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Transition metal complexes of bis(imidazol-2-yl) derivatives of dipeptides †

Katalin Ősz,[a] Katalin Várnagy,[a] Helga Süli-Vargha,[b] Daniele Sanna,[c] Giovanni Micera[d] and Imre Sóvágó *[a]

[a] *Department of Inorganic and Analytical Chemistry, University of Debrecen, P.O. Box 21, H-4010 Debrecen, Hungary. E-mail: sovago@delfin.klte.hu*

[b] *Research Group of Peptide Chemistry, Hungarian Academy of Sciences, P.O. Box 32, H-1518 Budapest 112, Hungary*

[c] *Istitutio C.N.R. di Chimica Biomolecolare, Sezione di Sassari, Trav. La Crucca 3 Reg. Baldinca, I-07040 Sassari, Italy*

[d] *Department of Chemistry, University of Sassari, Via Vienna 2, I-07100 Sassari, Italy*

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Copper(II), nickel(II) and zinc(II) complexes of the dipeptides containing chelating bis(imidazolyl) agents at the C-termini (GlyLeu-BIMA, LeuGly-BIMA, PheGly-BIMA and AlaPro-BIMA; BIMA = bis(imidazol-2-yl)-methylamine) were studied by potentiometric, UV-VIS, EPR and MALDI-MS techniques. The imidazole nitrogen donor atoms were described as the primary metal binding sites forming stable mono- and bis-(ligand) complexes in the acidic pH range. The formation of dinuclear complexes was detected in equimolar solutions of copper(II) and dipeptides and various isomeric forms of these species ($[Cu_2L_2]^{4+}$ and $[Cu_2H_{-2}L_2]^{2+}$) were indentified by EPR measurements. Deprotonation and co-ordination of the amide groups of dipeptides took place in slightly alkaline solution resulting in the formation of $[MH_{-2}L]$ complexes of copper(II) and nickel(II). Metal ion co-ordination of one of the imidazole nitrogen donor atoms promoted the ionization of the pyrrole type N(1)H group of imidazole in copper(II) complexes and resulted in the formation of a trinuclear complex ($[Cu_3H_{-6}L_2]$). The latter species contains negatively charged bridging imidazolato residues.

Introduction

Co-ordination chemical investigations of various peptide molecules help significantly in the understanding of the structure and function of the active sites of metalloenzymes. The results obtained for the metal complexes of the most common peptides containing histidine have already been reviewed [1,2] and the data provide unambiguous proof for the outstanding metal binding ability of the imidazolyl moieties of histidyl residues. Poly-imidazole ligands are also frequently used as structural models of the active sites of metalloenzymes.[3–8] One of the most important groups of these compounds is represented by the di- or poly-nuclear complexes, in which the metal ion co-ordination induces the deprotonation of the imidazole N(1)H group resulting in an imidazolato bridge between two metal ions. The aim of the investigation of these binary and/or ternary polynuclear complexes is to mimic the active site of superoxide dismutase[9–14] and tyrosinase enzymes.[15]

The simplest representatives of polyimidazole ligands are the derivatives of bis(imidazol-2-yl)methane (BIM), in which two imidazole rings are linked *via* a single tetrahedral carbon atom. The bis(imidazol-2-yl)methyl group was reported to be a very effective complexing agent for a great variety of transition metal ions forming stable six-membered chelates *via* the co-ordination of imidazole nitrogen atoms.[16–22] The co-ordination chemistry of the ligands containing two imidazole rings is more versatile when the chelating nitrogen donors are linked to other chelating ligands creating multi- and/or ambi-dentate ligands.[5,23–25]

In our previous papers we have reported results on the metal complexes of multidentate ligands containing the bis(imidazol-2-yl)methyl residues at the C- or N-termini of amino acids or peptides.[20,26–30] It is obvious from these studies that the imidazole nitrogen donor atoms are the primary metal binding sites in the copper(II), nickel(II) and zinc(II) complexes of these molecules. It was also proved that in the absence of a terminal amino group the peptide backbone cannot compete with the chelating side chains.[20,28] The results obtained on the metal complexes of Gly-BIMA, Phe-BIMA and His-BIMA (BIMA = bis(imidazol-2-yl)methylamine) containing an unprotected amino group revealed that the amino group has a significant impact on the co-ordination properties of the bis(imidazol-2-yl)methyl ligands.[26,29] Very stable dinuclear complexes were obtained in equimolar solutions of copper(II) and bis(imidazol-2-yl)methyl derivatives of amino acids, in which all metal ions were co-ordinated by [NH_2, N^-, N(Im) (Im = imidazolyl)] donors and the metal centres were joined *via* imidazole bridging. The presence of the histidyl side chain in His-BIMA, however, resulted in a further increase in the versatility of the complex formation reactions.[30] The existence of a dinuclear complex $[Cu_2L_2]^{4+}$ was described in slightly acidic solution with three isomeric forms. Deprotonation of the imidazole N(1)H donor functions was detected under slightly alkaline conditions. An excess of copper(II) ions shifted this reaction into the slightly acidic pH range and resulted in the formation of a trinuclear complex. The species $[Cu_3H_{-4}L_2]^{2+}$ contains imidazolato bridges between the adjacent metal ions and this structure is reminiscent of the active site of superoxide dismutase (SOD). The two lateral metal ions are, however, only tridentately co-ordinated in the trinuclear complex and the species $[Cu_3H_{-4}L_2]^{2+}$ of His-BIMA is very sensitive to hydrolytic reactions. Peptide derivatives of the bis(imidazol-2-yl)-methyl ligands can, however, easily saturate the co-ordination sphere of all metal ions and may provide stable conditions for the formation of imidazolato bridged polynuclear complexes.

Now, in this paper we report the results of combined potentiometric and spectroscopic (EPR, UV-VIS, MS, NMR) studies on the copper(II), nickel(II) and zinc(II) complexes of GlyLeu-BIMA, LeuGly-BIMA, PheGly-BIMA and AlaPro-BIMA (Scheme 1) and the results indicate that copper(II) complexes are promising structural models of the CuZnSOD enzymes.

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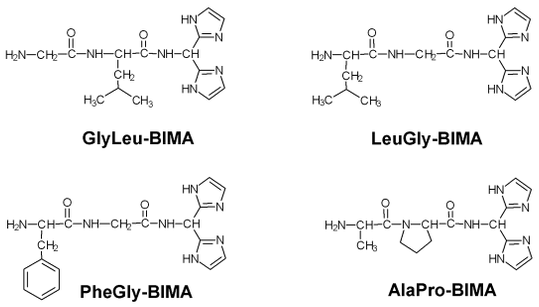


Scheme 1

Experimental

Synthesis

The synthetic procedure for the preparation of *N*-protected and free amino acids and peptides containing BIMA on their C-terminus have already been reported: GlyLeu-BIMA, LeuGly-BIMA, PheGly-BIMA and AlaPro-BIMA were prepared accordingly.[31] The purity of the above peptide derivatives was checked by TLC and HPLC, their structure was proved by ^{1}H NMR.

Potentiometric measurements

The pH-potentiometric titrations in the pH range 2.2–11.0 were performed on 4–6 cm^3 samples in the concentration range 2–8 × 10^{-3} mol dm^{-3} at metal ion to ligand ratios ranging between 1 : 3 and 2 : 1. Argon was bubbled through the samples to ensure the absence of oxygen and to stir the solutions. All measurements were carried out at 298 K and at a constant ionic strength of 0.2 mol dm^{-3} KCl with a Radiometer pHM84 pH-meter equipped with a 6.0234.100 combination glass electrode (Metrohm) and a Dosimat 715 automatic burette (Metrohm) containing carbonate-free potassium hydroxide of known concentration. The pH readings were converted into hydrogen ion concentration as described earlier.[32] Protonation constants of the ligands and the overall stability constants (log β_{pqr}) of the systems were calculated by means of a general computational program, PSEQUAD,[33] using eqn. (1) and (2).

$$pM + qH + rL \rightleftharpoons M_pH_qL_r \quad (1)$$

$$\beta_{pqr} = \frac{[M_pH_qL_r]}{[M]^p \cdot [H]^q \cdot [L]^r} \quad (2)$$

Spectroscopic measurements

UV-VIS spectra of the copper(II) and nickel(II) complexes were recorded on a Hewlett Packard HP 8453 or JASCO UVIDEC-610 spectrophotometer in the same concentration range as used for the potentiometry.

Anisotropic X-band EPR spectra (9.15 GHz) of frozen solutions were recorded at 120 K, using a Varian E-9 spectrometer after addition of 10% ethylene glycol to ensure good glass formation in frozen solutions. Copper(II) stock solution for EPR measurements were prepared from $CuSO_4 \cdot 5H_2O$ enriched with ^{63}Cu to get better resolution EPR spectra. For this purpose metallic copper (99.3% ^{63}Cu and 0.7% ^{65}Cu) was purchased from JV Isoflex, Moscow, Russia and converted into the sulfate.

Matrix-assisted-laser desorption/ionization mass spectrometry (MALDI-MS)

MALDI-MS spectra of the $[Cu_3H_{-6}L_2]$ complex (L = LeuGly-BIMA) were recorded on a Bruker BIFLEX III mass spectrometer equipment with a time-of-flight (TOF) analyzer. In all cases 19 kV acceleration voltage was used with pulsed ion extraction (PIE). The positive ions were detected in the reflectron mode (20 kV). A nitrogen laser (337 nm, 3 ns pulse width, 10^6–10^7 W cm^{-2}) operating at 4 Hz was used to produce laser desorption, and 50–60 shots were summed. The spectra were externally calibrated with poly(ethylene glycol) (PEG) standard (M_n = 1450 g mol^{-1}, MWD = 1.02) with linear calibration.[34–36]

Samples were prepared with 2,5-dihydroxybenzoic acid (DHB) and 2-amino-5-nitropyridine matrices (20 mg cm^{-3}). The aqueous solution of the complex (analyte; 3–5 mg cm^{-3}, pH adjusted to 8–9 using potassium hydroxide solution) was mixed in a 10 : 1 matrix to analyte (v/v) ratio. The solvent was ethanol : water 1 : 1. A volume of 0.5–1 μl of these solutions was deposited on the sample plate (stainless steel) and allowed to air-dry.

Measurement of magnetic properties

The Evans NMR method was used to determine the paramagnetic susceptibility of the complexes in solution. This method is based on the principle that the position of a given proton resonance in the NMR spectrum of a molecule is dependent on the bulk susceptibility of the medium in which the molecule is found.[37,38]

The special NMR tube used for the measurement consisted of precision-made coaxial outer and inner tubes. The sample in the inner tube was the solution of the paramagnetic copper(II) complex in D_2O with 2% *tert*-butyl alcohol, and the solution in the outer tube was 2% *tert*-butyl alcohol in D_2O. Proton NMR spectra of the samples were measured as a function of pD, which was determined by the use of a Radiometer pHM84 pH-meter equipped with a 6.0234.100 combination glass electrode (Metrohm) and addition of 0.4 to the pH-meter readings. The spectra were recorded on a Bruker MA 360 MHz instrument.

Results and discussion

The protonation constants of the dipeptides containing bis(imidazol-2-yl)methylamine residue (BIMA) are included in Table 1 together with some literature data of the amino acid derivatives for comparison.

The ligands have three dissociable protons and the protonation constants are similar to those of the corresponding bis(imidazol-2-yl)methyl ligands and the most common dipeptides. The p*K* values of the imidazole nitrogens are very similar to each other and to those of the amino acid derivatives. This means that the increase in the length of the side chains connected to the bis(imidazol-2-yl)methyl moiety and the presence of the bulky phenyl or alkyl group in the peptide does not effect the acid–base properties of the imidazole rings. Basicity of the terminal amino group is, however, significantly influenced by the amino acid sequence of the ligands. The bulky aromatic ring of N-terminal phenylalanine with a strong electron withdrawing effect decreases, while the presence of proline in the second position slightly increases the p*K* values of amino groups. These tendencies are in agreement with those observed for amino acid derivatives of bis(imidazol-2-yl)methyl ligands (Table 1) and dipeptides (LeuGly:[39] p$K(NH_2)$ = 8.24, PheGly:[40] p$K(NH_2)$ = 7.46, AlaPro:[40] p$K(NH_2)$ = 8.52).

Copper(II) complexes

The structures of three ligands (GlyLeu-BIMA, LeuGly-BIMA and PheGly-BIMA) are very similar to each other and five nitrogen donors can be potential metal binding sites: two imidazole nitrogens can form six-membered chelate ring, while the binding of terminal amino, two deprotonated amide and one of the imidazole nitrogens results in the formation of stable fused chelate rings. The prolyl residue of the fourth compound (AlaPro-BIMA) is, however, considered as a "breakpoint" in the peptide chain, because the secondary amide nitrogen

Table 1 Protonation constants of the ligands (T = 298 K; I = 0.2 mol dm^{-3}, standard deviations are in parentheses)

	GlyLeu-BIMA	LeuGly-BIMA	PheGly-BIMA	AlaPro-BIMA	Gly-BIMA [26]	Phe-BIMA [30]
$[HL]^+$	7.92(1)	7.76(1)	7.33(1)	8.11(1)	7.95	7.17
$[H_2L]^{2+}$	13.49(1)	13.35(2)	12.95(2)	13.63(3)	13.46	12.45
$[H_3L]^{3+}$	16.66(2)	16.53(3)	16.14(4)	16.60(5)	16.68	15.54
pK(Im)$_1$	3.17	3.18	3.19	2.97	3.22	3.09
pK(Im)$_2$	5.58	5.59	5.61	5.52	5.51	5.28
pK(NH_2)	7.92	7.76	7.33	8.11	7.95	7.17

Table 2 Stability constants (log β_{pqr}) of copper(II) complexes (T = 298 K; I = 0.2 mol dm^{-3}, standard deviations are in parentheses)

	GlyLeu-BIMA	LeuGly-BIMA	PheGly-BIMA	AlaPro-BIMA
$[CuH_2L_2]^{4+}$	30.13(2)	30.02(3)	30.11(3)	32.17(4)
$[CuHL_2]^{3+}$	22.82(7)	22.95(9)	23.57(5)	25.4(2)
$[CuHL]^{3+}$	15.76(2)	15.77(2)	16.13(3)	17.45(5)
$[CuL]^{2+}$	–	–	–	13.14(2)
$[Cu_2L_2]^{4+}$	24.96(4)	24.62(5)	25.37(6)	–
$[Cu_2H_{-2}L_2]^{2+}$	10.74(7)	12.42(7)	13.57(7)	–
$[CuH_{-2}L]$	−3.63(3)	−2.79(5)	−1.45(3)	–
$[CuH_{-3}L]^-$	−14.46(3)	−13.54(7)	−11.96(3)	–
$[Cu_2H_{-3}L]^+$	−3.77(2)	−2.55(2)	−1.45(3)	–
$[Cu_4H_{-8}L_2]$	−20.24(7)	−16.52(6)	−14.9(2)	–
$[Cu_3H_{-6}L_2]$	−9.49(4)	−7.38(5)	−5.15(9)	–
pK(MH_2L_2/MHL_2)	7.31	7.07	6.54	6.77
pK($MH_{-2}L$/$MH_{-3}L$)	10.69	10.75	10.51	–
log(K_1/K_2) [1]	1.39	1.52	2.21	2.73

[1] Calculated for the protonated complexes: 2 log β_{111} − log β_{122}.

cannot be a metal binding site. Former studies on the peptides containing proline in an intermediate position led to the conclusion that proline breaks the metal ion co-ordination to the subsequent amide nitrogen atoms, but it does not necessarily stop further metal–peptide binding.[41,42]

The stoichiometries and stability constants of copper(II) complexes formed with the dipeptide ligands (Table 2) show, that there are no significant differences in the co-ordination behaviour of the three ligands, while complex formation processes in the copper(II)–AlaPro-BIMA system are dissimilar. In acidic pH-range all four ligands bind the metal ion *via* bis(imidazol-2-yl)methyl groups. Similarly to amino acid derivatives,[26] mono- and bis-(ligand) complexes, $[CuHL]^{3+}$ and $[CuH_2L_2]^{4+}$ (see Fig. 1), are formed and the terminal amino groups are protonated. The formation of bis(ligand) complexes is not hindered by the length of the peptide chain connected to the bis(imidazol-2-yl)methyl group. The UV-VIS and EPR parameters (Table 3) also correspond to those of copper(II) complexes co-ordinated by two or four imidazole nitrogens.

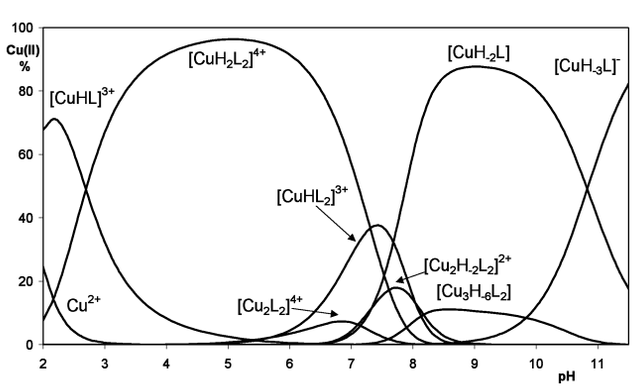


Fig. 1 Species distribution of the complexes formed in the copper(II)–GlyLeu-BIMA system (c_L = 4 × 10^{-3} mol dm^{-3}, c_M = 2 × 10^{-3} mol dm^{-3}).

Only 1 : 1 and polynuclear species are present following the deprotonation of the non-co-ordinated ammonium groups of $[CuHL]^{3+}$ and $[CuH_2L_2]^{4+}$ in the case of all four ligands, as is demonstrated by Fig. 1 for the copper(II) complexes of GlyLeu-BIMA. The deprotonation of $[CuHL]^{3+}$ and the formation of 1 : 1 complexes take place at much lower pH than the pK values of the terminal amino groups of the free ligands and this process is accompanied by a blue shift of the absorption maxima. The formation of dimeric complexes is supposed by EPR spectra (see Fig. 2) measured in the pH range 5–8. The species are not EPR silent, but the line broadening suggests a dipolar interaction between copper(II) ions and the presence of a mixture of copper(II) ions with different co-ordination environments.

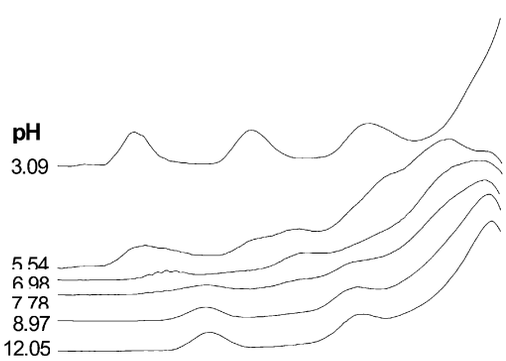


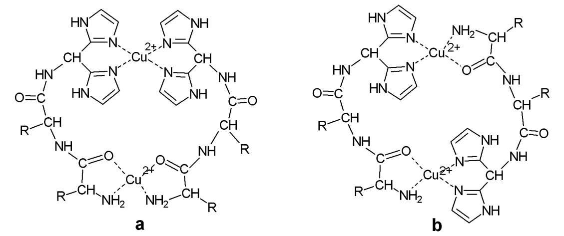
Fig. 2 EPR spectra obtained in an equimolar solution of copper(II) and LeuGly-BIMA at different pH values in frozen solution.

The concentration of the dinuclear complex $[Cu_2L_2]^{4+}$ is almost negligible in the presence of excess of ligand, but it is the major species in equimolar solution in the pH range 5–7. On the other hand, Fig. 2 reveals that a nine-line superhyperfine splitting can be observed in the parallel region of the EPR spectra in the same pH range, which is characteristic of copper(II) complexes co-ordinated by two bis(imidazol-2-yl)-methyl groups. The unusually broad absorption bands with maxima around λ 620 nm, however, probably derive from a mixture of various copper(II) complexes containing two, three and/or four N donor atoms in the co-ordination sphere. UV-VIS parameters of the possible co-ordination modes can be obtained from the literature ($[CuL_2]^{2+}$ of BIM (2 × (N(Im), N(Im)): λ_{max} = 578 nm;[20] $[CuL_2]^{2+}$ of glycyl-sarcosine (2 ×

Table 3 UV-VIS and EPR spectral parameters of copper(II) complexes

	GlyLeu-BIMA			LeuGly-BIMA			PheGly-BIMA			AlaPro-BIMA			Gly-BIMA[26]		
	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	$g_\parallel$	$A_\parallel$	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	$g_\parallel$	$A_\parallel$	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	$g_\parallel$	$A_\parallel$	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	$g_\parallel$	$A_\parallel$	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	$g_\parallel$	$A_\parallel$
$[CuH_nL_2]^{(n+2)+}$	599 (56)	2.235	193	595 (52)	2.235	193	595 (55)	2.235	192	601 (56)	2.234	193	590 (34)	2.236	201
$[CuHL]^{3+}$	695 (38)	2.297	171	690 (45)	2.297	173	694 (38)	2.297	172	698 (35)	2.299	168	690 (31)	2.299	175
$[Cu_2L_2]^{4+}$	627 (56)			617 (55)			621 (51)			620 (54)	2.233	191			
$[CuL]^{2+}$											2.284	179			
$[Cu_2H_{-2}L_2]^{2+}$	513 (138)			546 (70)			537 (71)						595 (91)		
$[CuH_{-2}L]$	508 (172)	2.178	204	514 (158)	2.174	205	513 (240)	2.178	204						
$[CuH_{-3}L]^-$				508 (185)	2.168	209									
$[Cu_2H_{-3}L]^+$				546 (65)											
$[Cu_4H_{-8}L_2]$				559 (157)											
$[Cu_3H_{-6}L_2]$				514 (163)											

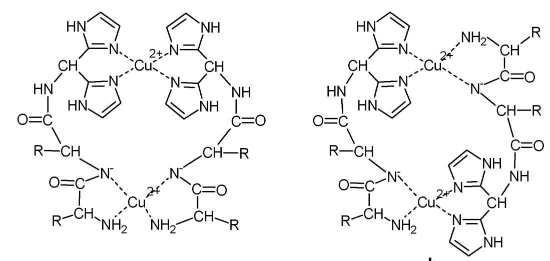
(NH$_2$, CO)): λ_{max} = 658 nm[43]) or can be calculated by empirical formula[44] (calculation for (NH$_2$, CO) + (N(Im),N(Im)) mixed co-ordination: λ_{max} = 616 nm). These observations can be explained by the assumption of a ligand bridged dimeric structure for the species $[Cu_2L_2]^{4+}$, which can exist in the form of two isomers (see Scheme 2). In principle, the ratio of the isomers could be detected by EPR spectroscopy, but the exact parameters cannot be calculated, because of the overlap and broadening of spectra. However, earlier studies on the copper(II) complexes of different nitrogen donors came to the conclusion that mixed ligand complex formation is unfavourable if both aromatic and aliphatic nitrogen donors are present.[45] As a consequence, the symmetrical arrangement (isomer "a" in Scheme 2) of the donor sites is favoured over the asymmetrical one (isomer "b") and is supported by the appearance of the superhyperfine splitting of EPR spectra.



$[Cu_2L_2]^{4+}$

Scheme 2

In the case of the copper(II) complexes of GlyLeu-BIMA, LeuGly-BIMA, PheGly-BIMA, an extra base consuming process starts above pH 6 and it is accompanied by the shift of absorption maxima to lower wavelengths. The deprotonation and co-ordination of one of the amide nitrogens and the formation of $[Cu_2H_{-2}L_2]^{2+}$ complex can be concluded. Although the stoichiometry of this dinuclear species is the same as that of the dinuclear complexes formed in the copper(II)–Gly-BIMA and –Phe-BIMA systems, the spectral parameters are significantly different suggesting that the metal binding sites are not the same. The absorption maxima of the dinuclear complexes of Gly-BIMA and Phe-BIMA were obtained at λ_{max} = 590 nm[29] and the binding sites were described *via* [NH$_2$, N$^-$, N(Im)] tridentate co-ordination of each ligand and imidazole bridging. The UV-VIS parameters characteristic for $[Cu_2H_{-2}L_2]^{2+}$ species are included in Table 3, but the values are slightly uncertain, because $[Cu_2H_{-2}L_2]^{2+}$ is not a major species at any metal ion to ligand ratios. The change of absorption maxima from ≈620 nm to ≈540 nm, upon the transformation of $[Cu_2L_2]^{4+}$ to $[Cu_2H_{-2}L_2]^{2+}$, however, indicates an increase in the number of co-ordinated nitrogen atoms. These observations suggest that the deprotonation is not able to break the ligand bridged structure of $[Cu_2L_2]^{4+}$, but the conversion of (NH$_2$, CO) chelate (Scheme 2) to (NH$_2$, N$^-$) chelate (Scheme 3) takes place in the dinuclear species. The literature data and calculated parameters on similar donor sets are also in agreement with this assumption: $[CuH_{-2}L_2]$ of glycinamide[45] (2 × (NH$_2$, N$^-$)): λ_{max} = 528 nm; calculated values[44] for the co-ordination of (NH$_2$, N$^-$) +



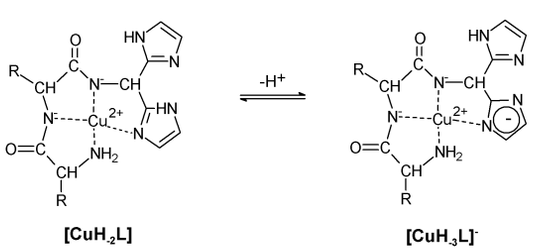
$[Cu_2H_{-2}L_2]^{2+}$

Scheme 3

(N(Im), N(Im)): λ_{max} = 549 nm. Taking into account the fact that the deprotonation of the amide nitrogens does not occur either in the N-protected tripeptide derivatives of bis(imidazol-2-yl)methyl ligands[27] or in the copper(II)–AlaPro-BIMA system, the deprotonation process should belong to the first amide nitrogen at the N-terminus. The ligand bridged structure of $[Cu_2H_{-2}L_2]^{2+}$ provides the possibility of isomeric forms and the appearance of the superhyperfine splitting of EPR spectra supports the predominant formation of the symmetrical arrangement (Scheme 3a).

As was mentioned earlier, the first amide nitrogen of AlaPro-BIMA on the N-terminus is not able to bind a metal ion and this results in a significant difference between the complex formation processes of AlaPro-BIMA and the other three dipeptides above pH 5. Deprotonation of the non-co-ordinated ammonium groups of $[CuHL]^{3+}$ and $[CuH_2L_2]^{4+}$ leads to the formation of 1 : 1 complexes the stoichiometry of which can be either $[CuL]^{2+}$ or $[Cu_2L_2]^{4+}$. Potentiometric measurements cannot distinguish between the mono- and di-nuclear species, but the EPR spectra strongly support the mononuclear complexes as being the major species. The EPR parameters (Table 3) of copper(II)–AlaPro-BIMA definitely show the existence of two types of copper(II) ions. One set of these parameters corresponds to a 2 × (N(Im), N(Im)) (for $[CuL_2]^{2+}$ of BIM[20] $g_{||}$ = 2.234, $A_{||}$ = 199 × 10^{-4} cm^{-1}) and the other corresponds to a 2 × (NH_2, CO) co-ordination (for $[CuL_2]^{2+}$ of glycineamide[45] $g_{||}$ = 2.276, $A_{||}$ = 171 × 10^{-4} cm^{-1}). Taking into account the 1 : 1 stoichiometry of the complexes these parameters cannot be interpreted by the formation of simple bis(ligand) complexes in the copper(II)–AlaPro-BIMA system. There are two possibilities for the resolution of this contradiction: these co-ordination modes may exist (i) in a ligand bridged dimeric complex with a symmetrical arrangement (see Scheme 2a) with large Cu(II)–Cu(II) distances caused by the more rigid proline residues, (ii) in a mononuclear complex containing a macrochelate in a strongly distorted environment from the same binding sites. It is important to emphasize that a further increase of pH does not cause any change in the co-ordination sphere of copper(II) ions in the species $[CuL]^{2+}$ (or $[Cu_2L_2]^{4+}$). The deprotonation of amide nitrogen cannot be observed, but the hydrolysis of the complex and precipitation occur similarly to the simplest bis(imidazol-2-yl)methyl compounds (*e.g.* BIM)[20] and tripeptide derivatives without free terminal amino groups.[27]

A new base consuming process starts in the case of copper(II)–GlyLeu-BIMA, –LeuGly-BIMA and –PheGly-BIMA systems above pH 7 both in equimolar solution and in the presence of excess ligand and the species $[CuH_{-2}L]$ predominates in the pH range 8–10 at any metal ion to ligand ratios. The EPR spectra indicate monomeric species with the parameters of $g_{||}$ = 2.174–2.178, $A_{||}$ = 204–205 × 10^{-4} cm^{-1} and their formation is accompanied with a blue shift in the absorption spectra (λ_{max} = 513–514 nm). The tetradentate co-ordination of the ligands can be supposed *via* amino, two amide and one imidazole nitrogens resulting in a very stable fused chelate rings system (Scheme 4). This binding mode of the ligands is very similar to that of GlyGlyHis in the $[CuH_{-2}L]^{-}$ complex, where the ligand is co-ordinated by (NH_2, N^-, N^-, N(HisIm)) donor set and its spectroscopic parameters[46] ($g_{||}$ = 2.178, $A_{||}$ = 209 × 10^{-4} cm^{-1}, λ_{max} = 525 nm) are very close to the data cited above.



Scheme 4

Another base consuming process takes place under strongly alkaline conditions (pH > 10), which may correspond to the deprotonation of pyrrole type nitrogen of imidazole ring or the formation of mixed hydroxo complexes. Taking into account the decrease of λ_{max} and $g_{||}$ and the increase of $A_{||}$ values (Table 3), the formation of $[CuH_{-3}L]^{-}$ should be the result of deprotonation of the N(1)H group (Scheme 4). The stable 4N structure with a saturated co-ordination sphere around the copper(II) ions hinders the hydrolysis of the complex and the appearance of precipitation. The deprotonation of the N(1)H group of the imidazole ring was observed in alkaline solutions of copper(II) complexes of various ligands containing histidyl residues.[30,47–49] The deprotonation generally took place in the pH range 8–12 (histidine:[47] pK = 11.7, GlyHis:[48] pK = 9.6, His-BIMA:[30] pK = 8.1 and 8.9) resulting in the formation of polymeric structures in some cases. The blue shift of the absorption maxima was also observed in parallel with the deprotonation of the pyrrole type N(1)H group.

The multidentate character of the ligands makes it possible to bind more than one metal ion and it is important to note that the N(1)H deprotonation creates a new chelating site, too. As a consequence, precipitation was not observed even in slightly basic solution up to 2 : 1 Cu(II) : L ratio and the formation of various polynuclear complexes was suggested as demonstrated in Fig. 3. It is clear from Table 2 and Fig. 3 that the species

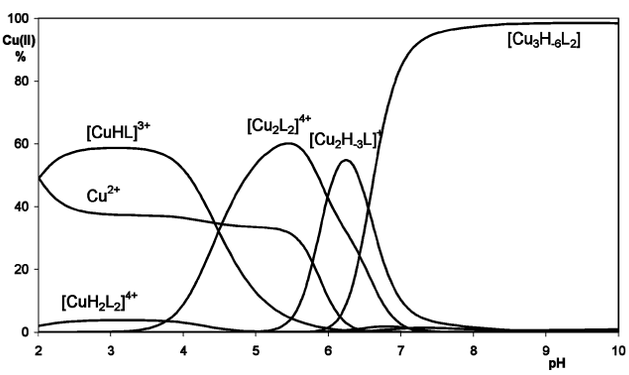
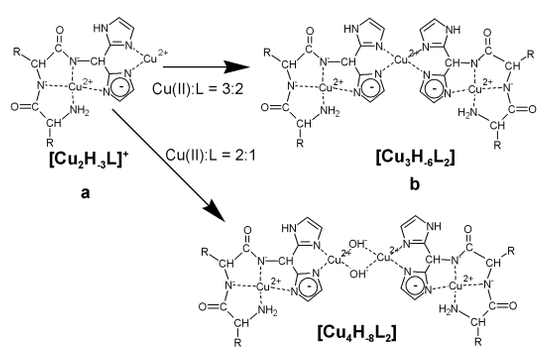


Fig. 3 Species distribution of the complexes formed in the copper(II)–GlyLeu-BIMA system (c_L = 4 × 10^{-3} mol dm^{-3}, c_M = 6 × 10^{-3} mol dm^{-3}).

$[Cu_2H_{-3}L]^{+}$, $[Cu_3H_{-6}L_2]$ and $[Cu_4H_{-8}L_2]$ are the major polynuclear complexes in alkaline solutions and their formation is represented by Scheme 5. Following the binding of the second amide group in $[CuH_{-2}L]$ the imidazole nitrogen atoms get to a sterically favourable position which promotes the deprotonation of the pyrrole type N(1)H group resulting in the formation of the $[Cu_2H_{-3}L]^{+}$ species (Scheme 5a.). It is also clear from the structure of $[Cu_2H_{-3}L]^{+}$, that the co-ordination sphere of the metal ion is not saturated yet, and this results in the formation of tri- or tetra-nuclear complexes depending on the metal to ligand ratio. At 3 : 2 metal to ligand ratio the trinuclear complex $[Cu_3H_{-6}L_2]$ predominates above pH 7, in which all



Scheme 5

copper(II) ions are co-ordinated by 4 N donor atoms (Scheme 5b), while at 2 : 1 metal to ligand ratio a mixed hydroxo complex $[Cu_4H_{-8}L_2]$ is formed connecting the $[Cu_2H_{-3}L]^+$ moieties *via* hydroxo bridges (Scheme 5c).

The existence of the trinuclear complexes is also supported by MALDI-MS. The major part of the MALDI-MS spectrum is shown in Fig. 4 and it unambiguously proves the presence of three copper(II) ions in the complex formed at pH 8 in solutions containing copper(II) and LeuGly-BIMA at 3 to 2 ratio. The isotopic distribution pattern obtained for the complex is in very good agreement with that calculated for the molecular ion $[Cu_3H_{-6}L_2]H^+ = [Cu_3C_{30}H_{40}O_4N_{14}]H^+$.

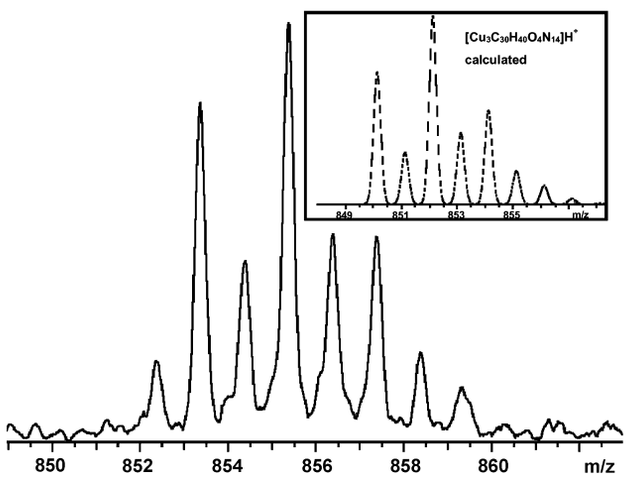


Fig. 4 MALDI-MS spectra obtained in the copper(II)–LeuGly-BIMA system at 3 : 2 ratio.

There is a difference, however, between the estimated (M($[Cu_3C_{30}H_{40}O_4N_{14}]H^+$ = 852.3) and measured (M = 855.3) molecular weights, which corresponds to the uptake of three protons. The higher values for the measured molecular weight could be explained by the reduction of Cu^{2+} to Cu^+ during the absorption/ionization process. Similar effects were observed and reported in the literature in the case of MALDI-MS studies of copper(II) complexes of cyclen and cyclam derivatives[50] and some pseudo-peptides.[51]

Measurements of magnetic moments were carried out by NMR to prove the formation of tetranuclear complexes containing hydroxo bridges in the copper(II)–LeuGly-BIMA = 2 : 1 systems. There was no change in the values of the magnetic moments in the pH range 2 to 5 indicating the presence of mononuclear copper(II) complexes with one unpaired electron (μ_{eff} = 1.73 μ_B). However, the number of paramagnetic centres started to decrease above pH 5 and μ_{eff} = 1.58 μ_B was calculated from NMR spectra detected at pH 6.2, which corresponds to the highest concentration of the species $[Cu_2H_{-3}L]^+$. Further increase of pH resulted in further decrease of magnetic moment and μ_{eff} = 1.41 μ_B was obtained at pH 10.0, where the tetranuclear complex $[Cu_4H_{-8}L_2]$ predominates. Both values indicate polynuclear species with antiferromagnetic coupling between copper(II) ions. These data together with the potentiometric results support the assumption that the base consuming process is not a simple deprotonation of the co-ordinated water molecules in $[Cu_2H_{-3}L]^+$, but the decrease of the magnetic moment could originate from the stronger antiferromagnetic coupling in $[Cu_4H_{-8}L_2]$ species containing hydroxo bridges. A similar effect was observed in other hydroxo-bridged dicopper(II) complexes.[52] The data strongly support the theory that the dihydroxo bridged tetranuclear complex is one of the major species formed in slightly alkaline solution in the presence of excess metal ion in the copper(II)–LeuGly-BIMA system. On the other hand, these results and the formation of precipitates at high pH values suggest the presence of other hydroxo species in solution.

Nickel(II) and zinc(II) complexes

Stability constants of nickel(II) and zinc(II) complexes of the three dipeptide derivatives (GlyLeu-BIMA, LeuGly-BIMA, PheGly-BIMA) were determined by potentiometric measurements and the data are included in Table 4.

The data reveal that the stoichiometry and stability constants of the complexes are independent of the dipeptide chain. In all three cases the metal ions bind to the bis(imidazol-2-yl)-methyl residue of the molecules in acidic media. The trend of the thermodynamic stability of bis(imidazol-2-yl)methyl co-ordinated complexes obeys the Irving–Williams series ($\log\beta[CuH_2L_2]^{4+}] > \log\beta[NiH_2L_2]^{4+}] > \log\beta[ZnH_2L_2]^{4+}]$ and $\log\beta[CuHL]^{3+}] > \log\beta[NiHL]^{3+}] > \log\beta[ZnHL]^{3+}]$).

The successive deprotonation of the mono- and bis-(ligand) complexes comes from the deprotonation of the non-co-ordinated terminal amino groups. The p*K* values are, however, lower than those of the free ligands suggesting weak axial interaction between the metal ions and amino groups. In contrast to the copper(II) complexes, the octahedral geometry of nickel(II) and zinc(II) complexes makes possible the tridentate co-ordination of ligands.

Although the bis(imidazol-2-yl)methyl group is considered as an effective binding site for both metal ions, only the nickel(II) ion is able to induce the deprotonation of amide nitrogens. These processes take place around pH 7.5 similar to the Ni(II)–GlyGlyHis[29] system. On the other hand, it is also clear from Table 4 that the deprotonation of the two amide functions very much overlap, which is a common feature of nickel(II)–tripeptide systems, although the p*K* values for the nickel(II) complexes of the bis(imidazol-2-yl) ligands are slightly lower than those reported for triglycine[53] (Ni(II)–GlyGlyGly: p*K* (ML/$MH_{-1}L$) = 8.8; p*K*($MH_{-1}L/MH_{-2}L$) = 7.7). As a consequence, the complex $[NiH_{-2}L]$ is the major species in a rather wide pH range both in equimolar solution and in the presence of excess of ligand (Fig. 5).

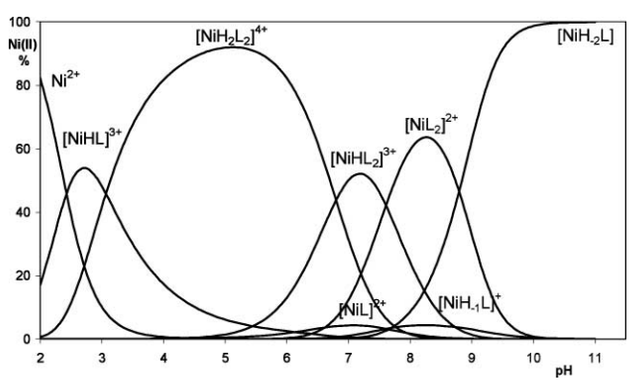


Fig. 5 Species distribution of the complexes formed in the nickel(II)–GlyLeu-BIMA system ($c_L = 4 \times 10^{-3}$ mol dm^{-3}, $c_M = 2 \times 10^{-3}$ mol dm^{-3}).

It is important to note that in parallel with the formation of $[NiH_{-2}L]$ complexes the solutions turn yellow and an intense absorption band develops at 393 nm in the UV-VIS spectra. These observations can be explained by the change of the co-ordination geometry from octahedral to square planar and the saturation of the co-ordination sphere around the nickel(II) ion in the $[NiH_{-2}L]$ complex similar to the corresponding copper(II) complexes as was shown in Scheme 4.

Complex formation reactions between zinc(II) ions and the dipeptide ligands are much simpler than those of the other two metal ions. The imidazole nitrogen atoms are the metal binding sites in slightly acidic solutions, but this type of co-ordination is not able to suppress hydrolytic reactions. It is also clear from

Table 4 Stability constants (log β_{pqr}) of copper(II) complexes (T = 298 K; I = 0.2 mol dm^{-3}, standard deviations are in parentheses)

	GlyLeu-BIMA		LeuGly-BIMA		PheGly-BIMA	
	Ni(II)	Zn(II)	Ni(II)	Zn(II)	Ni(II)	Zn(II)
$[MH_2L_2]^{4+}$	27.99 (2)	24.56 (4)	27.75 (2)	24.17 (5)	27.13 (3)	23.39 (7)
$[MHL_2]^{3+}$	21.18 (7)	17.77 (7)	20.73 (8)	17.30 (5)	20.74 (9)	17.20 (9)
$[ML_2]^{2+}$	13.59 (5)		13.51 (5)		14.13 (9)	
$[MHL]^{3+}$	14.36 (2)	12.45 (2)	14.16 (1)	12.28 (3)	13.65 (4)	11.97 (3)
$[ML]^{2+}$	8.22 (3)	5.1 (1)	8.12 (2)		8.48 (4)	
$[MH_{-1}L]^{+}$	0.62 (5)		1.02 (2)		1.10 (7)	
$[MH_{-2}L]$	−7.07 (3)		−6.55 (2)		−6.61 (5)	
pK(MH_2L_2/MHL_2)	6.81	6.79	7.02	6.87	6.39	6.19
pK(MHL_2/ML_2)	7.59		7.22		6.61	
pK(MHL/ML)	6.14	7.37	6.04		5.17	
pK(ML/$MH_{-1}L$)	7.60		7.10		7.38	
pK($MH_{-1}L$/$MH_{-2}L$)	7.69		7.57		7.71	
log(K_1/K_2) [l]	0.73	0.34	0.57	0.39	0.17	0.55

[l] Calculated for the protonated complexes: 2 log β_{111} − log β_{122}.

the potentiometric titration curves that the zinc(II) ion is not able to induce deprotonation of the amide nitrogen in any case and precipitation can be observed above pH 7 at all metal ion to ligand ratios.

Conclusions

The results presented in this paper clearly demonstrate that the bis(imidazol-2-yl)methyl residues linked to the dipeptides are the primary ligating sites for all three metal ions and significantly increase the metal binding ability of the ligands as compared to the simple dipeptides. The species $[MH_nL_2]^{(2+n)+}$ (n = 1–2) and $[MHL]^{3+}$ were formed in acidic media in all systems studied and spectroscopic data unambiguously prove that the nitrogen donor atoms of the bis(imidazol-2-yl)methyl residues are the exclusive metal binding sites under these conditions. In the case of copper(II) and nickel(II) complexes, however, the donor functions of the peptide chains can compete with the 4N binding mode and this results in a great versatility of complex formation reactions. It is especially true for the complex formation reactions of copper(II) where the formation of a large number of various di- and poly-nuclear complexes has been detected including the species $[Cu_2L_2]^{4+}$, $[Cu_2H_{-2}L_2]^{2+}$, $[Cu_2H_{-3}L]^{+}$, $[Cu_3H_{-6}L_2]$ and $[Cu_4H_{-8}L_2]$. The UV-VIS and EPR spectra of equimolar solutions indicate that the stoichiometry $[Cu_2L_2]^{4+}$ and $[Cu_2H_{-2}L_2]^{2+}$ corresponds to a mixture of several isomeric species including those represented by Schemes 2 and 3.

In the case of the copper(II) and nickel(II) complexes, with the exception of AlaPro-BIMA, the interaction of the terminal amino group promotes deprotonation and co-ordination of the –CONH– amide functions and the formation of $[MH_{-2}L]$ species with metal binding of terminal amino, two amide and one imidazole nitrogens. One of the most intriguing findings of this study is that the deprotonation of the N(1)H group of imidazole creates a new chelating site in the species $[CuH_{-2}L]$. This reaction provides a good base for the formation of various polynuclear complexes including $[Cu_2H_{-3}L]^{+}$, $[Cu_3H_{-6}L_2]$ and $[Cu_4H_{-8}L_2]$. The formation of the trinuclear complex $[Cu_3H_{-6}L_2]$ is especially favoured among them being a single species at 3 : 2 metal to ligand ratio. The existence of $[Cu_3H_{-6}L_2]$ was confirmed by UV-VIS and MALDI-MS measurements. The metal ions of this trinuclear complex are bridged *via* doubly deprotonated and negatively charged imidazole residues and the deprotonation and co-ordination of imidazole moieties take place at physiological pH. Metal ion promoted deprotonation of the pyrrole type N(1)H groups of imidazole has already been reported, but in the case of histidine and its peptides it took place only in basic solution.

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