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Copper(II) complexes of amino acid derivatives of the bis(imidazol-2-yl)methyl residue

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Copper(II) complexes of amino acid derivatives of bis(imidazol-2-yl)methylamine (BIMA), α-Asp–BIMA, α-Glu–BIMA, γ-Glu–BIMA and β-Ala–BIMA, were studied by potentiometric, UV-VIS and EPR techniques. The bis(imidazol-2-yl)methyl residue is the main binding site in strong acidic medium in all cases. The formation of ligand-bridged dinuclear $[Cu_2L_2]^{2+}$ species was detected in an equimolar solution of α-Asp–BIMA and γ-Glu–BIMA with binding of the N-terminal amino and carboxylate groups. The deprotonation of amide nitrogen takes place above pH 6 for the ligands containing an amide group in a chelatable position with terminal amino group (α-Asp–BIMA, α-Glu–BIMA and β-Ala–BIMA), resulting in the formation of dinuclear $[Cu_2H_{-2}L_2]$ species with imidazole bridging. For these 3 ligands, deprotonation of the pyrrole-type nitrogen was observed and the existence of a trinuclear species $[Cu_3H_{-4}L_2]$ was assumed in alkaline solution. This species contains negatively charged imidazolato bridges.

Introduction

The N(1)/N(3) donor atoms of histidyl and carboxylate groups of the aspartyl and glutamyl residues are especially preferred binding sites of metalloenzymes. Metal ions in the active centre of enzymes are very often bound by two or more imidazole rings[1–3] and in these cases the three-dimensional structures of the macromolecules make possible the coordination of different side chain donor atoms to the same metal ion. The active centre of these metalloenzymes can be mimicked by ligands containing two or more imidazole rings linked *via* an aliphatic carbon chain. On the other hand, various metalloenzymes contain the imidazole moiety as a bridging ligand, such as in CuZn-superoxide dismutase (CuZn-SOD) , where both nitrogen atoms of the negatively charged imidazolato residue take part in metal bridging.[4] In recent years the transition metal complexes of numerous polyimidazole ligands have been synthesized[5–7] and their enzyme activities—for example, SOD,[8,9] tyrosinase,[7] nitrite reductase[6] and hydrolytic[5]— were investigated. These studies reveal that the transition metal complexes of various ligands containing two or more imidazole rings may serve as structural and functional models of metalloenzymes binding the metal ions *via* N(3) and/or N(1) donor atoms of imidazole rings.

The simplest representatives of polyimidazole ligands are derivatives of bis(imidazol-2-yl)methane (BIM), in which two imidazole rings are linked *via* the methylene group. 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* coordination by the imidazole nitrogen atoms.[10] The coordination chemistry of ligands containing two imidazole rings is more versatile when the chelating agent is linked to another chelating ligand, creating multi- and/or ambidentate ligands.[11–15] It is obvious from studies on the copper(II) complexes of amino acid derivatives of the bis(imidazol-2-yl)methyl group (Gly–BIMA, Phe–BIMA, His–BIMA) that the potential donor atoms of the amino acids connected to the bis(imidazol-2-yl)methyl group are able to change the bis(imidazol-2-yl)methyl-like coordination mode.[11,13,14] The effect of the amino acid residue depends on the side chain donor groups. For example, the presence of the strongly coordinating histidyl imidazole nitrogens in His–BIMA results in a great variety of complex formation reactions of the ligand. The existence of a dinuclear complex $[Cu_2L_2]^{4+}$ with ligand bridging was described in slightly acidic solution with three isomeric forms. Deprotonation of the terminal amino group is followed by the deprotonation and coordination of the amide nitrogen, resulting in a stable imidazole-bridged dinuclear complex. Deprotonation of the imidazole N(1)H donor functions was detected under slightly alkaline conditions. 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 carboxylate group is another important binding site of metalloenzymes; for example, it takes part in the binding of zinc(II) ion in the CuZn-SOD enzyme. In contrast to imidazole, however, the carboxylate group exhibits weak ligation toward copper(II) ion. Studies on the copper(II) complexes of peptides containing an α-aspartyl moiety reveal that the effect of carboxylate groups on the complex formation processes depends on the location of the aspartyl residue in the peptide chain.

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The presence of α-aspartic acid at the N-terminus enhances the metal-binding ability of the ligands and slightly suppresses the deprotonation and coordination of the amide group as compared to oligoglycines.[16–19] An α-glutamic acid at the N-terminus, however, does not have the same effect and the complex formation processes of α-Glu–X dipeptides are analogous to those of Ala–X dipeptides.[18,19] On the other hand, the β-carboxylate group of aspartic acid and γ-carboxylate group of glutamic acid can be part of an amide bond connecting to other amino acid. In these cases there are amino-acid-like binding sites on the N-termini of the ligands, which are able to modify the coordination modes, resulting in the formation of stable mono- and bis(ligand) complexes.[18,19] For example, in the case of the ligands α-Asp–ε-Lys or γ-Glu–ε-Lys, the β-alanine- or α-alanine-like coordination, respectively, allows the formation of dimeric species with ligand bridging.[20,21]

Recently, we have investigated the effect of the side chain carboxylate group on the complexation of amino acid bis(imidazol-2-yl)methyl derivatives *via* the ligands *N*-α-aspartyl-bis(imidazol-2-yl)methylamine (α-Asp–BIMA), *N*-α-glutamyl-bis(imidazol-2-yl)methylamine (α-Glu–BIMA), *N*-γ-glutamyl-bis(imidazol-2-yl)methylamine (γ-Glu–BIMA) and, for comparison, *N*-β-alanyl-bis(imidazol-2-yl)methylamine (β-Ala–BIMA), all shown in Scheme 1. These studies give the possibility to compare the influence of the strongly coordinating imidazole ring and slightly coordinating carboxylate group on the complex formation ability of amino acid–BIMA ligands. The results of combined potentiometric and spectroscopic (EPR, UV-VIS) studies on the copper(II) complexes of the four ligands are reported in this paper.

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Experimental

Synthesis

The synthetic procedure for the preparation of N-protected and free amino acids and peptides containing BIMA on their C-terminus has already been reported: α-Asp–BIMA, α-Glu–BIMA, γ-Glu–BIMA and β-Ala–BIMA were prepared accordingly.[22] The purity of the above peptide derivatives was checked by TLC and HPLC and their structure was proven by ^{1}H NMR.

Potentiometric measurements

The pH potentiometric titrations in the pH range 2.2–11.0 were performed on 3–4 cm^3 samples in the concentration range of 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. 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 combined glass electrode (Metrohm) and a Dosimat 715 automatic burette (Metrohm) containing carbonate-free potassium hydroxide in known concentration. In some cases the potentiometric titrations were performed with a MOLSPIN pH meter equipped with a MOL-ACS microburette controlled by computer.

The number of experimental data points varied from 50 to 70 (cm^3/pH) for each titration curve. The pH readings were converted into hydrogen ion concentration as described earlier.[23]

Protonation constants of the ligands and the overall stability constants (log β_{pqr}) of the systems were calculated by means of general computational programs, PSEQUAD[24] and SUPERQUAD,[25] using eqns (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) complexes were recorded on Hewlett Packard HP 8453 or JASCO UVIDEC-610 spectrophotometers in the same concentration range as used for the potentiometry measurements.

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 was prepared from $CuSO_4 \cdot 5H_2O$ enriched in ^{63}Cu to get better resolution of the 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 to sulfate.

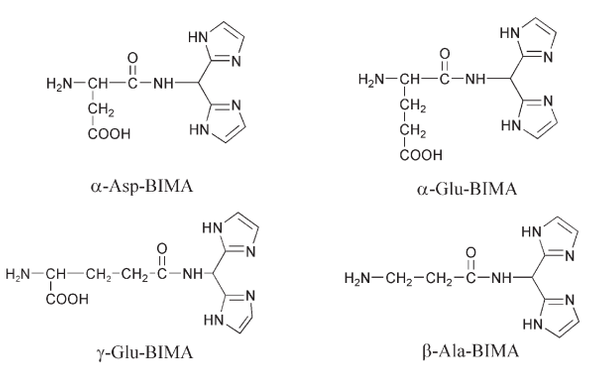
Results and discussion

Protonation equilibria of the ligands

The examined ligands can be divided into two groups on the basis of the number of dissociable groups present in the molecules. The ligand β-Ala–BIMA has only 3 acidic groups in the molecule (and therefore can be indicated as H_3L^{3+}), namely two imidazole nitrogens and the N-terminal amino nitrogen. The ligands in the second group have an extra free carboxylic group, either at the C-terminus or in the side chain (α-Asp–BIMA, α-Glu–BIMA and γ-Glu–BIMA) and can be indicated as H_4L^{3+}.

Protonation constants and p*K* values of the ligands α-Asp–BIMA, α-Glu–BIMA, γ-Glu–BIMA, β-Ala–BIMA are collected in Table 1 with the associated parameters for Gly–BIMA also shown for comparison. It is clear from Table 1 that the highest p*K* values belong to the deprotonation processes of terminal amino groups. The side chain carboxylate group decreases the basicity of the amino group, similarly to those of simple dipeptides [p*K*(NH_2): Gly–Gly: 8.13,[26] α-Asp–Gly: 7.94,[18] α-Glu–Val: 7.86[18]]. The p*K*(NH_3^+) value is much higher for the ligands β-Ala–BIMA and γ-Glu–BIMA due to the electron-releasing effect of the aliphatic chain. A similar trend was observed for γ-Glu–Val [p*K*(NH_2): 9.16[18]] and β-Ala–Gly [p*K*(NH_2): 9.44[26]] ligands.

The deprotonation of the bis(imidazol-2-yl)methyl nitrogen atoms takes place at lower pH than that of free imidazole, similarly to other bis(imidazol-2-yl)methyl derivatives.[10–12] The deprotonation of N donor atoms occurs separately and their p*K* values are in good agreement with each other and that of Gly–BIMA. The deprotonation processes of the first imidazole nitrogen and the side chain carboxyl group are , however, significantly overlapped.



Scheme 1

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Table 1 Stability constant (logβ) of the proton and copper(II) complexes of amino acid derivatives of the bis(imidazol-2-yl)methyl residue

	α-Asp–BIMA	α-Glu–BIMA	γ-Glu–BIMA	β-Ala–BIMA	Gly–BIMA[11]
HL	7.48(1)	7.53(1)	9.05(1)	9.23(1)	7.95
H_2L	12.97(2)	13.05(2)	14.88(2)	14.74(1)	13.46
H_3L	16.29(3)	16.79(3)	18.29(3)	17.87(2)	16.68
H_4L	18.61(5)	19.48(4)	20.08(5)	–	–
pK_1	2.32(5)	2.69(4)	1.79(5)	3.13(2)	3.22
pK_2	3.32(3)	3.74(3)	3.41(3)	5.51(1)	5.51
pK_3	5.49(2)	5.52(2)	5.83(2)	9.23(1)	7.95
pK_4	7.48(1)	7.53(1)	9.05(1)	–	–
CuH_4L_2	–	37.46(7)	–	–	–
CuH_3L_2	–	34.01(8)	–	–	–
CuH_2L_2	29.04(9)	29.84(5)	32.54(5)	32.73(6)	31.64
$CuHL_2$	23.11(36)	–	–	–	–
$CuH_{-1}L_2$	8.7(9)	–	–	–	11.12
CuH_2L	–	19.97(2)	–	–	–
CuHL	15.63(3)	16.38(12)	17.18(2)	17.28(5)	17.11
CuL	–	–	–	11.95(5)	–
$CuH_{-2}L$	–	–	–	−3.52(10)	−0.92
Cu_2L_2	25.25(16)	–	29.09(6)	–	–
$Cu_2H_{-2}L_2$	15.46(13)	16.70(3)	–	13.93(12)	18.43
$Cu_2H_{-3}L_2$	6.66(26)	8.26(12)	–	–	–
$Cu_2H_{-4}L_2$	−2.33(19)	−0.71(7)	–	–	–
$Cu_3H_{-4}L_2$	9.33(11)	7.97(11)	–	6.64(10)	–
$Cu_2H_{-1}L$	9.92(7)	10.06(5)	10.45(7)	–	–
pK(CuH_2L/CuHL)	–	3.59	–	–	–
pK(CuH_4L_2/CuH_3L_2)	–	3.45	–	–	–
pK(CuH_3L_2/CuH_2L_2)	–	4.17	–	–	–
pK(CuH_2L_2/$CuHL_2$)	5.93	–	–	–	–
pK($Cu_2H_{-2}L_2$/$Cu_2H_{-3}L_2$)	8.8	8.44	–	–	–
pK($Cu_2H_{-3}L_2$/$Cu_2H_{-4}L_2$)	8.99	8.97	–	–	–
$\log(K_1/K_2)^{H}$ [a]	2.22	2.92	1.82	1.83	2.58

[a] $\log (K_1/K_2)^{H} = 2 \log\beta(CuHL) - \log\beta(CuH_2L_2)$.

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Copper(II) complexes of the ligands

It is clear from the structure of the ligands that all four ligands have several different types of binding sites. The terminal amino, amide and bis(imidazol-2-yl)methyl nitrogens are in chelatable positions in the case of α-Asp–BIMA, α-Glu–BIMA and β-Ala–BIMA. For γ-Glu–BIMA, however, the amide bond is connected to the γ-carboxylate group and only the unfavourable 7 -membered ring could be formed. On the other hand, for α-Asp–BIMA and γ-Glu–BIMA the terminal amino and side chain carboxylate groups can also coordinate to a metal ion, forming a six- or five-membered chelate ring, respectively.

The stability constants of the copper(II) complexes and some selected equilibrium parameters are summarized in Table 1. These data show that the complex formation processes are very similar in all four cases in strong acidic media. Mono- and bis(ligand) complexes are formed with coordination of two or four imidazole nitrogens ([CuHL], $[CuH_2L_2]$).

The EPR parameters of the bis(ligand) complexes (Table 2) are very similar to each other and to those of Gly–BIMA and His–BIMA, supporting the same 4N bis(imidazolyl) binding sites in all bis(ligand) complexes. All these complexes are characterized by the presence of a well-resolved nine-line superhyperfine splitting in the first parallel band of the EPR spectra (Fig. 1), in agreement with the coordination of 4 equivalent nitrogen donors in the equatorial plane of the metal ion, while excluding (NH_2,COO^-) coordination in the $[CuH_2L_2]$ species in all cases. In the case of α-Glu–BIMA the species $[CuL_2H_4]^{4+}$ and $[CuL_2H_3]^{3+}$ are also formed with the same coordination as $[CuL_2H_2]^{2+}$; these are deprotonated with pK values of 3.45 and 4.17, respectively, which can be assigned to the carboxylic functions. Moreover, for the same ligand the species $[CuLH_2]^{3+}$ can also be formed; it deprotonates with a pK value of 3.59 assigned again to the carboxylic function. Since the EPR parameters of the species $[CuLH_2]^{3+}$ and $[CuLH]^{2+}$ are different (see Table 2) we suggest a weak coordination of the side chain carboxylate group, while $[CuLH_2]^{3+}$ has the usual [N(Im), N(Im)] coordination. This can probably be explained by the fact that the γ-carboxylate group of α-Glu–BIMA is further from the coordinated bis(imidazol-2-yl)methyl residue than that of α-Asp–BIMA and it is sterically more favoured to form a loop.

The comparison of the stepwise stability constants reveals some differences in the thermodynamic stability of the bis-chelated complexes. The $\log(K_1/K_2)^{H}$ values follow the order: α-Glu–BIMA (2.92) > α-Asp–BIMA (2.22) > β-Ala–BIMA (1.83) ≈ γ-Glu–BIMA (1.82), which reflects the increased stability of bis(ligand) complex formation. This order leads to the conclusion that a β- or a γ-amino acid linked to the bis-imidazolyl residue increases the stability of the bis-chelated complexes compared to ligands with α-amino acid residues linked to the same moiety. This means that the further the amino and carboxylate groups are from the coordinating donors, the more stable the bis-chelated complexes are.

The deprotonation of terminal amino group is, however, accompanied by new complex formation processes. The possibility to form five- or six membered amino acid-like chelate rings on the N-termini of ligands results in the existence of dinuclear $[Cu_2L_2]^{2+}$ species in the equimolar solution of α-Asp–BIMA and γ-Glu–BIMA. At least two isomeric forms of ligand-bridged structures (Scheme 2) can be postulated, with symmetric and asymmetric arrangements.

EPR spectra obtained in an equimolar solution of Cu(II)–α-Asp–BIMA in the pH range of existence of the dinuclear complex show a decrease in the intensity of the signals, but molecular models clearly indicate that a dinuclear species with

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Table 2 Spectral parameters of various copper(II) complexes of the amino acid derivatives of the bis(imidazol-2-yl)methyl residue

		α-Asp–BIMA	α-Glu–BIMA	γ-Glu–BIMA	β-Ala–BIMA	Gly–BIMA[d 11]	His–BIMA[15]
CuH_2L	$10^4\ A_{\parallel/cm^{-1}}$		168				176
	$g_\parallel$		2.298				2.298
	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})		687 (31)[a]				683 (32)
CuHL [N(Im), N(Im)][b]	$10^4\ A_{\parallel/cm^{-1}}$	171	180	172	170	175	
	$g_\parallel$	2.298	2.287	2.296	2.296	2.299	
	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	690 (46)	695 (25)/ 633 (36)[c]	688 (43)[a]	691 (41)[a]	681 (31)	
CuH_nL_2 (n = 2–4) [2·N(Im), N(Im)]	$10^4\ A_{\parallel/cm^{-1}}$	193	193	193	192	201	197
	$g_\parallel$	2.234	2.232	2.234	2.233	2.236	2.234
	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	601 (42)	596 (65)	592 (46)	592 (46)	590 (34)	605 (50)
$Cu_2H_{-2}L_2$ [NH_2,N^-,N(Im)] + N(Im) bridge	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	591 (113)	589 (104)	–	564 (93)	595 (91)	592 (111)
	Cu–Cu distance/pm	393	393	–	414	390	397
$Cu_2H_{-4}L_2$ [NH_2,N^-,N(Im)] + N(Im) bridge	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	567 (95)	562 (100)	–	–	–	565 (117)
	Cu–Cu distance/pm	386	385	–	–	–	384

[a] Because of the presence of more than one species, the electronic absorption parameters should be considered as approximate. [b] The species CuLH formed with α-Glu-BIMA has a different coordination: [Im(N), Im(N) + COO^-]. [c] The dimeric species $Cu_2H_{-2}L_2$ is also present in solution. [d] EPR spectra of Gly-BIMA were measured with natural copper.

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two [N(Im), N(Im)](NH_2, COO^-) coordinated metal ions can be formed. The spectral parameters, for the species $[Cu_2L_2]^{2+}$ formed by α-Asp–BIMA, are $g_\parallel = 2.244$ and $A_\parallel = 176 \times 10^{-4}$ cm^{-1}.

In the case of γ-Glu–BIMA the ligand-bridged dinuclear structure can also be assumed with amino acid-like and bis-(imidazol-2-yl)methyl-like coordination. Fig. 2 shows the distribution curves of complexes formed in an equimolar solution of Cu(II)–γ-Glu–BIMA, together with the distribution curves of complexes formed in the Cu(II)–glycine(= A)–BIM(= B) ternary system. It is clear from Fig. 2 that in the pH range of existence of the dinuclear $[Cu_2L_2]^{2+}$ species the $[CuAB]^+$ complex predominates in the ternary system. This fact supports the idea that the $[Cu_2L_2]^{2+}$ structure with a mixed (NH_2, COO^-) + [N(Im),N(Im)] coordination of the copper(II) ions is preferred. The EPR parameters of the $[Cu_2L_2]^{2+}$ species are also similar to those obtained for the $[CuAB]^+$ complex formed in the ternary system ($g_\parallel = 2.239$, $A_\parallel = 190 \times 10^{-4}$ cm^{-1}).

Similar complexes with coordination of both ends of the ligands were detected in the case of His–BIMA[14] and the dipeptide derivatives Leu–Gly–BIMA, Gly–Leu–BIMA and

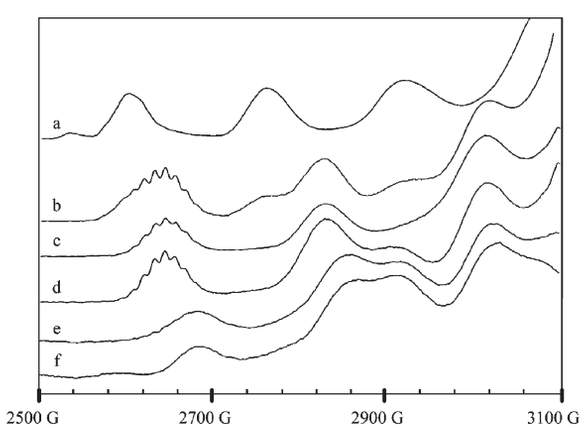
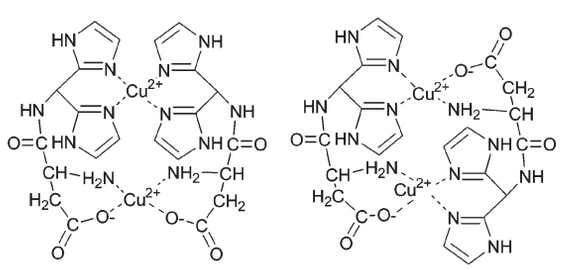
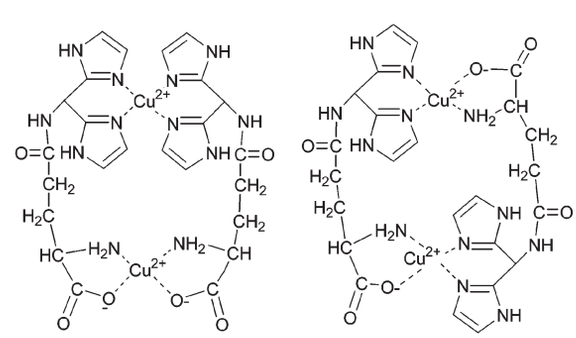


Fig. 1 Lowfield parallel region of the EPR spectra of the copper(II)–α-Asp–BIMA system at different pH values in frozen solution. Metal-to-ligand ratio 1:2. pH: (a) 2.51, (b) 3.00, (c) 4.90, (d) 6.00, (e) 7.55, (f) 8.80.



α-Asp-BIMA



γ-Glu-BIMA

Scheme 2

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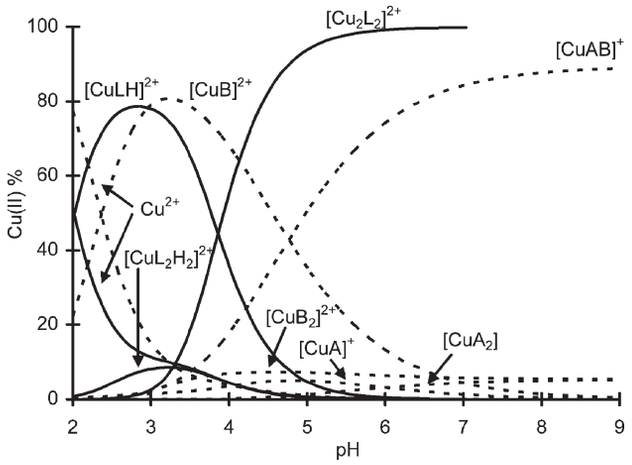

Fig. 2 Species distribution of the complexes formed in the copper(II)–γ-Glu-BIMA system (solid lines: $c_{Cu(II)} = c_L = 4 \times 10^{-3}$ mol dm^{-3}) and in the copper(II)–Gly(= A)–BIM(= B) ternary system (dotted lines: $c_{Cu(II)} = c_A = c_B = 4 \times 10^{-3}$ mol dm^{-3}) as a function of pH.

Phe–Gly–BIMA.[15] In the latter cases the terminal amino and carbonyl groups take part in the coordination. This binding mode, however, was not observed in the copper(II) complexes of Gly–BIMA or Phe–BIMA, probably because of steric effects. Similarly to the aliphatic amino acid derivatives, $[Cu_2L_2]^{4+}/[Cu_2L_2]^{2+}$ is not formed with either β-Ala–BIMA or α-Glu–BIMA. The species $[CuHL]^{3+}$ of β-Ala–BIMA deprotonates at the terminal amino group to give a complex, $[CuL]^{2+}$, with the same coordination.

In the case of α-Glu–BIMA the deprotonation of the terminal amino group occurs in parallel with deprotonation of the amide nitrogen. The deprotonation of the amide nitrogen also takes place in the α-Asp–BIMA and β-Ala–BIMA systems (Fig. 3). This process is accompanied with significant spectral changes. As a consequence, the $[Cu_2H_{-2}L_2]$ complex is formed in all cases—similarly to amino acid derivatives[11]—in which the terminal amino, deprotonated amide and one of the imidazole nitrogen donor atoms are the binding sites with a bridging imidazole from the other nitrogen atom of the bis(imidazolyl) residue. The EPR spectra obtained for the species $[Cu_2H_{-2}L_2]$ formed with α-Glu–BIMA and α-Asp–BIMA at pH 6.60 and 7.25, respectively, are more intense but very similar to those obtained for Gly–BIMA and His–BIMA, suggesting the same coordination environments for X–BIMA ligands. The spectra were simulated with the values $g_{\|} = 2.189$, $A_{\|} = 88 \times 10^{-4}$ cm^{-1}, $g_{\perp} = 2.055$ and $D = 0.0493$ cm^{-1}; these parameters are similar to those previously given for Gly–BIMA and His–BIMA. So we can assume that in all cases the bis(imidazolyl) residue acts as a bridging ligand, instead of the carboxylate group.

The formation of a dinuclear $[Cu_2H_{-2}L_2]^{2+}$ species can be observed in the β-Ala–BIMA system too. Its structure, however, cannot be exactly the same as previously observed because in this case a β-amino acid, instead of an α one, is linked to the bis(imidazol-2-yl)methyl residue and thus a six membered chelated ring is formed, instead of a five-membered one, involving amino and amide nitrogens. The spectral parameters of this species ($g_{\perp} = 2.057$, $D = 0.0388$ cm^{-1}) are therefore different from those measured previously in the case of Gly–BIMA. These differences can be explained by considering the differences in copper–copper distance (Table 2) and angles between the two dimeric structures, the first with 6- and the second with 5-membered chelated rings. The dimeric species formed with β-Ala–BIMA is, however, less stable than those formed with Gly–BIMA. The presence of a 6-membered chelated ring leads to an arrangement more relaxed toward a planar geometry, as it was previously observed in the case of

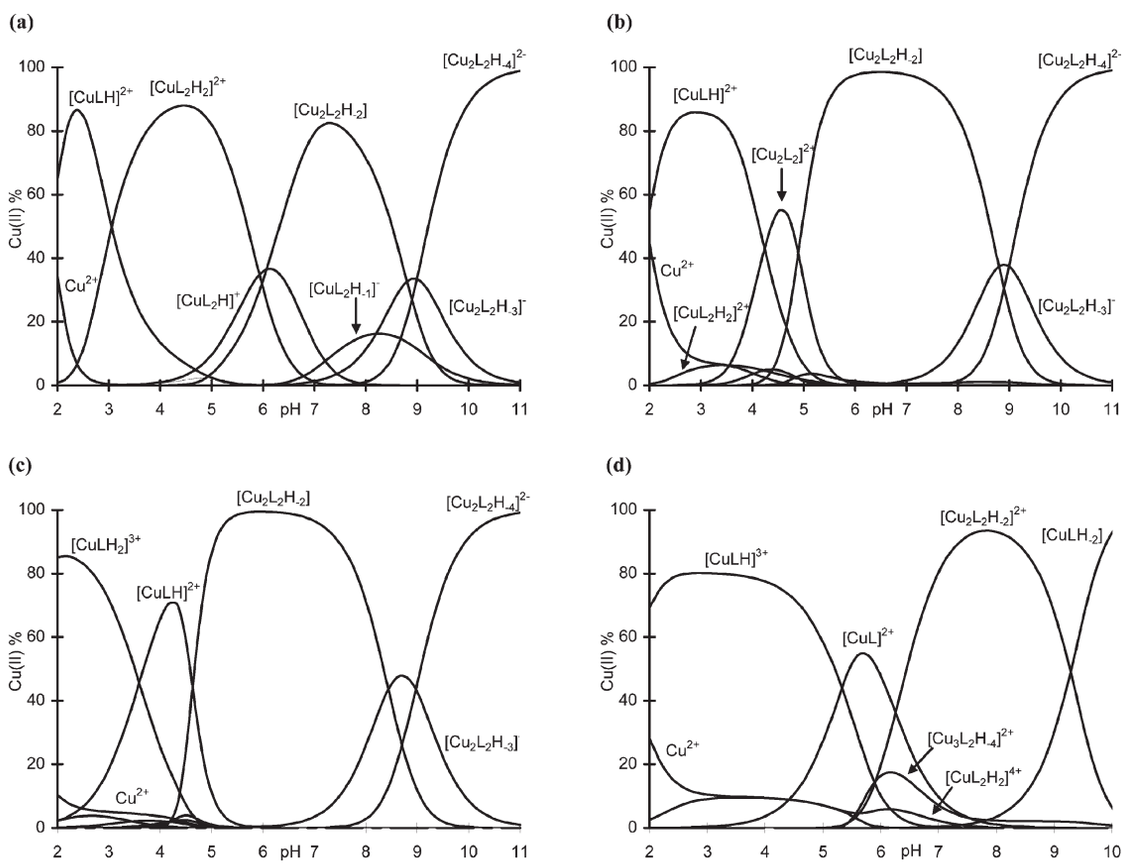

Fig. 3 Species distribution of the complexes formed in the (a and b) copper(II)–α-Asp–BIMA, (c) copper(II)–α-Glu–BIMA and (d) copper(II)–β-Ala–BIMA systems as a function of pH. $c_{Cu(II)} = c_L = 4 \times 10^{-3}$ mol dm^{-3} in all cases except for (a) for which $c_{Cu(II)} = 2 \times 10^{-3}$ mol dm^{-3}.

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oligopeptides containing β-Ala in various positions along the chain.[27] For all three ligands this dimeric species predominates, both in equimolar solution and in the presence of an excess of ligand, around the physiological pH range.

With an excess of α-Asp–BIMA above pH 7 another complex can be observed in solution, having the composition $[CuH_{-1}L_2]^-$. The unusual parameters measured for this species ($g_{\|} = 2.217$, $A_{\|} = 173 \times 10^{-4}$ cm^{-1}) suggest the tridentate equatorial [NH_2, N^-, N(Im)] coordination of one ligand and monodentate equatorial or bidentate axial-equatorial coordination of the bis(imidazolyl) nitrogen donors of the second ligand. The same behaviour was previously observed in the case of His–BIMA[14] and Gly–BIMA.[11]

The deprotonation of the amide nitrogen, however, cannot be observed in the Cu(II)–γ-Glu–BIMA system. Above pH 7 a blue precipitate appears in the solution. Taking into account that in this pH range the bis(amino acid)-like coordination is able to prevent the hydrolysis of copper(II) ion, we can conclude that this precipitate is a Cu(II) complex with polymeric structure.

The complex formation processes of α-Asp–BIMA and α-Glu–BIMA are similar to those of His–BIMA[14] and significantly different from those of β-Ala–BIMA and Gly–BIMA[11] under alkaline conditions. The major differences can be observed in equimolar solutions at high pH values. With β-Ala–BIMA and Gly–BIMA ligands the $[Cu_2H_{-2}L_2]^{2+}$ dinuclear complexes were transformed into mixed hydroxo species above pH 8. The same base-consuming process is observed with α-Asp–BIMA and α-Glu–BIMA, but it is accompanied by a significant blue shift of the absorption spectra (see Table 2) and characteristic changes in the EPR signals. The EPR spectra provide an unambiguous proof that the species $[Cu_2H_{-4}L_2]^{2-}$ detected with α-Asp–BIMA and α-Glu–BIMA are still dinuclear complexes, but the blue shift of the d-d bands suggests that the deprotonation cannot be a simple mixed hydroxo complex formation. These data can be explained by assuming that the base-consuming process arises from the deprotonation of the pyrrole-type N(1)H groups of coordinated imidazole residues. The coordination of charged nitrogen donors generally results in a blue shift of absorption spectra (*e.g.*, in peptide complexes) and a shortening of the bond distances compared to neutral nitrogen donors. Both the Cu(II)–Cu(II) distances and spectroscopic parameters (Table 2) are very similar to those of His–BIMA,[14] which establish the dimeric structure with deprotonated pyrrole-type nitrogens.

Fortunately, the EPR spectrum of the species $[Cu_2H_{-4}L_2]^{2-}$, formed by α-Asp–BIMA and α-Glu–BIMA, exhibits well-resolved triplet features, typical of an axial symmetry. Two sets of seven parallel ($2nI + 1 = 7$) well-resolved lines (separated by $2D$, where D is the zero field splitting parameter) due to copper hyperfine interactions, superimposed onto two perpendicular lines (separated by D), are observed (see Fig. 4); the spectrum was satisfactorily simulated by taking $g_{\|} = 2.183$, $A_{\|} = 90 \times 10^{-4}$ cm^{-1}, $g_{\perp} = 2.052$ and $D = 0.0523$ cm^{-1}. The half-field signal (centred at $g = 4.44$) is well-resolved and is distinctive of a dinuclear copper(II) complex.

With the previously examined ligands (Gly–BIMA, Phe–BIMA and His–BIMA)[11,13,14] it was possible to observe the perpendicular resonances, together with the hardly distinguishable seven parallel lines at lower fields, in the EPR spectra of the dimeric species $[Cu_2H_{-2}L_2]^{2+}$. Since it was impossible, at least under those experimental conditions, to observe the seven hyperfine lines at higher fields, it was also impossible to measure the D parameter from these signals and to extract its value. Therefore we used the two perpendicular lines. These lines are very broad and so we extracted only approximate values of the parameters. With α-Asp–BIMA and α-Glu–BIMA we obtained more intense EPR spectra for both dinuclear species ($[Cu_2H_{-2}L_2]$ and $[Cu_2H_{-4}L_2]^{2-}$) and it was possible to simulate the spectra with D values extracted from the parallel lines, which give a more precise value for this parameter. Consequently, only for these two ligands were we able to obtain reliable structural information directly from the EPR spectra.

The fully deprotonated dinuclear complex is the major species in alkaline solution, even in the presence of an excess of ligand, as it is demonstrated by Fig. 3. The deprotonation of $[Cu_2H_{-2}L_2]$ species occurs in two steps ($[Cu_2H_{-3}L_2]^-$, $[Cu_2H_{-4}L_2]^{2-}$) and the pK values for the deprotonation steps are 8.44 and 9.97 for α-Glu–BIMA, 8.80 and 8.99 for α-Asp–BIMA, values that are close to those of His–BIMA (8.13 and 8.93).

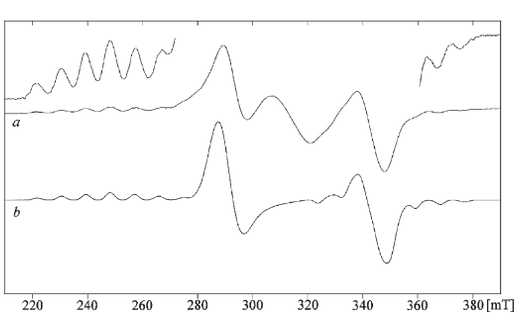

Fig. 4 Anisotropic X-band EPR spectrum (trace a, experimental; trace b, simulated) of the species $[Cu_2H_{-4}L_2]^{2-}$ formed by α-Asp–BIMA (pH 11.6, L/M = 2). The simulated spectrum was obtained with the simulation program Bruker Win-EPR SimFonia (see text for the parameters).

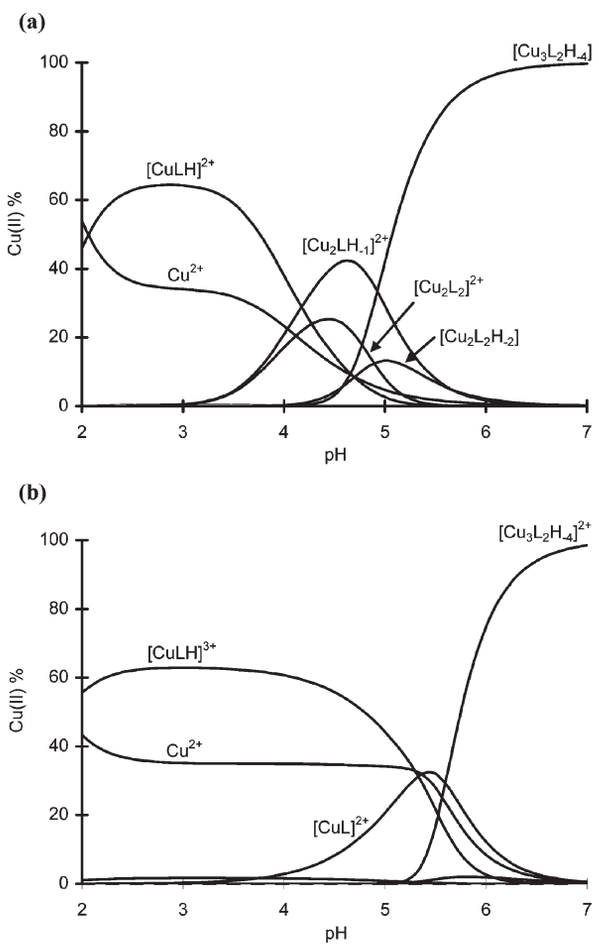

Fig. 5 Species distribution of the complexes formed in the (a) copper(II)–α-Asp-BIMA and (b) copper(II)–β-Ala-BIMA systems as a function of pH. $c_{Cu(II)} = 6 \times 10^{-3}$ mol dm^{-3}, $c_L = 4 \times 10^{-3}$ mol dm^{-3}.

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Another important consequence of the deprotonation of the N(1)H groups is that they can be considered as additional metal binding sites in the presence of excess metal ions. In agreement with this expectation there is no precipitation in solutions containing copper(II) and α-Asp–BIMA or α-Glu–BIMA in a molar ratio of 3:2. (Fig. 5.).

In the case of α-Asp–BIMA and α-Glu–BIMA the dinuclear complex $[Cu_2H_{-1}L]^{2+}$ is also formed. In this species the two copper ions are bound to the N- and C-terminal sides of the ligands with a likely [N(Im), N(Im)] and [NH_2, N^-, N(Im)] coordination, but in this case EPR spectroscopy cannot be used to assign the coordination because the spectra are poorly resolved.

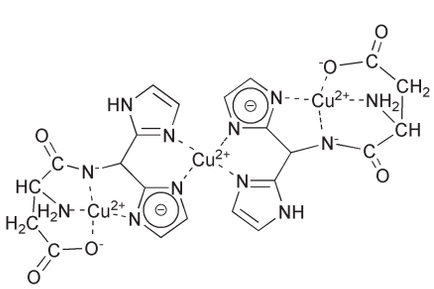
The extra base-consuming process appears between pH 5 and 6, resulting in the trinuclear $[Cu_3H_{-4}L_2]^{2+}$ complex for β-Ala–BIMA and $[Cu_3H_{-4}L_2]$ for α-Asp–BIMA and α-Glu–BIMA. Fig. 4 shows that the species $[Cu_3H_{-4}L_2]$ is formed almost exclusively at pH 7 under these conditions. The d-d absorption band of this species is rather wide with a maximum around 580 nm and shows broad unresolved EPR spectra, suggesting a significant dipolar interaction between the copper ions. These spectral features suggest that the trinuclear complex contains copper(II) ions in at least two different environments, as shown by Scheme 3. Two of the copper(II) ions are coordinated by (NH_2, N^-, N^-, N(Im)) donor functions, while the third copper(II) ion occupies the central position with 4 imidazole-N coordination by two tetradentately coordinated residues.This type of binding requires the deprotonation of one imidazole-N(1)H group in each tetradentately coordinated unit in the pH range 5–6. Similar processes can be observed in the case of Cu(II)–His–BIMA at a 3:2 molar ratio and it should be considered that deprotonation is accompanied by metal ion coordination. Imidazolato-bridged di- or polynuclear complexes have already been prepared by several authors and they generally were formed around the physiological pH range with potential SOD activity.[28,29]

Conclusion

The results of studies on copper(II) complexes of four amino acid ligands containing a bis(imidazol-2-yl)methyl residue are presented in this paper. These results provide further information about the complex formation processes of this type of ligands, the effect of side chain donor groups and allow the comparison of the behaviour of amino acid derivatives with a carboxylate group in the side chain (present work) with amino acid derivatives having a non-coordinating side chain and with an imidazole ring in the side chain.[11,14]

Our conclusions are the following. (i) The complex formation processes start in very acidic solution (pH ≤ 2), with the bis(imidazol-2-yl)methyl residue being the exclusive binding site in this pH region. (ii) The side chain donor groups of amino acids form six- or five membered chelate rings with the terminal amino group and this results in the formation of isomers of dinuclear complexes with ligand bridging. It is observed in the case of α-Asp–BIMA, γ-Glu–BIMA and His–BIMA. (iii) For all amino acid derivatives in which the terminal amino and deprotonated amide nitrogen can form five- or six membered chelates, the deprotonation of the amide nitrogen takes place at slightly acidic pH (above pH 6) and the coordination of the [NH_2, N^-, Im(N)] donor set is able to break the bis(imidazol-2-yl)methyl coordination mode. It results in the formation of dinuclear $[Cu_2H_{-2}L_2]$ species, in which one of the imidazole rings of the bis(imidazol-2-yl)-methyl moiety serves as a bridge. This process, however, does not take part in the case of γ-Glu–BIMA, in which the amino and amide nitrogens could only form a seven-membered chelate ring. (iv) A new base-consuming process can be observed in alkaline solution. This process can be explained by mixed hydroxo complex formation in the case of amino acid derivatives with a non-coordinating side chain (β-Ala–BIMA and Gly–BIMA) and the deprotonation of the pyrrole-type nitrogen of ligands containing a side chain donor group (α-Asp–BIMA, α-Glu–BIMA and His–BIMA). As a consequence, the presence of side chain donor group can prevent the hydrolysis of the copper(II) complex, probably because of its weak interaction with the copper(II) ion. (v) The deprotonation of the pyrrole-type nitrogen creates a new metal ion binding site, which leads to the formation of a trinuclear $[Cu_3H_{-4}L_2]$ complex with negatively charged imidazolate bridge in the Cu(II)–α-Asp–BIMA and Cu(II)–α-Glu–BIMA systems at a 3:2 molar ratio. The formation of trinuclear species also can be assumed for Cu(II)–β-Ala–BIMA at an excess of metal ion.

To sum up, it can be stated that the presence of a coordinating donor group in the amino acid side chain can influence the complex formation processes of the amino acid–BIMA ligands. The formation of the trinuclear complex is characteristic of both α-Asp–BIMA and His–BIMA systems and the coordination sphere of copper(II) ions with negatively charged imidazolato bridges resembles the active site of the CuZn-SOD enzyme.



$[Cu_3H_{-4}L_2]$

Scheme 3

Acknowledgements

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References

1 I. Bertini, C. Luchinat and A. Scozzafava, *Struct. Bonding*, 1982, **48**, 45.
2 F. A. Qyichio and W. N. Lipscomb, *Adv. Protein Chem.*, 1971, **25**, 1.
3 P. M. Collman, H. C. Freeman, J. M. Guss, M. Murata, V. A. Norris, J. A. M. Ramshwa and M. P. Venkatapp, *Nature (London)*, 1978, **272**, 319.
4 J. A. Tainer, E. D. Getzoff, K. M. Beem, J. S. Richardson and D. C. Richardson, *J. Mol. Biol.*, 1982, **160**, 181.
5 T. Gajda, R. Krämer and A. Jancsó, *Eur. J. Inorg. Chem.*, 2000, 1635.
6 M. Beretta, E. Bouwman, L. Casella, B. Douziech, W. L. Driessen, L. Gutierres-Soto, E. Monzani and J. Reedijk, *Inorg. Chim. Acta*, 2000, **310**, 41.
7 W. Andrade, I. A. Bagatin and A. M. Da Costa Ferreira, *Inorg. Chim. Acta*, 2001, **321**, 11.
8 H. Ohtsu, S. Itoh, S. Nagatomo, T. Kitagawa, S. Ogo, Y. Watanabe and S. Fukuzumi, *Inorg. Chem.*, 2001, **40**, 3200.
9 S.-P. Yang, Y.-X. Tong, H.-L. Zhu, H. Chao, X.-M. Chen and L.-N. Ji, *Polyhedron*, 2001, **20**, 223.
10 K. Várnagy, I. Sóvágó, K. Ágoston, Z. Likó, H. Süli-Vargha, D. Sanna and G. Micera, *J. Chem. Soc., Dalton Trans.*, 1994, 2939.
11 K. Várnagy, I. Sóvágó, W. Goll, H. Süli-Vargha, G. Micera and D. Sanna, *Inorg. Chim. Acta*, 1998, **283**, 233.
12 K. Várnagy, I. Sóvágó, H. Süli-Vargha, D. Sanna and G. Micera, *J. Inorg. Biochem.*, 2000, **81**, 35.

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13 I. Sóvágó, K. Várnagy and K. Ősz, *Comments Inorg. Chem.*, 2002, **23**, 149.
14 K. Ősz, K. Várnagy, H. Süli-Vargha, D. Sanna, G. Micera and I. Sóvágó, *Inorg. Chim. Acta*, 2002, **339**, 373.
15 K. Ősz, K. Várnagy, H. Süli-Vargha, D. Sanna, G. Micera and I. Sóvágó, *J. Chem. Soc., Dalton Trans.*, 2003, 2009.
16 B. Decock Le-Reverand, F. Liman, C. Livera, L. D. Pettit, S. I. Pyburn and H. Kozlowski, *J. Chem. Soc., Dalton Trans.*, 1988, 887.
17 J.-F. Galey, B. Decock Le-Reverand, A. Lebkiri, L. D. Pettit, S. I. Pyburn and H. Kozlowski, *J. Chem. Soc., Dalton Trans.*, 1991, 2281.
18 I. Sóvágó, E. Farkas, C. Bertalan, A. Lebkiri, T. Kowalik-Jankowska and H. Kozlowski, *J. Inorg. Biochem.*, 1993, **51**, 715.
19 H. Kozlowski, A. Lebkiri, C. O. Onindo and L. D. Pettit, *Polyhedron*, 1995, **14**, 211.
20 P. G. Daniele, E. Prenesti and G. Ostacoli, *J. Inorg. Biochem.*, 1996, **61**, 165.
21 Cs. Kállay, K. Várnagy, I. Sóvágó, D. Sanna and G. Micera, *J. Chem. Soc., Dalton Trans.*, 2002, 92.
22 Z. Likó and H. Süli-Vargha, *Tetrahedron Lett.*, 1993, **34**, 1673.
23 H. Irving, G. Miles and L. D. Pettit, *Anal. Chim. Acta*, 1967, **38**, 475.
24 L. Zékány and I. Nagypál, in *Computational Methods for the Determination of Stability Constants*, ed. D. Leggett, Plenum, New York, 1985, pp. 291–299.
25 P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1196.
26 I. Sóvágó, D. Sanna, A. Dessí and K. Várnagy, *J. Inorg. Biochem.*, 1996, **63**, 99.
27 D. Sanna, C. G. Ágoston, G. Micera and I. Sóvágó, *Polyhedron*, 2001, **20**, 3079.
28 G. Kolks, C. R. Frihart, H. N. Rabinowitz and S. J. Lippard, *J. Am. Chem. Soc.*, 1976, **98**, 5720.
29 G. Tabbi, W. L. Driessen, J. Reedijk, R. P. Bonomo, N. Veldman and A. L. Spek, *Inorg. Chem.*, 1997, **36**, 1168.

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