Equilibrium and structural studies on transition metal complexes of amino acid derivatives containing the bis(pyridin-2-yl)methyl residue

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The stoichiometries, stability constants and structures of the complexes formed in the reaction of copper(II), nickel(II) and zinc(II) ions and *N*-glycylbis(pyridin-2-yl)methylamine (Gly-BPMA) and *N*-histidylbis(pyridin-2-yl) methylamine (His-BPMA) ligands have been determined by potentiometric, UV-VIS and EPR spectroscopic methods. The formation of four-coordinate 1 : 1 copper(II) complexes and octahedral mono and bis(ligand) nickel(II) and zinc(II) complexes was detected. The bis(pyridin-2-yl)methyl moiety is the main binding site in acidic media, while deprotonation and coordination of the amide nitrogen take place in the physiological pH range. The stability of the complexes of the bis(pyridin-2-yl)methyl ligands is lower than that of analogous bis(imidazol-2-yl)methyl compounds. The weaker metal binding ability of the ligands containing the bis(pyridin-2-yl)methyl group is also reflected in the fact that, in contrast with analogous bis(imidazol-2-yl)methyl ligands, the non-coordinated pyridine nitrogens cannot act as a bridge-forming polymeric species.

Introduction

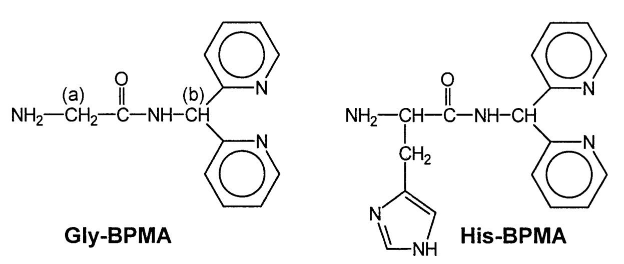
The complex formation processes of oligopeptides with palladium(II), copper(II) and nickel(II) ion are generally characterised by successive deprotonation and coordination of amide nitrogens.[1–4] However, the amide group is a very weak acid[1,5] and its metal ion coordination usually requires the presence of an anchoring donor group forming a chelate ring with the amide nitrogen. The terminal amino group is considered the most common anchor. The metal binding ability of peptides, however, is influenced significantly by the presence of various side chain donor groups. An effective side chain donor atom (like imidazole N or mercapto S) on the N-terminus of the peptide molecule can prevent the deprotonation and coordination of the amide nitrogen. On the other hand, the presence of these donor groups in chelatable positions with the amide nitrogens promotes the binding of the amide functions.[6–8] Glycylhistidine and glycylglycylhistidine are among the most extensively studied peptides, and in these cases cobalt(II) and zinc(II) ions were also reported to induce deprotonation and coordination of peptide amide groups in the physiological pH range.[6,9,10]

The bis(imidazol-2-yl)methyl moiety containing two imidazole rings linked by a methylene group can form especially stable complexes with 3d transition metal ions resulting in a six-membered chelate ring.[11] Its presence as a side chain group in peptides results in considerable changes in the complex formation processes. This group is the main binding site for copper(II), nickel(II) and zinc(II) ions in peptide derivatives containing it either on the C- or N-termini. In the case of tripeptide derivatives with a protected terminal amino group, amide nitrogen donor atoms of the peptide backbone do not take part in metal ion coordination suggesting that the bis(imidazol-2-yl)methyl residue (BIMA) is not an anchoring group for amide binding.[11,12] However, in the case of the glycine derivative (Gly-BIMA) the presence of the terminal amino group facilitates metal ion induced deprotonation and coordination of the amide nitrogen, breaking the six-membered chelate ring of the bis(imidazol-2-yl)methyl group.[13]

The pyridine nitrogen has a lower metal binding ability than the imidazole nitrogen, but various ligands containing two or more pyridine rings in chelating positions form stable complexes with transition metal ions. The equilibrium and X-ray diffraction studies of the complexes in solution[14] and in the solid state,[15–17] respectively, revealed that the other donor atoms of the ligands (*e.g.* amino or/and carboxylate groups) can also take part in coordination, but the pyridine nitrogens remain the main binding sites.

The bis(pyridin-2-yl)methyl group is analogous to the bis(imidazol-2-yl)methyl group. It was concluded from studies on complexes of the simplest derivatives of the bis(pyridin-2-yl)methyl residue[18–21] that this group is also an effective binding site for transition metal ions, similarly to the bis(imidazol-2-yl)methyl group. The bis(ligand) complexes of bis(pyridin-2-yl)methane with copper(II) ion were also prepared in the solid state[22] and the X-ray data show the binding of four pyridine nitrogens to the metal ion in the equatorial plane.

In the amide adduct of bis(pyridin-2-yl)methyl with proline (Pro-BPMA) the aromatic nitrogen donor atom is in a chelatable position with the amide nitrogen. The results obtained for the copper(II), nickel(II) and zinc(II) complexes of this compound[12] reflect that coordination of the bis(pyridin-2-yl)



Scheme 1

group occurs in the acidic pH range and this is followed by coordination of terminal amino, amide and pyridine nitrogens, similarly to Gly-BIMA.

In our previous papers[11–13] we reported the results of equilibrium and spectroscopic studies on copper(II) and zinc(II) complexes of tripeptides and glycine derivatives of bis(imidazol-2-yl)methyl and proline derivatives of bis(pyridin-2-yl)methyl residues. As the imidazolyl side chain of histidine can significantly influence the coordination ability of the peptide molecules, we continued our work with a study of bis(pyridin-2-yl)methyl derivatives of histidine. In the present paper the equilibrium, UV-VIS and EPR spectroscopic results are reported for copper(II), nickel(II) and zinc(II) complexes of *N*-histidylbis(pyridin-2-yl)methylamine (His-BPMA) (Scheme 1). These results are completed by the characterisation of complexes of the simplest amino acid derivative with the same group (*N*-glycylbis(pyridin-2-yl)methylamine, Gly-BPMA, Scheme 1) in order to compare its complex forming abilities with those of Gly-BIMA.

Experimental

1 Materials

D_2O, DCl, NaOD (Isotec Inc.), $CuCl_2$, $NiCl_2$, $ZnCl_2$, $Zn(NO_3)_2$, KCl, KOH (Reanal), histamine (imidazole-4-ethanamine) (Sigma) were purchased from commercial sources, and used without further purification. Metal ion stock solutions were prepared from analytical grade reagents and their concentration was checked gravimetrically.

Merck Kieselgel-precoated sheets No. 5553 were used for thin layer chromatography (TLC) with eluents A ethyl acetate–pyridine–acetic acid–water (120 : 20 : 6 : 11) and B butanol–pyridine–acetic acid–water (4 : 1 : 1 : 1). For staining the chromatograms, beside ninhydrin, the chlorine–tolidine reaction[23] has been used, slightly modified for TLC. The plates developed with ninhydrin, then chlorinated for one minute and aerated, were sprayed with the following reagent: 2 g of tolidine dissolved in 5 mL warm acetic acid were diluted with 100 mL of water, then 100 mL of 0.05 mol dm^{-3} KI solution and 100 mL ethanol were added. High-performance liquid chromatography (HPLC) was performed on a Knauer instrument using an analytical Vydac C18 column for analysis and a Waters C18 column (550 × 35 mm) for purification (detection at 220 nm). NMR spectra were measured on Bruker DRX equipment.

2 Synthesis of ligands

2.1 *N*-Glycylbis(pyridin-2-yl)methylamine (H-Gly-BPMA). *Boc-Gly-BPMA*. Boc-Gly-OH (Boc = *tert*-butyloxycarbonyl) (0.631 g, 3.6 mmol) was dissolved in dry dichloromethane (15 ml) and at $-5\,°C$ *N*-methylmorpholine (0.4 ml, 3.6 mmol) and ethyl chlorocarbonate (0.4 ml, 3.9 mmol) were added dropwise under stirring. Stirring was continued at $-5\,°C$ for 15–20 min, then a solution of 0.62 g (3.35 mmol) BPMA[24] in dichloromethane (DCM) (8 ml) was added. The pH of the solution was adjusted to 8 with *N*-methylmorpholine and stirring was continued at room temperature for 2 h. Then the reaction mixture was extracted with saturated $NaHCO_3$ and NaCl solutions, respectively, dried over Na_2SO_4 and evaporated *in vacuo*. The oily residue was triturated with diethyl ether and filtered. Yield of Boc-Gly-BPMA 0.772 g (67.4%). R_f 0.67 (A).

Boc-Gly-BPMA (0.558 g, 1.63 mmol) was dissolved in trifluoroacetic acid (TFA) (6 ml) and stirred at room temperature for 40 min. The reaction mixture was evaporated and dried *in vacuo*. The crude product was purified by HPLC using an acetonitrile gradient (0 to 20% over 40 min) in 0.1% TFA. Yield of H-Gly-BPMA 0.250 g (63.4%). R_f 0.67 (B). NMR: ^{13}C (DMSO), δ 40.5 (methylene), 60.1 (methine), 123.1 (Py-3), 123.9 (Py-5), 138.6 (Py-4), 149.4 (Py-6), 159.5 (Py-2), 166.7 (carbonyl); ^1H (DMSO), δ 6.26 (1H, d, methine), 7.30 (2H, t, Py-5H), 7.51 (2H, t, Py-3H), 7.81 (2H, t, Py-4H), 8.49 (2H, d, Py-6H), 9.41 (1H, d, amide NH), CH_2 signal hidden by the HDO signal of the solvent.

2.2 *N*-Histidylbis(pyridin-2-yl)methylamine (H-His-BPMA). *Boc-His-N_2H_3*. Hydrazine hydrate (0.75 g, 15 mmol) was added to a solution of $(Boc)_2$His-OMe[25] (1.1 g, 3.3 mmol) in methanol (6 ml), and the reaction mixture stirred at room temperature for 48 h. After evaporation *in vacuo*, the yellow oily residue was triturated with diethyl ether and filtered. Yield of Boc-His-N_2H_3 0.58 g (65.2%). R_f 0.37 (A).

Boc-His-BPMA. A solution of Boc-His-N_2H_3 (0.58 g, 2.15 mmol, in 4 ml DMF) was cooled to $-5\,°C$. At this temperature HCl solution (6 M, 0.93 ml, 6.45 mmol) and $NaNO_2$ (0.166 g, 2.47 mmol) were added, and the reaction mixture was stirred for 5 min. It was then poured into a solution of BPMA (0.425 g, 2.3 mmol) and triethylamine (TEA) (1.04 ml, 6.45 mmol) in DMF (2 ml) at $-5\,°C$. The pH was adjusted to 8 with TEA, and the mixture was stirred for 1 h at $-5\,°C$ and then allowed to stand overnight in a refrigerator. The precipitated TEA · HCl was filtered off, and the remaining solution evaporated *in vacuo*. The product was dissolved in ethyl acetate and extracted with water (2 × 3 ml). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. Yield of Boc-His-BPMA 0.650 g (66.6%). R_f 0.55 (A).

Boc-His-BPMA (0.52 g, 1.15 mmol) was dissolved in TFA (5 ml) and stirred for 20 min. Diethyl ether was added and the precipitate filtered off. Yield of H-His-BPMA] 0.300 g (81.1%). R_f 0.43 (B). The crude product was further purified by HPLC using an acetonitrile gradient (0 to 40% over 40 min) in 0.1% TFA. NMR: ^{13}C (DMSO), δ 27.4 (methylene), 40.4 (His-methine), 60.2 (methine), 118.9 (Im-5), 123.1 (Py-3), 123.8 (Py-5), 127.6 (Im-4), 135.2 (Im-2), 138.2 (Py-4), 149.7 (Py-6), 159.4 (Py-2), 167.8 (carbonyl); ^1H (DMSO), δ 4.38 (1H, t, His methine), 6.23 (1H, d, methine), 7.29 (2H, t, Py-5), 7.41 (1H, s, His-5), 7.52 (2H, t, Py-3), 7.72 (2H, t, Py-4), 8.48 (2H, d, Py-6), 8.96 (1H, s, Im-2), 9.43 (1H, s, Im NH), 14.5 (1H, d, amide NH).

3 Potentiometric measurements

The pH-metric titrations in the pH range 2.2–11.0 were performed on 4 ml samples in the concentration range 2×10^{-3}–4×10^{-3} mol dm^{-3} at metal ion to ligand ratios ranging between 1 : 1 and 1 : 3. 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 pHM 84 pH-meter equipped with a 6.0234.100 combination glass electrode (Metrohm) and a Dosimat 715 automatic burette (Metrohm) containing carbonate-free potassium hydroxide in known concentration. The pH readings were converted into hydrogen ion concentration as described earlier.[26] Protonation constants of the ligands and the overall stability constants (log β_{pqr}) of the binary and ternary systems were calculated by means of a general computational program, PSEQUAD,[27] using eqn. (1) and (2).

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

$$\beta = [M_pH_qL_r]/[M]^p[H]^q[L]^r \quad (2)$$

4 Spectroscopic measurements

UV-VIS spectra of the copper(II) and nickel(II) complexes were recorded on Hewlett Packard HP 8453 or JASCO UVIDEC-610 spectrophotometers in the same concentration range as used for 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. ^{1}H NMR spectra of zinc(II) complexes were recorded on a Bruker AM360 spectrometer using TSP [sodium 3-(trimethylsilyl)propionate] as an internal standard.

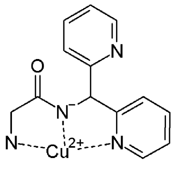
Results and discussion

The protonation constants of the ligands are included in Table 1 together with some literature data for comparison. The bis(pyridin-2-yl)methyl group has two nitrogen donors, but in the amino acid derivatives the basicity of one of the pyridine nitrogens is very low and the exact protonation constants cannot be obtained by potentiometric measurements. This suggests that the presence of amide substituents on the central carbon atom results in a significant decrease in the basicity of the pyridine nitrogens, and is consistent with the results for bis(pyridin-2-yl)methylamine (BPMA)[19] in which the presence of the amino substituent on the linking carbon atom decreases the protonation constants of the pyridine nitrogens. The decrease in the basicity of pyridine nitrogen donors is especially pronounced in the case of His-BPMA and it is also reflected in the lower pK of the imidazolyl group as compared to that of His-Gly. Protonation constants of the terminal amino groups of Gly-BPMA and His-BPMA correspond well to those of simple dipeptides, Gly-Gly and His-Gly, respectively.

Gly-BPMA

The stability constants of the metal complexes of Gly-BPMA and bis(pyridin-2-yl)methane (BPM) are summarised in Table 2. Equilibrium data of the copper(II) complexes of Gly-BIMA are also given for comparison. The stability constants and the metal ion speciation of the copper(II)–Gly-BPMA system clearly indicate that only 1 : 1 complexes are formed in the whole pH range. The two aromatic nitrogen donors are the metal binding sites in the acidic pH range forming the species $[\mathrm{Cu(HL)}]^{3+}$, while the formation of bis(ligand) complexes with coordination of four aromatic rings was detected in the case of BPM ($[\mathrm{CuL_2}]^{2+}$) and Gly-BIMA ($[\mathrm{CuH_2L_2}]^{4+}$). This difference might be due to a steric effect caused by the amino acid residue and the lower basicity of the pyridine nitrogens with respect to the imidazole nitrogens.

An extra base consuming process can be seen by increasing the pH, which corresponds to the deprotonation and coordination of amide nitrogen. The spectroscopic parameters of the species $[\mathrm{CuL}]^{2+}$ and $[\mathrm{CuH_{-1}L}]^{+}$ support the coordination of three nitrogens in the equatorial plane. This means that the coordination of the two pyridine nitrogens is replaced by that of terminal amino, amide and one of the pyridine nitrogen donor atoms (Scheme 2). A similar coordination of nitrogen donors was observed in the case of Pro-BMPA.[13] In the $[\mathrm{CuL}]^{2+}$ species the non-bonded pyridine donor atom is protonated, and its deprotonation results in the formation of $[\mathrm{CuH_{-1}L}]^{+}$. The pK value for amide deprotonation is 3.85, which is much lower than that of Gly-BIMA and Gly-Gly



Scheme 2

Table 1 Protonation constants of the ligands [T = 298 K; I = 0.2 mol dm^{-3} (KCl)]

	Gly-BPMA	His-BPMA	BPM	BPMA[19]	Gly-BIMA[13]	His-Gly[6]
log β_{011}	7.91(5)	7.31(3)	5.11(1)	7.32	7.95	7.59
log β_{021}	11.25(3)	12.74(3)	7.72(3)	≈9.0	13.46	13.53
log β_{031}	—	15.65(5)	—	—	16.68	16.49
pK_{ar1}	<1.5	<1.5	2.61	—	3.22	—
pK_{ar2}	3.34	2.91	5.11	≈1.7	5.51	—
pK_{His}	—	5.43	—	—	—	5.94
p$K_{\mathrm{NH_3^+}}$	7.91	7.31	—	7.32	7.95	7.59

Table 2 Stability constants (log β_{pqr}) of copper(II), nickel(II) and zinc(II) complexes [T = 298 K; I = 0.2 mol dm^{-3} (KCl)]

	Cu$^{\mathrm{II}}$			Ni$^{\mathrm{II}}$		Zn$^{\mathrm{II}}$	
Complex	Gly-BPMA	BPM	Gly-BIMA[13]	Gly-BPMA	BPM	Gly-BPMA	BPM
$[\mathrm{M(HL)}]^{3+}$	11.96(8)	—	17.11	10.97(5)	7.63(9)	—	7.35(7)
$[\mathrm{ML}]^{2+}$	8.12(5)	6.67(1)	—	—	4.72(3)	—	3.20(8)
$[\mathrm{MH_2L_2}]^{4+}$	—	—	31.64	21.21(1)	—	—	—
$[\mathrm{MHL_2}]^{3+}$	—	—		15.88(5)	—	—	—
$[\mathrm{ML_2}]^{2+}$	—	11.61(1)	18.97	9.46(4)	9.57(5)	6.66(7)	6.39(8)
$[\mathrm{MH_{-1}L}]^{+}$	4.27(6)	0.10(2)	—	−1.46(2)	−3.57(2)	−3.23(1)	—
$[\mathrm{MH_{-2}L}]$	−5.19(7)	—	−0.92	−11.17(3)	—	−11.94(1)	—
$[\mathrm{MH_{-1}L_2}]^{+}$	—	—	11.12	—	—	−0.7(1)	—
$[\mathrm{MH_{-2}L_2}]$	—	—	—	−5.03(3)	—	−9.12(7)	—
$[\mathrm{M_2H_{-2}L_2}]^{2+}$	—	—	18.43	—	—	—	—
$\overline{\mathrm{p}K}^{\mathrm{ML_2}}_{\mathrm{MH_{-1}L_2}}$		—	—	7.25	—	7.89	—
$\mathrm{p}K^{\mathrm{ML}}_{\mathrm{MH_{-1}L}}$	3.85						
$\overline{\mathrm{p}K}^{\mathrm{MHL}}_{\mathrm{ML_{-1}L}}$		—	—	6.22	—	—	—

(Gly-BIMA, pH ≈ 4.8;[13] Gly-Gly, $\mathrm{p}K_{\mathrm{amide}} = 4.23$[28]) and indicates that the stronger metal ion binding capability of the bis(imidazol-2-yl)methyl group can hinder coordination of the amide nitrogen. Moreover, the formation of joined chelates with three nitrogen donor atoms (amino, amide and pyridine nitrogens) in the case of Gly-BPMA is more favoured than the dipeptide-like binding of Gly-Gly *via* terminal amino, amide nitrogens and the carboxylate group. The $[\mathrm{CuH_{-1}L}]^+$ complex dominates in the physiological pH range, and in contrast with the dimeric species present in the Cu(II)–Gly-BIMA system the formation of a monomeric complex is concluded from the analysis of EPR spectra. This reflects the weaker metal binding ability of the pyridine nitrogen donor atom, which rules out bridging *via* pyridyl groups.

The $[\mathrm{CuH_{-2}L}]$ complex has a coordination set ($\mathrm{NH_2}$, $\mathrm{N^-}$, $\mathrm{N_{pyr}}$)($\mathrm{OH^-}$). Indeed the change of spectral parameters (Table 3) accompanying the formation of this species, *e.g.* a decrease of the $A_{\parallel} : g_{\parallel}$ ratio, is the same as that observed for hydrolysis of Cu(II)–GlyGly.[29]

It is clear from the stability constants in Table 2 that the metal binding ability of the bis(pyridin-2-yl)methyl group decreases in the order copper(II) > nickel(II) > zinc(II) in agreement with the Irving–Williams series. The bis(pyridin-2-yl) methyl group is the main binding site in the acidic pH range in the case of nickel(II), but the concentration of the $[\mathrm{Ni(HL)}]^{3+}$ complexes is lower than that of $[\mathrm{Cu(HL)}]^{3+}$ in the copper(II)–Gly-BPMA system. The octahedral geometry of nickel(II) complexes, however, results in the formation of bis(ligand) complexes.

An extra base consuming process can be observed in the titration curves of the $\mathrm{Ni^{II}}$–Gly-BPMA and $\mathrm{Zn^{II}}$–Gly-BPMA systems (Fig. 1), which corresponds to amide deprotonation and coordination.

Nickel(II) ion is able to induce deprotonation of the amide nitrogen around pH 6.2 at a 1 : 1 ratio ($\mathrm{p}K^{\mathrm{MHL}}_{\mathrm{MH_{-1}L}} = 6.22$) and an octahedral $[\mathrm{NiH_{-1}L}]^+$ complex is present above pH 6. The ligand is bound *via* amino, amide and one of the pyridine nitrogens. The much lower value compared to that of $\mathrm{Ni^{II}}$–Gly-Gly ($\mathrm{p}K_{\mathrm{amide}} = 8.88$[30]) suggests that the tridentate coordination of this ligand is more favoured than that of Gly-Gly, similarly to the copper(II)–Gly-BPMA system. The coordination of two ligands in the bis(ligand) complexes slightly hinders the deprotonation of the amide nitrogen, and the octahedral $[\mathrm{NiH_{-2}L_2}]$ complexes coordinated with two tridentate ligands dominate only above pH 9. The formation of a $[\mathrm{NiH_{-2}L}]$ mixed-ligand hydroxo complex is detected above pH 10 and is accompanied by the appearance of a new absorption band at 452 nm ($\varepsilon = 96$ $\mathrm{dm^3\ mol^{-1}\ cm^{-1}}$) in the visible spectra. The change in the geometry of the complexes is caused by deprotonation of the coordinated water molecules, which results in saturation of the square planar coordination sphere around nickel(II) ion. This is in agreement with a conclusion published earlier:[31] at least three nitrogen donors and a fourth donor atom stronger than $\mathrm{H_2O}$ are required to stabilize the square planar nickel(II) peptide complexes in aqueous solution.

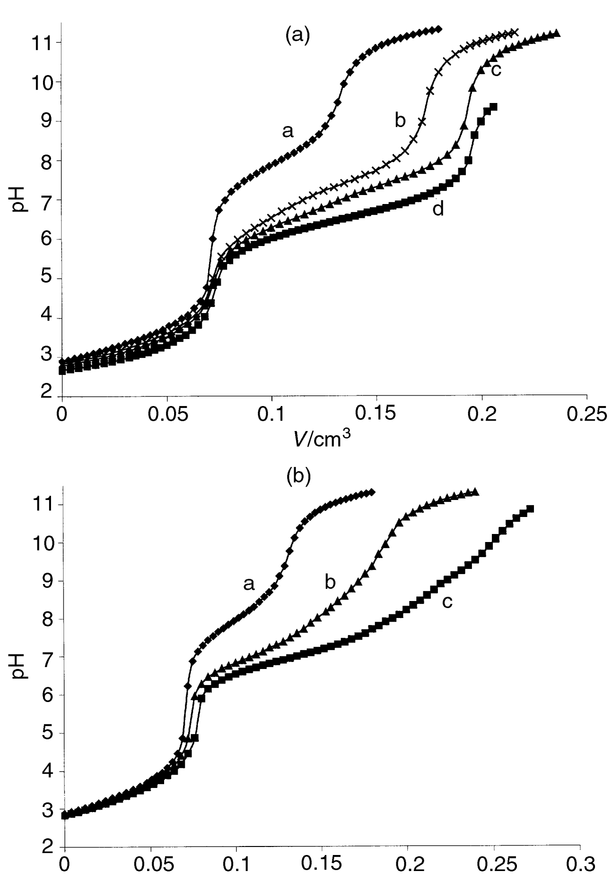


Fig. 1 Titration curves of (a) the nickel(II)–Gly-BPMA system (a free ligand, $c_{\mathrm{L}} = 4 \times 10^{-3}$ M; metal ion to ligand molar ratio b 1 : 3; c 1 : 2; d 1 : 1) (b) the zinc(II)–Gly-BPMA system (a free ligand, $c_{\mathrm{L}} = 3.93 \times 10^{-3}$ M; metal ion to ligand molar ratio b 1 : 2.5; c 1 : 1).

The pH-potentiometric data of the zinc(II)–Gly-BPMA system can be fitted assuming mono and bis(ligand) deprotonated complexes, in which the coordination mode is probably the same as in the corresponding nickel(II) complexes. It is known that the zinc(II) ion usually cannot promote deprotonation of the amide nitrogen of simple dipeptides. This process, however, was reported to occur in the zinc(II)–Gly-His system.[6] In the case of Gly-BPMA the pyridine nitrogen, similarly to the imidazole nitrogen of Gly-His, can behave as an anchoring group to induce binding of the amide nitrogen. The coordination mode is supported by ^{1}H NMR spectroscopic measurements.

As can be seen from the structural formula of the ligand (Scheme 1), the chemical shifts of the CH group between the two pyridine rings are affected by deprotonation of the bis(pyridin-2-yl)methyl group and amide nitrogen. The formation of $[\mathrm{ZnH_{-1}L}]^+$ is accompanied with an upfield shift of

Table 3 Spectral parameters of copper(II) complexes [$T = 298$ K; $I = 0.2$ mol $\mathrm{dm^{-3}}$ (KCl)]

	Gly-BPMA				His-BPMA			
	λ_{max}/nm	$\varepsilon_{\mathrm{max}}$/$\mathrm{dm^3\ mol^{-1}\ cm^{-1}}$	$g_{\parallel}$	$A_{\parallel}/10^{-4}\ \mathrm{cm^{-1}}$	λ_{max}/nm	$\varepsilon_{\mathrm{max}}$/$\mathrm{dm^3\ mol^{-1}\ cm^{-1}}$	$g_{\parallel}$	$A_{\parallel}/10^{-4}\ \mathrm{cm^{-1}}$
$[\mathrm{Cu(H_2L)}]^{4+}$							2.284	177
$[\mathrm{Cu(HL)}]^{3+}$	658	56	2.295	172	645	82	2.278	172
$[\mathrm{CuL}]^{2+}$	611	105	2.225	197	611	115	2.220	187
$[\mathrm{CuH_{-1}L}]^{+}$	602	133	2.225	197	—	—	—	—
$[\mathrm{Cu_2H_{-2}L_2}]^{2+}$	—	—	—	—	585	140	—	—
$[\mathrm{CuH_{-2}L}]$	595	116	2.225	174	590	133	2.213	175

the methylene protons as compared to the free ligand ($[ZnH_{-1}L]^+$, 5.977; H_2L^{2+}; 6.610; L; 6.244 ppm). A similar upfield shift can be observed in the proton resonances of the CH_2 group ($[ZnH_{-1}L]^+$; 3.272; H_2L^{2+}; 4.010; L; 3.474 ppm). This indicates that the ligand is bound tridentately *via* amino, amide and one pyridine nitrogen and the zinc(II) complex hydrolyses above pH 9, resulting in a stable mixed-ligand hydroxo $[ZnH_{-2}L]$ complex, preventing precipitation of zinc(II) hydroxide.

His-BPMA

His-BPMA (Scheme 1) is an ambidentate ligand which can bind to metal ions in two different ways forming six-membered chelate rings in both cases: (i) "histamine-like" coordination through the terminal amino and histidine imidazole nitrogens, (ii) *via* the bis(pyridin-2-yl)methyl residue. These two types of modes make the formation of multinuclear or bis(ligand) complexes possible with mixed-ligand coordination spheres. Data reported for the mixed-ligand complexes of various nitrogen donors[32,33] suggest that the mixed-ligand coordination mode could be more favourable for all three metal ions than the binding of four aromatic residues. Moreover, a tridentate "Gly-BPMA-like" coordination of the ligand is possible *via* terminal amino, amide and one pyridine nitrogen. In this case the non-bonded imidazole donor group can also act as a bridging ligand, resulting in dimeric or polymeric structures.

The results show that, in spite of the various coordination possibilities, the characterisation of the copper(II)–, nickel(II)– and zinc(II)–His-BPMA systems is less complicated than expected. The stoichiometry and stability constants of the complexes formed in the three systems are summarised in Table 4.

Similarly to the metal complexes of Gly-BPMA, only 1 : 1 species are present in the copper(II)–His-BPMA system. In the $[Cu(HL)]^{3+}$ complex the ligand is probably bound *via* two pyridine nitrogens and there is a weak interaction between the histidine imidazole nitrogen and copper(II). The EPR parameters are slightly different from those of the $[Cu(HL)]^{3+}$ species of Gly-BPMA showing a small change in the coordination sphere around copper(II). A further species is detected by EPR at pH values lower than 3. Its stoichiometry is $[Cu(H_2L)]^{4+}$, and magnetic parameters suggest a structure similar to that of $[Cu(HL)]^{3+}$ but with protonated imidazole. The ternary system Cu^{II}–BPM(A)–histamine(B) provided further support for the binding sites of His-BPMA in the copper(II) complexes.

It is obvious from the species distribution curves (Fig. 2) of the ternary system that the $[CuA]^{2+}$ complex with the coordination of two pyridine rings is present at acidic pH. $[CuB]^{2+}$ with "histamine-like" coordination is formed only at pH > 5.

Table 4 Stability constants (log β_{pqr}) of copper(II), nickel(II) and zinc(II) complexes of His-BPMA [$T = 298$ K; $I = 0.2$ mol dm^{-3} (KCl)]

Complex	Cu^{II}	Ni^{II}	Zn^{II}
$[MH_4L_2]^{6+}$	—	31.0(1)	—
$[MH_3L_2]^{5+}$	—	—	25.62(3)
$[MH_2L_2]^{4+}$	—	23.12(4)	20.82(2)
$[ML_2]^{2+}$	—	12.64(4)	9.98(1)
$[MH_{-2}L_2]$	—	−4.84(6)	—
$[MH_2L]^{4+}$	—	15.7(1)	—
$[M(HL)]^{3+}$	14.64(1)	—	—
$[ML]^{2+}$	10.36(1)	7.16(3)	4.75(3)
$[MH_{-1}L]$	—	—	−2.33(8)
$[M_2H_{-2}L_2]^{2+}$	12.58(3)	3.92(7)	—
$[MH_{-2}L]$	−4.47(2)	—	—

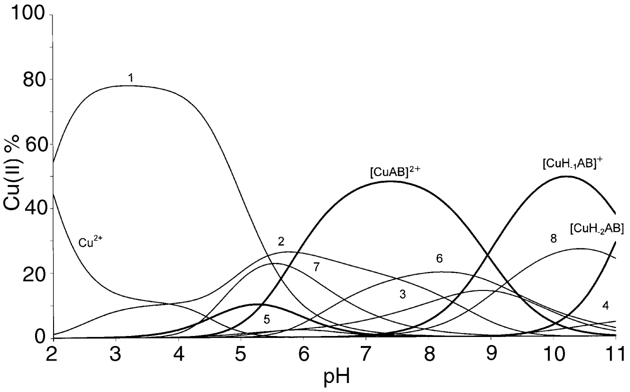
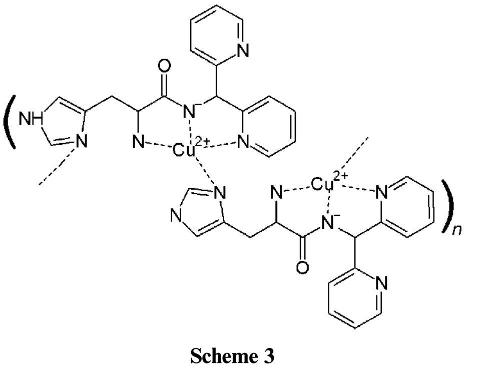


Fig. 2 Species distribution of the complexes formed in the copper(II)–BPM(A)–histamine(B) ternary system as a function of pH ($c_{Cu(II)} = c_A = c_B = 4 \times 10^{-3}$ M). Species: 1 $[CuA]^{2+}$; 2 $[CuA_2]^{2+}$; 3 $[CuH_{-1}A]^+$; 4 $[CuH_{-2}A]$; 5 $[CuHB_2]^{3+}$; 6 $[CuB_2]^{2+}$; 7 $[CuB]^{2+}$; 8 $[Cu_2H_{-2}B_2]^{2+}$.

Fig. 2 also shows that the $[CuAB]^{2+}$ ternary species is present in the pH range 6–10 suggesting that the formation of the bis(ligand) complexes with [(NH_2, N(im)) + (N(pyr),N(pyr)] coordination is expected only above pH 6. Amide deprotonation, however, can be observed at pH > 4 and it rules out bis(ligand) complex formation. Following deprotonation of the amide nitrogen the potentiometric data can be fitted by the assumption of $[CuL]^{2+}$ and either $[CuH_{-1}L]^+$ or $[(CuH_{-1}L)_2]^{2+}$ complexes. On the basis of spectroscopic parameters, the ligand is bound *via* amino, amide and one pyridine nitrogen in the $[CuL]^{2+}$ complex, while the non-coordinated histidine imidazole ring is protonated. The EPR spectra obtained in the Cu^{II}–His-BPMA system (1 : 1 ratio) at pH > 4 are not well resolved suggesting the presence of polymeric species. In this complex the ligand is coordinated *via* terminal amino, amide and one pyridine nitrogen donor atom, while the imidazole nitrogen atom is bound to the fourth coordination site of another copper(II) ion (Scheme 3). This structure explains the blue shift in the absorption spectra as compared to that of $[CuH_{-1}L]^+$ for Gly-BPMA (Table 3).

These observations also support the stronger binding ability of imidazole nitrogen donor atoms in comparison with pyridine nitrogen donors. The latter are not able to act as a bridging ligand forming polymeric structures, while histidine imidazole nitrogen, similarly to Gly-BIMA,[13] can act as a bridging ligand. This bond is not stable enough to prevent hydrolysis and the mixed-ligand hydroxo $[CuH_{-2}L]$ complex dominates above pH 9.5. Its spectroscopic parameters are in acceptable agreement with those of the $[CuH_{-2}L]$ species formed by Gly-BPMA.

The bis(pyridin-2-yl)methyl residue is the binding site for nickel(II) ion too in acidic media. The regular octahedral geometry of nickel(II) ion results in the formation of various bis(ligand) complexes: $[NiH_4L_2]^{6+}$, $[NiH_2L_2]^{4+}$ and $[NiL_2]^{2+}$. In these species both ligands are coordinated *via*



Scheme 3

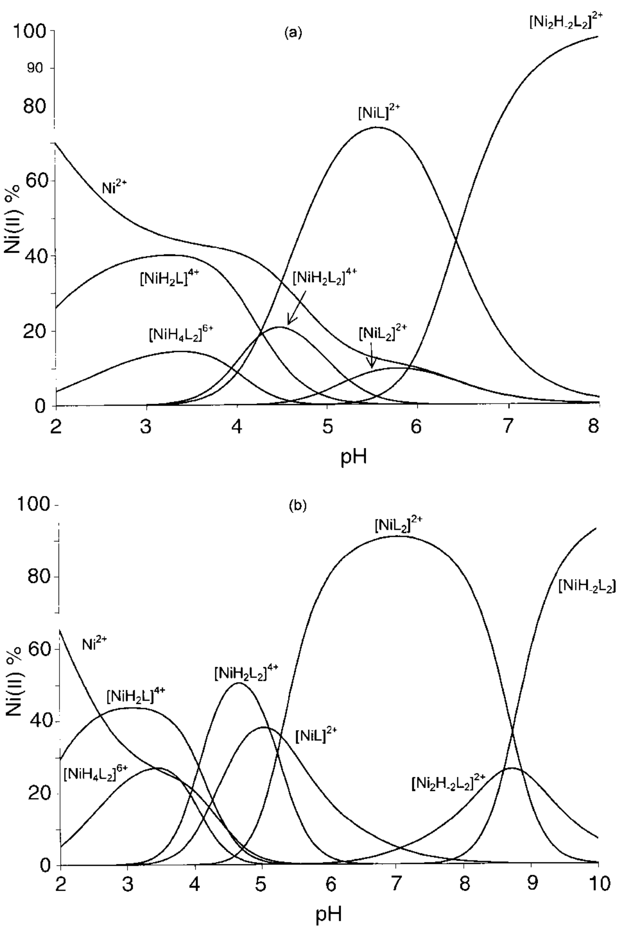


Fig. 3 Species distribution of the complexes formed in the nickel(II)–His–BPMA system at different ratios: (a) $c_{\mathrm{Ni(II)}} = 4 \times 10^{-3}$; $c_{\mathrm{L}} = 4 \times 10^{-3}$ M; (b) $c_{\mathrm{Ni(II)}} = 2 \times 10^{-3}$; $c_{\mathrm{L}} = 4 \times 10^{-3}$ M.

the bis(pyridin-2-yl)methyl moiety ([NiH$_4$L$_2$]$^{6+}$) or one ligand *via* this residue and the other *via* terminal amino and imidazole nitrogen donor atoms ([NiH$_2$L$_2$]$^{4+}$, [NiL$_2$]$^{2+}$). The possibility of formation of the latter species is supported by the results for the nickel(II)–BPM–histamine ternary system in which the ternary [NiAB]$^{2+}$ complex with coordination of N(pyr), N(pyr) + NH$_2$, N(im) is present in the pH range 6–10.

The deprotonation of the amide nitrogen can be observed at higher pH, as compared to the copper(II) containing system. It is shown in Fig. 3 that the [NiH$_{-2}$L$_2$] complex exists above pH 8, in which both ligands are coordinated *via* terminal amino, amide and one pyridine nitrogen donor atom. At 1 : 1 metal to ligand ratio the deprotonation of the amide nitrogen is not hindered, and tridentate 3N coordination of His-BPMA takes place around pH 6. The presence of the dimeric complex [(NiH$_{-1}$L)$_2$]$^{2+}$ is assumed similarly to Cu$^{\mathrm{II}}$–His-BPMA system, but the binding of histidine imidazole nitrogens cannot be decided unambiguously on the basis of pH-potentiometric and spectroscopic data. The spectroscopic data, however, support an octahedral geometry of the metal ion in all complexes formed in the nickel(II)–His-BPMA system.

The stability constants of complexes formed in the zinc(II)–His-BPMA system are smaller than those of Cu$^{\mathrm{II}}$–His-BPMA or Ni$^{\mathrm{II}}$–His-BPMA. Mono and bis(ligand) complexes are present in which the coordination of bis(pyridin-2-yl)methyl and/or histamine moieties can be assumed. In the [Zn(HL)]$^{3+}$ and [ZnH$_2$L$_2$]$^{4+}$ complexes the ligand is probably bound tridentately, which cannot promote deprotonation of the amide nitrogen, and prevent hydrolysis and precipitation above pH 7.

Conclusions

The study of the copper(II), nickel(II) and zinc(II) complexes of these amino acid derivatives containing two pyridine rings in chelatable position clearly demonstrates that the chelating group significantly influences the complexation of the ligands. The bis(pyridin-2-yl)methyl group of Gly-BPMA is the main binding site for all three metal ions in the acidic pH range, but the interaction between the metal ion and two pyridine nitrogens is weaker than that of the bis(imidazol-2-yl)methyl residue.[11,13] The binding ability decreases in the order Cu$^{\mathrm{II}}$ > Ni$^{\mathrm{II}}$ > Zn$^{\mathrm{II}}$ in agreement with the Irving–Williams series.

Similarly to analogous Gly-BIMA systems, all of the three metal ions are able to induce deprotonation and coordination of the amide nitrogen and the ligand is bound *via* amino, amide and one pyridine nitrogen forming two joined chelate rings. The non-coordinated pyridine ring, however, cannot act as a bridging ligand in contrast with copper(II)–Gly-BIMA complexes. In the case of copper(II) ion only mononuclear 1 : 1 complexes are formed, while in the nickel(II)– and zinc(II)–Gly-BPMA systems octahedral bis(ligand) complexes also are present.

The imidazole ring in the side chain of the histidine derivative of the bis(pyridin-2-yl)methyl residue (His-BPMA) significantly influences the complex formation process of the ligand. The deprotonation of the amide nitrogen and formation of NH$_2$, N$^-$, N(pyr) coordinated species take place at higher pH in the case of copper(II) and nickel(II), while it is prevented for zinc(II). At the same time the non-coordinated histidine imidazole nitrogen acts as a bridging residue resulting in the formation of dimeric or polymeric copper(II) and nickel(II) complexes.

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