) were described by the equatorial coordination of 4N donor atoms, while the regular octahedral geometry of $[NiH_2L_2]^{4+}$ and $[ZnH_2L_2]^{4+}$ provides a more flexible coordination environment. On the other hand, the relatively high stability of bis(ligand) complexes slightly suppresses amide deprotonation in the corresponding nickel(II) and zinc(II) complexes as compared to that of copper(II) species.

CONCLUSIONS

The results obtained on the metal complexes of ligands containing C-terminal histidyl residues or bis(imidazolyl) chelating agents provide further evidence that imidazole nitrogen donor atoms are very effective and specific binding sites for the late transition elements.

In the case of the simple oligopeptides containing C-terminal histidyl residues the imidazole nitrogen atoms were identified as the primary metal binding sites. In the metal complexes of GlyHis and GlyGlyHis (and related di- and tripeptides) the overall number of nitrogen donor atoms does not exceed the number of equatorial coordination sites and it results in the formation of fused 5- and 6-membered chelate rings *via* $[NH_2,N^-,N(Im)]$ and $[NH_2,N^-,N^-,N(Im)]$ coordinations, respectively. As a consequence, these peptide sequences have an outstanding metal binding ability, especially for copper(II), nickel(II) and some other d[8] transition elements. The biological significance of these molecules are emphasized by the fact that they mimic the N-terminal metal binding sites of human serum albumin[17] (ATCUN motif). Deprotonation and coordination of the amide nitrogens of these ligands are promoted not only by the above mentioned elements, but even with zinc(II) or cobalt(II).[9] In the case of GlyGlyHis (and related peptides) the deprotonation of two amide functions takes place in a cooperative manner and the imidazole nitrogen donor functions can effectively compete with metal ion hydrolysis even at high pH values.

In the case of tetra-, penta- and hexapeptides containing the histidyl residues at the C-termini (Gly_nHis, n = 3,4,5) the total number of possible nitrogen donor atoms exceeds the number of equatorial coordination sites, which makes the complex formation processes of these ligands more versatile and more complicated. Both previous literature data and our recent studies on the copper(II) and nickel(II) complexes of these ligands show that both N-and C-termini contribute to the high metal binding abilities of the molecules. The species $[ML]^+$ is formed first by increasing pH and its thermodynamic stability is much higher than those of other peptide complexes with the same stoichiometry. The binding sites of these species are represented by **(1)**, which shows the formation of a macrochelate with the involvement of both termini in metal ion coordination. The same binding mode is generally not observed with di- or tripeptides, but seems to be a preferred coordination mode for longer peptides having effective side chain donor functions. An increase in pH results in successive deprotonation and subsequent coordination of amide groups and, in the case of copper(II)-Gly_nHis systems, all species, $[CuH_{-n}L]^{(n-1)-}$ (n = 1–3), can be detected in measurable concentration.

The stoichiometry of the final species is $[MH_{-3}L]^{2-}$ for both copper(II) and nickel(II) with all ligands. Doubtlessly the metal binding sites are described by 4N coordination in all cases. However, the location of the coordinated amide functions can be a matter of debate, because either the terminal amino group or the imidazole nitrogen atom can act as an anchor for the metal binding of three amide nitrogens. Our potentiometric and spectroscopic measurements provide clear evidence that the terminal amino group is the more effective anchor in the molecules. As a consequence, the binding sites of the various species were described by Structures **(2)** – **(5)**, in which the amino nitrogen donor atoms are always coordinated. On the other hand, it is clear form these structures that the imidazole donor atoms are also important binding sites, but only in the form of macrochelates and the imidazole moiety will be a non-coordinated residue in the species $[MH_{-3}L]^{2-}$.

The other group of derivatives of peptides reported in this paper contained the bis(imidazolyl) residue, which is a very effective, 6-membered chelating agent. The results obtained on the copper(II), nickel(II) and zinc(II) complexes reveal that the nitrogen donor atoms of the bis(imidazolyl) residues are the primary metal binding sites of the molecules and dramatically enhance the metal binding ability of simple peptide molecules. The corresponding 2N and 4N coordinated mono- and bis(ligand) complexes are formed under strongly acidic conditions and can be easily identified by the characteristic spectral parameters.

Potentiometric and spectroscopic measurements on the copper(II) complexes of Ac-ProLeuGly-BIMA and BIP-IleAlaGly-OEt gave clear evidence that the N-protected peptide backbone can not compete with the metal ion coordination of the bis(imidazolyl) chelating agents. The coordination chemistry of these ligands is always characterised by 2N or 4N coordinations of the imidazoles and the peptide linkages can be considered as the non-coordinated side chains of the bis(imidazolyl) ligands. Even the insertion of histidyl residues into peptide sequence will not change the major metal binding sites. Monodentate coordination of the side chain histidyl imidazole-N donor atoms is not able to break the binding of bis(imidazolyl) residues and promote deprotonation and coordination of amide functions. The presence of the extra imidazole nitrogen atoms in these peptides, however, provides further enhancement in the thermodynamic stability of copper(II) complexes. The increased stability of the mono- and bis(ligand) complexes can be explained by the equatorial and axial coordinations of the side chain histidyl residues, respectively.

The presence of terminal amino group, however, results in a completely different coordination chemistry of the bis(imidazolyl) ligands.

The chelating agent remains the primary metal binding site under strongly acidic conditions, but it is cleaved by the tridentate coordination of the amino, amide and one of the imidazole nitrogen donor atoms by increasing pH. In the copper(II)-Gly-BIMA (and Phe-BIMA) system it results in the predominant formation of a dimeric species containing tridentate ligands and imidazole bridging (**22**). It is also important to note that the change of binding sites, namely the deprotonation and coordination of amide nitrogens takes place in the corresponding nickel(II) and zinc(II) complexes, too. These observations provide further support that imidazole nitrogen donor atoms of peptides are important metal binding sites both *via* monodentate coordination and especially chelation, but the terminal amino groups are more effective donor functions or anchors for amide binding.

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