Impact of the peptide sequence on the coordination abilities of albumin-like tripeptides towards Cu^{2+}, Ni^{2+} and Zn^{2+} ions. Potential albumin-like peptide chelators†

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Thermodynamic and spectroscopic studies have shown that the insertion of α-hydroxylmethylserine (HmS) residues into the N-terminal peptide motif of human serum albumin results in a very powerful chelating agent for Cu^{2+} and Ni^{2+} ions. The insertion of two HmS residues results in the HmS–HmS–His–OH/NH_2 sequence, which is the most effective chelating agent based on an albumin-like sequence for both studied metal ions, especially when the *C*-terminal carboxylate is protected by an amide function.

Our recent studies on the binding ability of the peptides containing α-hydroxymethylserine (HmS)[1–4] have shown that insertion of this residue into the peptide sequences significantly increases the stability of their complexes with Cu^{2+}, Ni^{2+} and Zn^{2+} ions. Although the basicity of the amino nitrogen of HmS is distinctly lower than that of the other amino acid residues, the insertion of its nitrogen into the peptide bond enhances the π-electron contribution to the metal–nitrogen bond. As a consequence, a considerable increase in the stability of the formed complexes is observed.[4] The results reported recently have shown that insertion of two α-hydroxymethylserine residues in an N-terminal albumin-like sequence leads to the most effective peptide chelating agent for Cu^{2+} and Ni^{2+} ions.[4] The albumin-like sequence is one of the basic structures used in metal ion binding, not only by albumin species but also by several other proteins.[5] This inspired us to undertake systematic studies on the coordination ability of oligopeptides having a histydyl residue in the third position and various amino acid residues at its N-terminal side, including that of the human serum albumin sequence, Asp–Ala–His–OH/NH_2. Tripeptides with an unprotected C-terminal carboxylate and its amide analogues were studied.

Experimental

Synthesis of peptides

Fmoc–His(Trt)–OH attached to *p*-hydroxybenzyl alcohol resin was purchased from Chem-Impex (USA) (grain size 100–200 mesh, substitution 0.6 meg g^{-1}, 1% DVB). *N*-9 fluorenylmethylcarbonyl (Fmoc) protected amino acids (Ser, Asp, Ala) and coupling reagents (TBTU, HATU) were obtained from commercial sources. The reactive side chains of amino acids were protected as follows: Ser, with *t*-butyl (O-*t*-Bu ether); Asp, with *t*-Butyl (O-*t*-Bu ester); HmS (α-hydroxymethylserine) was used as its *N*-9-fluroenylmethylcarbonyl-*O*, *O*-izopropylidene derivative [Fmoc–HmS(Ipr)–OH] and was synthesized according to published data.[6–8]

The peptides were constructed using the SPPS method and the Wang resin as the solid support. Standard TBTU coupling protocol was used for all amino acids except for HmS. In this case HATU was found to be a more efficient coupling reagent. All residues were added as the Fmoc-protected amino acid (3 equiv., 3 mmol) with the coupling agent (3 equiv.) and DIEA as base (3 equiv.). All coupling reactions have been performed in DCM and with reaction times of at least 12 h (overnight) to ensure completeness.

Cleavage of the Fmoc-protecting group has been achieved by the use of 20% piperidine in DMF for 2 and then 15 min after which the cleavage product was washed with DMF and DCM. Final cleavage of the peptidyl resin and side chain deprotection required 50% trifluoroacetic acid (TFA) in DCM for 2 h. The acidic solutions of peptides were evaporated under reduced pressure, then dissolved in water and extracted with ether. Lyophilization of the aqueous layer yielded crude peptides, which were purified by preparative HPLC (LCD Analytical) on a reverse-phase support (Vydac C_{18} column, 250 mm × 25 mm) with 0–30% or 0–100% gradients of B in A (B: 0.030% TFA in 90% acetonitrile–water. A: 0.05% TFA in water) in 40 min with a flow rate of 4 mL min^{-1}, $l = 214$ nm. Homogenity of purified peptides was checked by analytical HPLC (Vydac C_{18} column, 250 mm × 4.6 mm, 0–20% gradient of B in A, in 25 min, flow rate 1 mL min^{-1}) on a Thermo Separation HPLC system equipped with an AS 3000 auto sampler, P4000 pumps and scanning Spectra Focus detector (HPLC purity > 99%). The structures of the pure peptides were confirmed by FAB-MS. FAB mass spectra were recorded on an APO Electron Model MI 1200 1E mass spectrometer equipped with a FAB ion source.

† Electronic supplementary information (ESI) available: Tables S1–S3 and Fig. S1–S4 described in the text. See http://www.rsc.org/suppdata/nj/b1/b107412c/

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Potentiometric measurements

Stability constants for proton, Cu^{2+}, Ni^{2+} and Zn^{2+} complexes were calculated from titration curves carried out at 25 °C using a total volume of 1.5 cm^3. Alkali was added from a 0.250 cm^3 micrometer syringe, which was calibrated by both weight titration and the titration of standard materials. For the copper complexes the ligand concentration was $1.5–1.8 \times 10^{-3}$ and the Cu^{2+}-to-ligand ratio 1 : 1.2. Due to slow kinetics of the formation of the Ni^{2+} square-planar complexes at the 1 : 1 molar ratio an excess of ligand was used to improved precision and shorten complex formation equilibrium times.[9] Ligand concentration was $1.2–1.8 \times 10^{-3}$ M and the metal-to-ligand ratio 1 : 2.0–2.6.

A stock solution containing 1.35×10^{-3} M peptide was used in the zinc system and two metal-to-ligand ratios were used: 1 : 1.2 and 1 : 2.6. The pH-metric titrations were performed at 25 °C in 0.1 mol dm^{-3} KNO_3 on a MOLSPIN pH-meter system using a Russel CMAW 711 semi-micro combined electrode, calibrated in hydrogen ion concentration using HNO_3.[10] Three titrations were performed for each molar ratio, and the SUPERQUAD computer program was used for stability constant calculations.[11] Standard deviations quoted were computed by SUPERQUAD, and refer to random errors only. They are, however, a good indication of the importance of a particular species in the equilibrium.

Spectroscopic studies

Absorption spectra were recorded on a Beckman DU 650 spectrophotometer. Circular dichroism (CD) spectra were recorded on a Jasco J 715 spectropolarimeter in the 800–245 nm range. Metal concentrations in CD and UV-VIS spectroscopic measurements was adjusted to $0.5–1.5 \times 10^{-3}$ mol dm^{-3} and metal-to-ligand ratios were 1 : 1.2–2.6. EPR spectra were recorded on a Bruker ESP 300E spectrometer at X-band frequency (9.3 GHz) at 120 K. The spectroscopic parameters were obtained at the maximum concentration of the particular species from the potentiometric calculations. The ^{1}HNMR spectra were recorded on a Bruker AMX spectrometer at 300 MHz in D_2O solution using TSP-[$^2H_4$] (trimethylsilylpropanesulfonic acid sodium salt) as an internal standard.

Combined spectrophotometric and pH-metric determination of stability constants of Ni^{2+} complexes

Due to very slow co-ordination equilibria in the Ni^{2+}/Ser–HmS–His–NH_2 system the calculations based on the potentiometric data were less accurate. To evaluate the stability constant of the major complex a spectroscopic method was used. Stock solution containing 0.72×10^{-3} M of peptide (Ser–HmS–His–NH_2), 0.5×10^{-3} M of $Ni(NO_3)_2$ and 0.1 M KNO_3 were acidified with HNO_3 to pH 2.8. Samples were stored under nitrogen. Still under nitrogen, 0.1 M NaOH was added by portions to individual samples of stock solutions, and the resulting mixtures were monitored by the absorption band of the CD spectra at 478 nm until equilibrium was achieved. Spectra of equilibrated samples were recorded and the final pH values were measured. The absorption band at 478 nm was used as a measure of the concentration of $NiH_{-2}L$ (4N) complex at a given pH, assuming that the average value of the absorption observed for the samples in the pH range 7–9 corresponds to 100% of the complex.

Results and discussion

Protonation constants

Protonation constants for all studied tripeptides and their amides are collected in Table 1. As it was already reported earlier,[3,4] the two CH_2OH groups in the HmS side chain make its amino group the most acidic among those studied here. The acidity of the amino group of Ser1 is also relatively low compared to that of Asp1, for example.

Cu^{2+} complexes

All tripeptides with free C-terminal carboxylate form two complexes with Cu^{2+} ions, a minor CuL species with $\{NH_2, N_{imid}\}$ binding mode[12] and the major $CuH_{-2}L$ 4N complex with $\{NH_2, 2 \times N^-, N_{imid}\}$ coordination. The coordination mode is clearly supported by the spectroscopic data (see ESI Table S1). The d–d energy of the 4N species around 520 nm, as well as the EPR parameters with $A_{\|}$ around 200, are typical for the coordination of a four nitrogen donor system.[1,3,4,12,13]

The co-ordination equilibria in Cu^{2+}/Asp–Ala–His–OH/NH_2 system are distinctly different from those observed for all other tripeptides (Fig. 1, Table 2). The major difference concerns the high stability of the CuL species observed for the Asp–Ala–His–OH/NH_2 ligand (particularly for its carboxyl analogue). The presence of the β-carboxylate in the Asp1 residue induces the formation of the tridentate co-ordination involving the $\{NH_2, \beta\text{-}COO^-, N_{imid}\}$ donor set. The high stability of the CuL complex indicates the involvement of the Asp side chain in the metal ion binding in CuL species. The CuL complex dissociates two protons and the $CuH_{-2}L$, 4N, species is formed. It is interesting to note that the formation of the 4N complex is much easier for amide than for the carboxyl analogue (Fig. 1). The formation of two major species: CuL and $CuH_{-2}L$ with $\{NH_2, \beta\text{-}COO^-, N_{imid}\}$ and $\{NH_2, 2 \times N^-, N_{imid}\}$ binding modes is clearly seen in the absorption spectra, indicating formation of two major complexes with the d–d transition around 700 (CuL, 2N) and 520 nm ($CuH_{-2}L$, 4N) (ESI Fig. S1, Table S1). This equilibrium is also well seen in the CD spectra where both complexes give very characteristic spectra (ESI Table S1).

The involvement of β-carboxylate in the 4N species is less likely as the stability of this species is very close to that of Gly–Gly–His–OH[14] for example (Table 3, *vide infra*).

Table 1 Protonation constants for Xaa–Yaa–His–OH/NH_2 tripeptides at 298 K and $I = 0.10$ M (KNO_3)

	HL	H_2L	H_3L	H_4L	$\log K_{NH_2}$	$\log K_{imid}$	$\log K_{COO^-}$	$\log K_{COO^-(Asp)}$
Asp–Ala–His–OH	7.98(1)	14.96(1)	18.28(1)	20.88(1)	7.98	6.98	2.60	3.32
Ser–Ser–His–OH	7.48(1)	14.10(1)	16.81(1)		7.48	6.62	2.71	
Ser–HmS–His–OH	7.46(1)	14.02(1)	16.72(1)		7.46	6.56	2.70	
HmS–Ser–His–OH	7.16(1)	13.34(1)	15.92(1)		7.16	6.18	2.58	
Asp–Ala–His–NH_2	7.68(1)	14.18(1)	17.11(1)		7.68	6.50		2.93
Ser–Ser–His–NH_2	7.08(1)	13.24(1)			7.08	6.16		
Ser–HmS–His–NH_2	7.13(1)	13.27(1)			7.13	6.14		
HmS–Ser–His–NH_2	6.74(1)	12.65(1)			6.74	5.91		

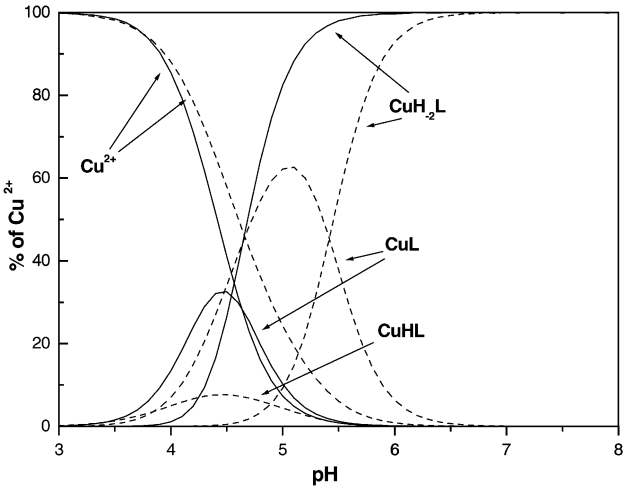

Fig. 1 Concentration distribution of the complexes formed in the systems: Cu(II) Asp–Ala–His–OH (dashed lines) and Cu(II) Asp–Ala–His–NH_2 (solid lines) as a function of pH. Metal-to-ligand molar ratio 1 : 1; $[Cu^{II}] = 1 \times 10^{-3}$ mol dm^{-3}.

Table 2 Stability constants of complexes formed by Xaa–Yaa–His–OH/NH_2 tripeptides with Cu(II) ions

$\log\beta$ [a]	CuHL	CuL	$CuH_{-2}L$	$CuH_{-3}L$
Asp–Ala–His–OH	12.85(4)	9.00(1)	−1.83(1)	
Ser–Ser–His–OH		7.72(2)	−1.05(1)	
Ser–HmS–His–OH		8.34(2)	−0.10(1)	
HmS–Ser–His–OH		7.73(1)	−0.71(1)	
Asp–Ala–His–NH_2		8.48(1)	−0.61(1)	
Ser–Ser–His–NH_2		7.22(6)	0.15(1)	−10.84(1)
Ser–HmS–His–NH_2		7.76(3)	0.84(1)	
HmS–Ser–His–NH_2			0.31(1)	−11.03(4)

[a] $\log\beta(CuH_iL) = [CuH_iL]/\{[Cu^{2+}][H^+]^i[L]\}$ Standard errors on the last digits are included in parentheses.

Comparison of the binding ability for Cu^{2+}–albumin-like peptide systems

The major complex formed in all the studied Cu^{2+}–tripeptide systems is the 4N species with $\{NH_2, 2\times N^-, N_{imid}\}$ binding mode. The $\log K^*$ constant ($\log K^* = \log\beta_{CuH_{-2}L} - \log\beta_{HnL}$, Table 3) was found to be a very useful parameter for evaluating the chelating power of peptides.[12] The $\log K^*$ values obtained here clearly indicate that the 4N complexes formed by the amide analogue are distinctly stronger than those obtained with peptides having an unprotected carboxylate (Tables 2, 3 and Fig. 1, 2).

The important factor, which influences the complex stability, is the basicity of the bound nitrogens. According to earlier work of Sigel and Martin[17] the N-terminal amino group has some impact on the stability of simple glycine-like complexes, while the effect of the basicity of the amino groups involved in amide bond formation is less evident. In order to evaluate the effect of the basicity of the amino groups involved in the peptide bonds we have correlated the $\log K^*$ and average pK values of imidazole and amino groups of the residues inserted in the peptide sequence (Table 3).[4] The pK values for the second residue of a tripeptide were taken as those obtained for its amino group with the residue placed at the first position in a dipeptide molecule (Table 4). It should be mentioned here that the use of such approach for Cu(II) and Ni(II)–dipeptide systems to evaluate the amide nitrogen binding ability lead to very complicated relations.[17,23]

Table 3 pK_{av} and $\log K^*$ obtained for Cu(II) complexes

Ligand	$\log K^*$ [a]	pK_{av} [b]
Asp–Ala–His–OH	−16.79	8.04
Ser–Ser–His–OH [c]	−15.15	7.64
Ser–HmS–His–OH [c]	−14.12	7.58
HmS–Ser–His–OH [c]	−14.05	7.45
HmS–HmS–His–OH [d]	−13.12	7.37
Gly–Gly–His–OH [e]	−16.43	8.05
Asp–Ala–His–NH_2	−14.79	7.46
Ser–Ser–His–NH_2	−13.09	7.04
Ser–HmS–His–NH_2	−12.43	7.01
HmS–Ser–His–NH_2	−12.34	6.89
HmS–HmS–His–NH_2 [d]	−11.05	6.77
Val–Ile–His–Asn–OH [f]	−15.63	7.53
Gly–Gly–His–NMA [g]	−14.95	7.57
Asp–Ala–His–NMA [g]	−14.84	7.49
Asp–Ala–His–Lys–NH_2 [f]	−14.42	7.37
Arg–Thr–His–Gly–Asn–NH_2 [h]	−14.24	7.05

[a] $\log K^* = \log\beta(CuH_{-2}L) - \log\beta(H_2L)$. [b] $pK_{av} = [pK_{(NH_2\text{–}X\text{–}Y\text{–}His)} + pK_{(NH_2\text{–}Y\text{–}His\ or\ \text{–}Y\text{–}Gly)} + pK_{(NH_2\text{–}His\ or\ \text{–}His\text{–}Gly)} + pK_{(NHimidazole\ (X\text{–}Y\text{–}His))}]/4$. [c] Ref. 4. [d] Ref. 1. [e] Ref. 14. [f] Ref. 15. [g] Ref. 5 (NMA = *N*-methyl amide). [h] Ref. 16.

Table 4 The pK data for amine groups of the dipeptides used for the calculation of the pK_{av} values of the discussed peptides

Peptide	pK_{NH_2}
HmS–His [a]	7.19
Ala–His [b]	8.08
Ile–Gly [c]	8.07
His–Gly [d]	7.59
Gly–His [d]	8.22
His [e]	9.12
Thr–Gly [f]	7.34
Ser–Gly [f]	7.33

[a] Ref. 3. [b] Ref. 18. [c] Ref. 19. [d] Ref. 20. [e] Ref. 21. [f] Ref. 22.

The plot of $\log K^*$ for the Cu^{2+} complexes *vs.* pK_{av} is shown in Fig 2. The obtained relations are rather surprising. The plots show an almost linear relation between $\log K^*$ and the average value of the amino group acidity. The relations plotted in Fig. 2 indicate also that lower basicity of the amide nitrogen results

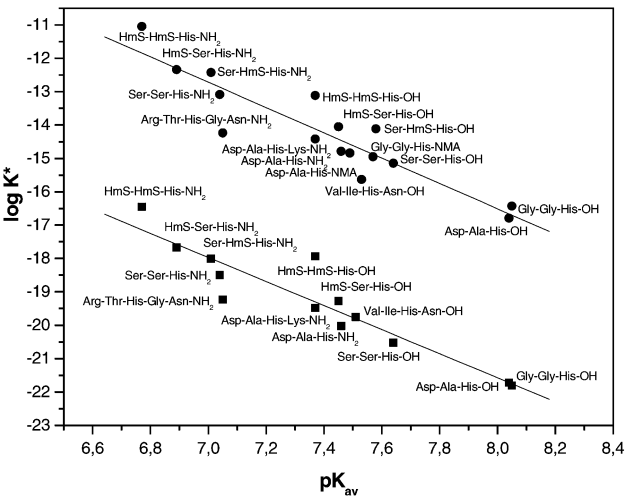

Fig. 2 The relation between $\log K^*$ and the amino group basicity expressed by the pK_{av} value of the amino group of the residues inserted into the peptide sequence for copper(II) (●) and nickel(II) (■) complexes.

in higher stability of the complexes formed. The lower pK_{av} value makes the amide proton less competitive for the metal ion and the deprotonation with simultaneous amide nitrogen binding by the metal ion occurs at lower pH. However, its binding to the metal ion should be more efficient when the amide nitrogen is more basic.

The data presented in Fig. 2 indicate the minor effect of the hydrophobic amino acid side chains on the complex stability.[24]

According to the plot shown in Fig. 2 the native human albumin sequence Asp–Ala–His–OH is the least effective in metal ion binding among the peptides studied. This may exclude any involvement of Asp β-COO^- in the metal ion coordination in the 4N complex in the latter peptide. The highest $\log K^*$ values are recorded for HmS and Ser containing residues (Table 3, Fig. 2). The amide analogues are distinctly better chelating agents for Cu^{2+} ions than the peptides with free carboxylate.

The $\log K^*$ values do not give, however, information about the total binding ability of a particular ligand. In order to illustrate the chelating power of the peptides studied the plots showing competition between selected ligands were calculated (ESI Fig. S2). When two ligands compete for Cu^{2+} ions in aqueous solution and only 4N species are taken into account, both HmS–HmS–His–NH_2 and Ser–Ser–His–NH_2 are more effective than Asp–Ala–His–NH_2. At pH 7.4 HmS–HmS–His–NH_2 binds around 93% of Cu^{2+}, while Asp–Ala–His–NH_2 is able to bind only 7% of total metal. The same values for Ser–Ser–His–NH_2 and Asp–Ala–His–NH_2 are 78 and 22%, respectively (ESI Fig. S2). These results clearly indicate that both mentioned analogues are distinctly more efficient chelating agents than the native human albumin N-terminal metal ion binding motif.

The pK values of $CuH_{-3}L + H^+ \rightleftharpoons CuH_{-2}L$ protonation reactions are around 11. The most likely protonation site in the $CuH_{-3}L$ complex seems to be the unbound imidazole nitrogen (pyrrole-NH).

Ni^{2+} complexes

Ni^{2+} forms only one major complex with all tripeptides studied (Table 5, ESI Table S2). The minor complexes like NiL or $NiH_{-1}L$ obtained in the calculations based on potentiometric data were not seen in the spectroscopic measurements and are not discussed below. The square-planar 4N complexes behave similarly to those discussed above for Cu^{2+}. The most stable 4N complexes are formed with HmS and Ser containing peptides and the amides are more efficient in metal ion binding than the tripeptides with unprotected carboxylate (Table 6, Fig. 2). The plot of $\log K^*$ *vs.* pK_{av} is very similar to that discussed above for Cu^{2+} (Fig. 2) and the reasons for the relation between the peptide sequence and complex stability are similar to those presented above. The competition plots obtained for HmS–HmS–His–NH_2 and Ser–Ser–NH_2 *vs.* Asp–Ala–His–NH_2 (ESI Fig. S3) show very similar relations to those obtained above for Cu^{2+}. At pH 7.4 HmS–HmS–His–NH_2 binds around 92% of Ni^{2+} while Asp–Ala–His–NH_2 only 8% of total metal. The respective numbers for Ser–Ser–His–NH_2 and the latter amide are 74 and 26% (ESI Fig. S3).

In the case of Ni^{2+} complexes proton dissociation from the $NiH_{-2}L$ species is also observed. This deprotonation occurs most likely from unbound imidazole nitrogen. Strong support for this assumption comes from proton NMR data. The chemical shift of imidazole protons moves to higher fields by up to $\approx$0.2 ppm (ESI Fig. S4, Table S3) when pH increases above 10.5, indicating proton dissociation from the imidazole ring.

Table 5 Stability constants of complexes formed by Xaa–Yaa–His–OH/NH_2 tripeptides with Ni(II) ions

	$\log \beta$[a]			
	NiL	$NiH_{-1}L$	$NiH_{-2}L$	$NiH_{-3}L$
Asp–Ala–His–OH	5.68(3)		− 6.76(2)	
Ser–Ser–His–OH			− 6.42(2)	
HmS–Ser–His–OH	4.72(7)		− 5.93(2)	− 17.35(7)
Asp–Ala–His–NH_2	5.27(3)		− 5.84(1)	
Ser–Ser–His–NH_2			− 5.26(2)	− 16.42(7)
Ser–HmS–His–NH_2			− 4.74(9)	
HmS–Ser–His–NH_2		− 0.43(8)	− 5.01(2)	− 16.07(5)

[a] $\log \beta(NiH_iL) = [NiH_iL]/\{[Ni^{2+}][H^+]^i[L]\}$ Standard errors on the last digits are included in parentheses.

Table 6 pK_{av} and $\log K^*$ obtained for Ni(II) complexes

Ligand	$\log K^*$[a]	pK_{av}[b]
Asp–Ala–His–OH	− 21.72	8.04
Ser–Ser–His–OH	− 20.52	7.64
HmS–Ser–His–OH	− 19.27	7.45
HmS–HmS–His–OH[c]	− 17.93	7.37
Gly–Gly–His–OH[d]	− 21.81	8.05
Asp–Ala–His–NH_2	− 20.02	7.46
Ser–Ser–His–NH_2	− 18.50	7.04
Ser–HmS–His–NH_2	− 18.01	7.01
HmS–Ser–His–NH_2	− 17.67	6.89
HmS–HmS–His–NH_2[c]	− 16.45	6.77
Val–Ile–His–Asn–OH[e]	− 19.75	7.50
Asp–Ala–His–Lys–NH_2[f]	− 19.48	7.37
Arg–Thr–His–Gly–Asn–NH_2[g]	− 19.23	7.05

[a] $\log K^* = \log \beta(NiH_{-2}L) - \log \beta(H_2L)$. [b] $pK_{av} = [pK_{(NH_2\text{–}X\text{–}Y\text{–}His)} + pK_{(NH_2\text{–}Y\text{–}His \text{ or } \text{–}Y\text{–}Gly)} + pK_{(NH_2\text{–}His \text{ or } \text{–}His\text{–}Gly)} + pK_{(NHimidazole\ (X\text{–}Y\text{–}His))}]/4$. [c] Ref. 4. [d] Ref. 14. [e] Ref. 24. [f] Ref. 15. [g] Ref. 16.

Zn^{2+} complexes

Our earlier work on Zn^{2+} complexes with HmS–His[3] has shown that insertion of the HmS residue enhances very significantly the ability of Zn^{2+} ion to deprotonate an amide nitrogen to form $ZnH_{-1}L$ species with $\{NH_2,N^-,N_{imidazole}\}$ binding mode. In order to check whether zinc ion is also able to deprotonate amide nitrogens in the HmS–HmS–His–NH_2 peptide we have studied the coordination equilibria in the Zn^{2+} HmS–HmS–His–NH_2 system. The potentiometric data (Table 7) indicate the formation of two complex species, $ZnH_{-1}L$ and $ZnH_{-2}L$. The stability constant of the major complex $ZnH_{-2}L$ is very close to that found for Gly–Gly–His–NMA (Fig. 3).[25] The proton NMR spectra show that only imidazole C2 and C4 protons are shifted upon metal ion binding (Fig. 4). This indicates that Zn^{2+} is unable to depro-

Table 7 Protonation constants for ligands and stability constants of complexes formed by HmS–HmS–His–NH_2 and Gly–Gly–His–NMA with Zn(II) ions

	$\log \beta$				
	HL	H_2L			
Gly–Gly–His–NMA[a]	7.87	14.19			
HmS–HmS–His–NH_2[b]	6.636	12.322			
	ZnHL	ZnL_2	$ZnH_{-1}L$	$ZnH_{-2}L$	$ZnH_{-2}L_2$
Gly–Gly–His–NMA[a]	10.20	6.58	− 5.02	− 12.70	− 11.78
HmS–HmS–His–NH_2			− 5.45(4)	− 12.24(1)	

[a] Ref. 25 (NMA = *N*-methyl amide). [b] Ref. 1.

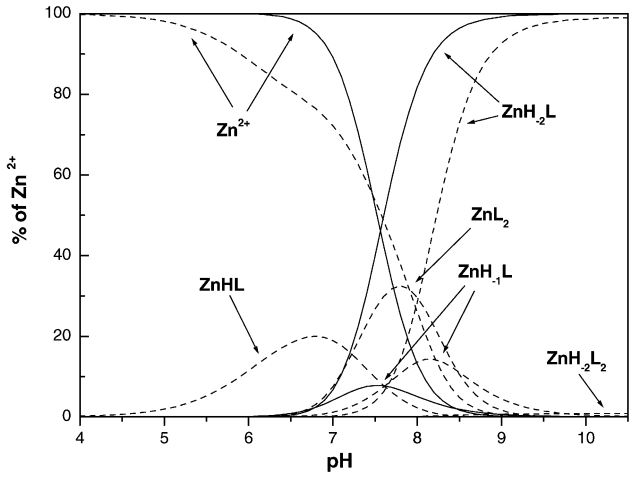

Fig. 3 Concentration distribution of the complexes formed in the systems: Zn(II) HmS–HmS–His–NH_2 (solid lines) and Zn(II) Gly–Gly–His–NMA (dashed lines) as a function of pH. Metal-to-ligand molar ratio 1 : 2; $[Zn^{II}] = 1 \times 10^{-3}$ mol dm^{-3}.

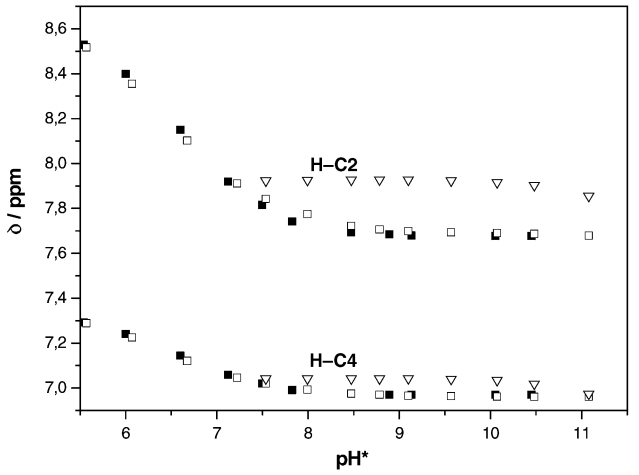

Fig. 4 pH* dependence of the chemical shift of imidazole C2-H, C4-H protons in D_2O solution containing 7.35×10^{-3} mol dm^{-3} HmS–HmS–His–NH_2 without $Zn(SO_4)_2$ (■) and with 4.64×10^{-3} mol dm^{-3} $Zn(SO_4)_2$ (□-free ligand, ∇-bound ligand). (pH* uncorrected for the isotopic effect.)

tonate amide nitrogens in tripeptides and a liberation of protons observed for two calculated complexes may derive from co-ordinated water molecules.

Conclusions

The X-ray structure of albumin[26] has shown that the N-terminal metal binding site of protein does not have any defined structure. Therefore, two residues at the N-terminal side of His3 will fundamentally influence the metal binding ability. All proteins with the human albumin-like N-terminal motif are specific for binding of Cu^{2+} and Ni^{2+} ions and the side chain donor system of the N-terminus dipeptide fragment (*e.g.*, Asp or Arg) does not seem to be important for the formation of complexes with the N-terminal sequence. The essential reason for the chelating power of this motif may be simply the acidity of the amino groups of two N-terminal amino acid residues. This increased acidity will make both nitrogens more available for metal ions, which substitute the nitrogen bound protons. In the case of amide nitrogen easier removal of the proton is accompanied by higher involvement of the π-bond in the metal amide co-ordination. The hydrophobic side chains (*e.g.* Val or Ile) could protect the metal ion against hydrolysis.[16] However, the data presented here suggest that hydrophobic interactions may not be a major factor affecting complex stability.

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