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Impact of α-hydroxymethylserine (HmS) residue on the binding ability of the histidyl residue in the HmS-His dipeptide towards Cu^{II}, Ni^{II} and Zn^{II}

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Potentiometric and spectroscopic data have shown that α-hydroxymethylserylhistidine is a very efficient ligand for Cu^{II}, Ni^{II} and Zn^{II}. The stabilities of the complexes formed are considerably higher than those obtained for Gly-His or Ala-His dipeptides. Copper(II) and Ni^{II} form very stable tetrameric complexes $M_4H_{-8}L_4$. According to ^1H NMR spectra the nickel tetrameric complex is of C_2 symmetry with two pairs of different imidazole rings. Zinc(II), on the other hand, forms only monomeric species but due to the second His residue it forms very stable $ZnH_{-1}L$ species *via* a $\{NH_2,N^-_{amide},N_{imidazole}\}$ donor set.

α-Hydroxymethylserine (HmS) is a non-protein amino acid, found in the antibiotic peptides antrimycin[1] and cirratiomycin.[2] It has an additional CH_2OH side chain at the serine α-carbon. This modification of the serine residue has very strong impact on the binding ability of peptides having a HmS residue inserted in their sequences. The most striking feature of the HmS residue is very strong enhancement of the binding abilities of peptides without direct involvement of the alcoholic groups in the metal ion co-ordination,[3–5] although in specific cases the direct involvement of alcoholic groups is likely.[6]

The most interesting results were obtained for the albumin-like HmS-HmS-His tripeptide chelator. Two HmS residues inserted on the N-terminal side of the His residue led to the most effective peptide chelator ever found for copper(II) and nickel(II) ions,[5] although no direct interaction between the metal ion and HmS side-chain could be recorded. The enhancing effect on complex stability was observed when bulky and hydrophobic Val and Ile residues were inserted into the Xaa-Yaa-His sequences,[7] although positively charged Arg could be even more effective.[8]

The details of the HmS impact on complex stability are not yet clear and in this work we have studied the complexes of Cu^{II}, Ni^{II} and Zn^{II} with the HmS-His dipeptide by potentiometry and absorption, CD, EPR and NMR spectroscopies. Zinc(II) ions are able to deprotonate and co-ordinate the amide nitrogen in the peptide bond only for specific peptides having a His residue, *e.g.* Xaa-His dipeptides.[9,10] This binding mode could be of importance for the zinc(II) binding by hemoglobin.[9]

Experimental

Peptide synthesis

The peptide HmS-His (×2 TFA (*i.e.* 2 mol TFA per mol peptide)) was prepared as follows. A sample of 2-chlorotrityl chloride resin (from Novabiochem) was loaded with Fmoc-His-(Boc)-OH according to the literature procedure.[11] α-Hydroxymethylserine was incorporated in the form Fmoc-HmS(Ipr)-OH (Ipr = *O*,*O*-isopropylidene).[12] The coupling was performed using HATU (*O*-7-azabenzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate)[13] reagent in DMF. Removal of the Fmoc group was accomplished by means of 20% piperidine in DMF (15 min). Peptidyl resin was treated with AcOH–TFE–CH_2Cl_2 (1 : 1 : 8; TFE = 2,2,2-trifluoroethanol)[11] for 30 min to yield the side chain protected compound HmS(Ipr)-His(Boc) (in acetate form). After final deprotection by means of 95% aqueous trifluoroacetic acid (TFA) (30 min) the required compound was precipitated with diethyl ether, redissolved in water, frozen and lyophilized.

TLC: R_f 0.53 (2-propanol–25% aqueous ammonia 7 : 3). HPLC: purity 96.0%, R_t 2.903 min (linear gradient over 25 min: 0–30% of acetonitrile in water +0.1% TFA). FAB-MS (*m*/*z*): 273, $[MH]^+$; 295, $[MNa]^+$; and 311, $[MK]^+$; calc. for $C_{10}H_{16}N_4O_5$ 272.

Potentiometric studies

Stability constants for proton and copper(II) 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. The ligand concentration was 2×10^{-3} mol dm^{-3} and the metal-to-ligand ratios were 1 : 2 and 1 : 3. 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 concentrations using HNO_3.[14] Three titrations were performed for each molar ratio, and the SUPERQUAD computer program was used for stability constant calculations.[15] 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

The EPR spectra were recorded on a Bruker ESP 300E spectrometer at X-band frequency (9.3 GHz) at 120 K, absorption spectra on a Beckman DU 650 spectrophotometer and circular dichroism (CD) spectra on a JASCO J 600 spectropolarimeter in the 750–250 nm range. The metal concentration in EPR, CD and UV-VIS spectroscopic measurements was adjusted to 2×10^{-3} mol dm^{-3} and metal to ligand ratios were 1 : 1 and 1 : 2. The spectroscopic parameters were obtained at the

Table 1 Protonation constants for HmS-His and comparable peptides at 298 K and $I = 0.10$ mol dm^{-3} (KNO_3)

Peptide	log β			log K		
	HL	H_2L	H_3L	NH_2	N_{Im}	CO_2^-
HmS-His	**7.19 ± 0.01**	**13.39 ± 0.01**	**16.01 ± 0.01**	**7.19**	**6.20**	**2.62**
Gly-His [a]	8.22	14.99	17.50	8.22	6.77	2.51
Ala-His [b]	8.08	14.84	17.57	8.08	6.76	2.73
Gly-Hist [c]	8.04	14.82		8.04	6.78	
Gly-His-Gly [d]	7.99	14.49	17.57	7.99	6.50	3.08

[a] Ref. 19. [b] Ref. 9. [c] Ref. 20. [d] Ref. 16.

Table 2 Stability constants (log β) of Cu^{II}, Ni^{II} and Zn^{II} complexes of Hms-His and comparable peptides. Concentration constants at 298 K and $I = 0.10$ mol dm^{-3} (KNO_3)

Species	**HmS-His**			Gly-His			Gly-Hist		Ala-His	Gly-His-Gly
	Cu^{2+}	**Ni^{2+}**	**Zn^{2+}**	Cu^{2+} [a]	Ni^{2+} [b]	Zn^{2+} [b]	Cu^{2+} [c]	Ni^{2+} [d]	Zn^{2+} [e]	Cu^{2+} [f]
MHL				12.45	11.34	10.87	11.84	10.46	10.30	
ML	**8.93 ± 0.01**	**3.57 ± 0.04**		9.06	4.68	3.98	7.19	4.20	3.50	9.35
$MH_{-1}L$	**4.76 ± 0.01**	**−1.97 ± 0.01**	**−3.13 ± 0.01**	4.91	−1.35	−2.75	3.99	−2.69	−3.60	5.66
$MH_{-2}L$			**−12.87 ± 0.01**			−12.66	−5.46	−12.22		−3.70
$MH_{-3}L$							−16.76			
ML_2				15.96	9.64	8.03	14.50	7.73	7.8	15.96
$MH_{-1}L_2$	**7.45 ± 0.02**		**−0.16 ± 0.02**	8.02	2.07	0.37	6.90	−0.16	0.40	8.32
$M_2H_{-3}L_2$	**3.27 ± 0.03**									
$M_4H_{-8}L_4$	**−9.26 ± 0.04**	**−37.17 ± 0.04**					−14.53	−37.68		−7.20
$\log K_{ML}^{MH_{-1}L}$ [g]	**−4.17**	**−5.53**		−4.15	−6.03	−6.73	−3.20	−6.89	− 7.10	−3.69
$\log K_{MH_{-1}L}^{MH_{-2}L}$ [h]			**−9.74**			−9.91	−9.45	−9.53		−9.36
$\log K_1^*$ [i]	**−4.46**	**−9.82**		−5.93	−10.31	−11.01	−7.63	−10.62	−11.34	−5.14
$\log K_2^*$ [j]	**−8.63**	**−15.36**	**−16.52**	−10.08	−16.34	−17.74	−10.83	−17.51	−18.44	−8.83
$\log K^{olig}$ [k]	**−7.07**	**−7.32**					−7.62	−6.73		−7.46

[a] Ref. 19. [b] Ref. 10. [c] Ref. 20. [d] Ref. 23. [e] Ref. 9. [f] Ref. 16. [g] For reaction $ML \rightleftharpoons MH_{-1}L + H^+$. [h] For reaction $MH_{-1}L \rightleftharpoons MH_{-2}L + H^+$. [i] $\log K_1^* = \log \beta(ML) - \log \beta(H_2L)$. [j] $\log K_2^* = \log \beta(MH_{-1}L) - \log \beta(H_2L)$. [k] $\log K^{olig} = \frac{1}{4} \log \beta(M_4H_{-8}L_4) - \log \beta(MH_{-1}L)$.

maximum concentration of the particular species from the potentiometric calculations. The ^1H NMR spectra were recorded on a Bruker AMX spectrometer at 300 MHz in D_2O using TSP (sodium (3-trimethylsilyl)-2,2,3,3-tetradeuteriopropionate) as internal standard, spectra for the metal ion concentration range 0.0075–0.01 mol dm^{-3} and metal : ligand molar ratios of 1 : 1 for Ni^{II} and 1 : 1.7 for Zn^{II}.

Results and discussion

Protonation constants for HmS-His (H_2L) and related peptides are given in Table 1. Two CH_2OH side chains on the HmS residue make the amino group distinctly the most acidic among those collected in the table. Also the pK value of the imidazole nitrogen deprotonation in HmS-His is the lowest one (Table 1).

Copper(II) complexes

Five metal complex species can be fitted to the experimental titration curves obtained for the Cu^{II}–HmS system (Fig. 1, Table 2), including three monomeric complexes, CuL, $CuH_{-1}L$, and $CuH_{-1}L_2$. Above pH 7 the $Cu_2H_{-3}L_2$ dimer is formed and above pH 9 the major complex is the $Cu_4H_{-8}L_4$ tetramer species (Fig. 1).

The co-ordination of metal ion starts at pH around 3 and the CuL complex is formed. The d–d transition energy of 618 nm and the presence in CD spectra of two charge transfer transitions $NH_2 \rightarrow Cu^{2+}$, $N_{Im} \rightarrow Cu^{2+}$ are consistent with {NH_2, N_{Im}} co-ordination (Table 3). The high value of the stability constant of the CuL species (Table 2) may suggest the involvement of the oxygen of the carbonyl group rather than the nitrogen of the amide group in the co-ordination of the metal ion.[16] This co-ordination mode was suggested for the Zn^{2+} complex of Gly-His from NMR measurements.[10]

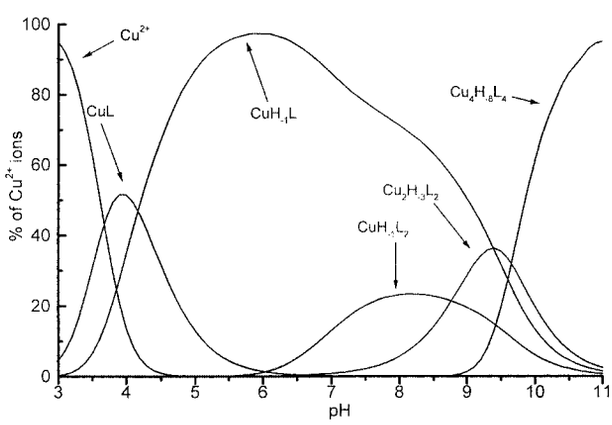

Fig. 1 Concentration distribution of the complexes formed in the Cu^{II}–HmS-His system as a function of pH. Metal to ligand molar ratio 1 : 2; [Cu^{II}] = 1×10^{-3} mol dm^{-3}.

The major complex formed at pH 4–9 is the $CuH_{-1}L$ monomeric species with {NH_2,N^-,N_{im}} binding sites. This co-ordination mode is clearly seen in the spectroscopic data. The d–d band at 588 nm and three charge transfer transitions at 345 ($N_{im} \rightarrow Cu^{II}$), 309 ($N^- \rightarrow Cu^{II}$) and 288 nm ($NH_2 \rightarrow Cu^{II}$) are consistent with the above binding mode.[17] It is interesting that the d–d transition energy of this $CuH_{-1}L$ species (588 nm) is distinctly higher than that observed *e.g.* for the Gly-His ligand (602 nm). This indicates the formation of the more effective metal ion–nitrogen co-ordination by HmS containing peptides.

The formation of the oligomeric species shifts the d–d transition towards higher energy (557 nm, Table 3) suggesting that the bridging donor is another nitrogen. The only possibility is the involvement of the second imidazole nitrogen to bind the adjacent copper(II) species. Thus, the significant blue shift in the

Table 3 Spectroscopic data for copper(II) and nickel(II) complexes of HmS-His dipeptide

	UV-VIS		CD		EPR	
Species	λ/nm	ε/dm^3 mol^{-1} cm^{-1}	λ/nm	$\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1}	$A_{\parallel}$	$g_{\parallel}$
Cu^{2+} Complexes						
CuL	618[a]	68	656[a]	−0.237	184	2.227
			508[a]	−0.023		
			342[b]	+0.063		
			285[c]	−0.582		
$CuH_{-1}L$	588[a]	80	730[a]	+0.052	199	2.213
			569[a]	+0.086		
			491[a]	−0.011		
			345[b]	+0.108		
			309[d]	−0.063		
			288[c]	+0.095		
$Cu_4H_{-8}L_4$	557[a]	93	566[a]	+0.081	No spectrum	
			476[a]	−0.083		
			341[b]	+0.127		
			290[d]	+0.109		
Ni^{2+} Complexes						
$NiH_{-1}L$	807[a]	11				
	740[a]	9				
	569[a]	15				
	352[a]	42				
$Ni_4H_{-8}L_4$	448[a]	140	485[a]	−1.460		
			425[a]	+1.832		
			306 (sh)	+0.390		

[a] d–d transition. [b] $N_{Im}\rightarrow Cu^{2+}$ charge transfer transition. [c] $NH_2\rightarrow Cu^{2+}$ charge transfer transition. [d] $N^-\rightarrow Cu^{2+}$ charge transfer transition.

absorption band can be interpreted by the formation of tetrameric $Cu_4H_{-8}L_4$, in which the fourth co-ordination site of the Cu^{II} is occupied by the deprotonated $N(1)_{Im}$ donor atom. A more detailed description of the tetrameric species will be given in discussion of the respective nickel(II) complex.[18]

The formation of oligomeric species is also supported by the EPR measurements. The formations of the dimer and tetramer complexes lead to vanishing of the EPR spectrum resulting from the strong metal–metal antiferromagnetic coupling in the complexes formed.

Tetrameric polynuclear complexes of type $Cu_4H_{-8}L_4$ in which four copper(II) ions are bridged by imidazole rings *via* both N(3) and N(1) nitrogens have also been proposed with glycylhistidine,[19] glycylhistidylglycine,[16] glycylhistamine[20] and carcinine {3-amino-*N*-[2-(imidazol-4-yl)ethyl]propanamide}[21] in the alkaline pH range (>9). The minor $CuH_{-1}L_2$ and $Cu_2H_{-3}L_2$ species cannot be characterised by the spectroscopic parameters due to their low concentrations.

Nickel(II) complexes

According to potentiometric data nickel(II) ion gives with HmS-His two major complexes, monomeric $NiH_{-1}L$ and tetrameric $Ni_4H_{-8}L_4$ (Table 2, Fig. 2). Absorption spectra indicate that Ni^{2+} ions in $NiH_{-1}L$ species are in an octahedral environment (Table 3). The ^{1}H NMR spectra of nickel(II)-containing systems show very broad lines between pH 5 and 10, due to the paramagnetic line broadening. The co-ordination mode in the $NiH_{-1}L$ complex is analogous to that found in the copper(II) species, *i.e.* involving the $\{NH_2,N^-,N_{Im}\}$donor set. At pH above 10, ^{1}H NMR measurements confirm the formation of a diamagnetic complex because the proton lines become narrow. The colour of the solution containing nickel(II) ions changes from green to yellow, and in absorption and CD spectra the d–d transitions of diamagnetic species are observed (Table 3). The formation of diamagnetic species and the increase of $\Delta\varepsilon$ values for d–d transitions in CD spectra suggest the involvement of 4 nitrogen atoms in co-ordination.[16] The structure of the diamagnetic tetrameric complex can be

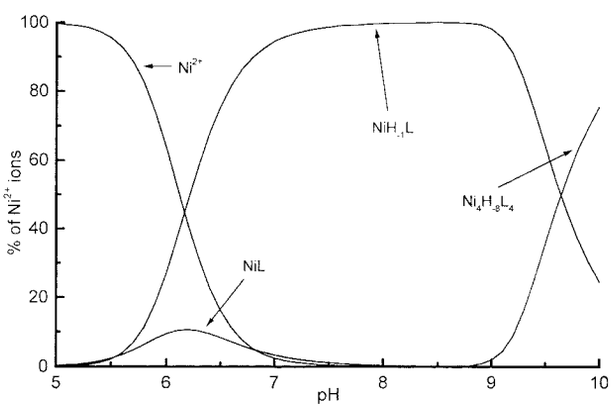

Fig. 2 Concentration distribution of the complexes formed in the Ni^{II}–HmS-His system as a function of pH. Metal to ligand molar ratio 1:2; $[Ni^{II}] = 1 \times 10^{-3}$ mol dm^{-3}.

convincingly assigned with use of ^{1}H NMR spectra (Table 4). The formation of the tetramer results in two sets of the proton spectra of the same intensity (Fig. 3) indicating an equimolar complex with C_2 symmetry (see Chart 1). The proton chemical shifts upon metal ion co-ordination indicate the involvement of both imidazole ($N(1)_{Im}$ and $N(3)_{Im}$) nitrogens in metal ion co-ordination with two, however, different symmetries for bound

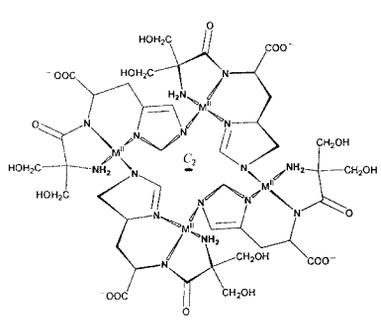

Chart 1

Table 4 The ^{1}H NMR data for HmS-His metal-free peptide at pH 10.9 and for the tetrameric $Ni_4H_{-8}L_4$ complex at pH 12.0. Chemical shifts are given in ppm relative to TSP

	$H(2)_{Im}$	$H(2)_{Im}$	$H(4)_{Im}$	$H(4)_{Im}$	α-CH_{His}	α-CH_{His}	CH_2 HmS(1)	CH_2 HmS(2)	CH_2 HmS(3)	CH_2 HmS(4)
HmS-His pH 10.9										
δ	7.673		6.942		4.441		3.632	3.601		
δ_A [a]							3.754	3.692		
δ_B [a]							3.510	3.510		
J_{AB}/Hz							11.58	11.58		
$\Delta\delta_{AB}$							0.244	0.182		
$Ni_4H_{-8}L_4$ pH 12.0										
δ	6.828	6.812	6.387	4.979	4.049	3.878	3.664	3.670	3.643	3.689
δ_A [a]							3.773	3.773	3.939	3.966
δ_B [a]							3.555	3.567	3.348	3.412
J_{AB}/Hz							11.95	11.98	11.95	11.56
$\Delta\delta_{AB}$							0.218	0.206	0.591	0.554
$\Delta\delta_L - \Delta\delta_C$ [b]	0.845	0.861	0.555	1.963	0.392	0.563				

[a] The chemical shifts for A and B of the CH_2 protons of the HmS residue. [b] The difference of chemical shift of the protons of HmS-His for metal-free peptide and for the $Ni_4H_{-8}L_4$ complex.

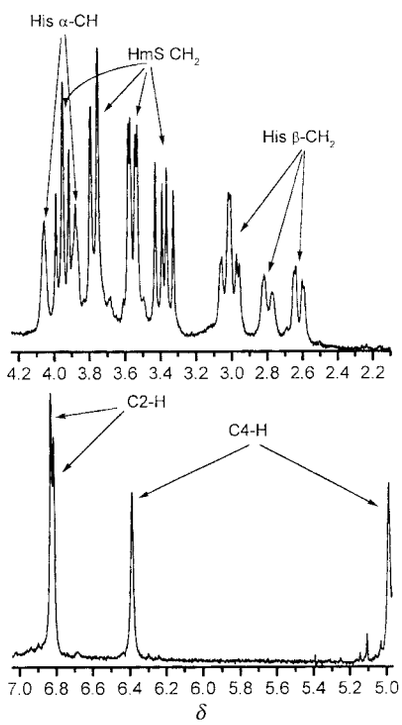

Fig. 3 The ^{1}H NMR spectrum of the Ni^{II}–HmS-His system in D_2O at pD 10.9. [L] = 1.1, [Ni^{II}] = 0.01 mol dm $^{-3}$.

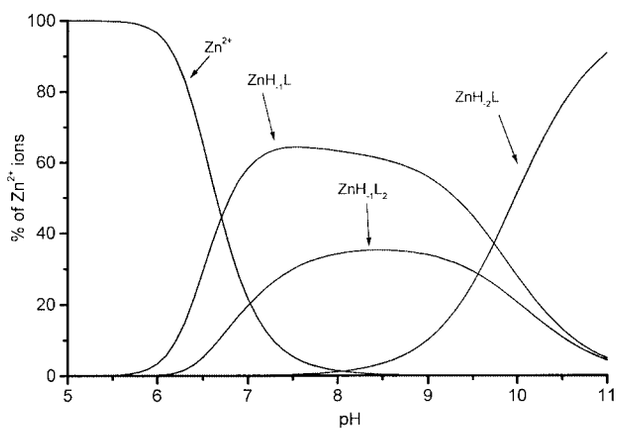

Fig. 4 Concentration distribution of the complexes formed in the Zn^{II}–HmS-His system as a function of pH. Metal to ligand molar ratio 1 : 2; [Zn^{II}] = 1×10^{-3} mol dm^{-3}.

imidazoles. The same type of complex was obtained for gold(III) with Gly-His.[22] In the latter case the crystal structure of the tetrameric complex was cyclic with metal ions having 4 nitrogen co-ordination including two imidazole groups at each metal bound in *cis* position.

This is presumably the first ^{1}H NMR spectroscopic evidence directly supporting the structure of the $Ni_4H_{-8}L_4$ species in solution, in good agreement with the distribution curves deduced from pH-metric measurements and the crystal structure obtained for the gold(III) complex.[22]

Tetrameric complexes of the type $Ni_4H_{-8}L_4$ have also been proposed with glycylhistidine,[18] glycylhistamine and sarcosylhistamine.[23]

It is interesting that the C_2 symmetry of the nickel(II) tetramer was never observed in the earlier works.[23]

Zinc(II) complexes

From potentiometric data calculations, three monomeric complexes are found for the Zn^{II}–HmS-His system (Table 2, Fig. 4). The $ZnH_{-1}L$ species dominates in the 6–10 pH range and it corresponds to the {NH_2,N^-,N_{im}} donor set involved in the metal ion binding.[9,10] The $ZnH_{-1}L_2$ complex is the second major species with the second ligand co-ordinated most likely *via* amino and imidazole nitrogens. Above pH 9 the major species is the equimolar hydroxy complex $Zn(OH)H_{-1}L$ ($ZnH_{-2}L$ in Fig. 4). The zinc(II) binding sites can be convincingly established from the ^{1}H NMR spectra. The formation of the $ZnH_{-1}L$ complex changes distinctly the proton chemical shift at imidazole and His α-CH protons (Fig. 5). These chemical shift changes clearly indicate the involvement of deprotonated amide nitrogen in the metal ion co-ordination. The formation of $ZnH_{-1}L$ species is accompanied by a distinct upfield shift of α-CH and imidazole protons due to binding to vicinal nitrogen donors.

Enhancement of the stability constants by HmS residue

The values of log K* in Table 2 are the protonation corrected stability constants which are useful to compare the ability of various ligands to bind a particular metal ion.[24,25] The data collected in Table 2 for HmS-His and four other related peptides clearly indicate that HmS-His is the most effective in metal ion binding when monomeric complexes are taken into account. The log K_2* values are more than one order of magnitude higher for α-hydroxymethylserine ligand for all metal ions studied here. Also the tetrameric complex of Cu^{II} is the most stable species for HmS dipeptide, while in the case of Ni^{II} Gly-Hist seems to form more stable species than for HmS-His (Table

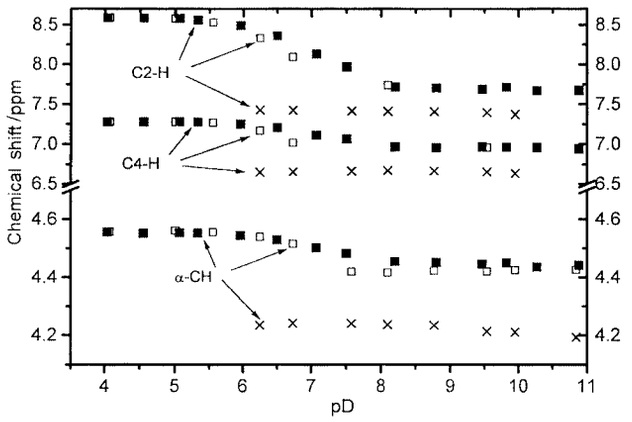

Fig. 5 pD Dependence of the chemical shift of imidazole C2-H, C4-H protons and the histidyl α-CH proton in D_2O solution containing 7.5×10^{-3} mol dm^{-3} Hms-His (■), and 7.5×10^{-3} mol dm^{-3} HmS-His and 4.4×10^{-3} mol dm^{-3} $Zn(NO_3)_2$ (×; □).

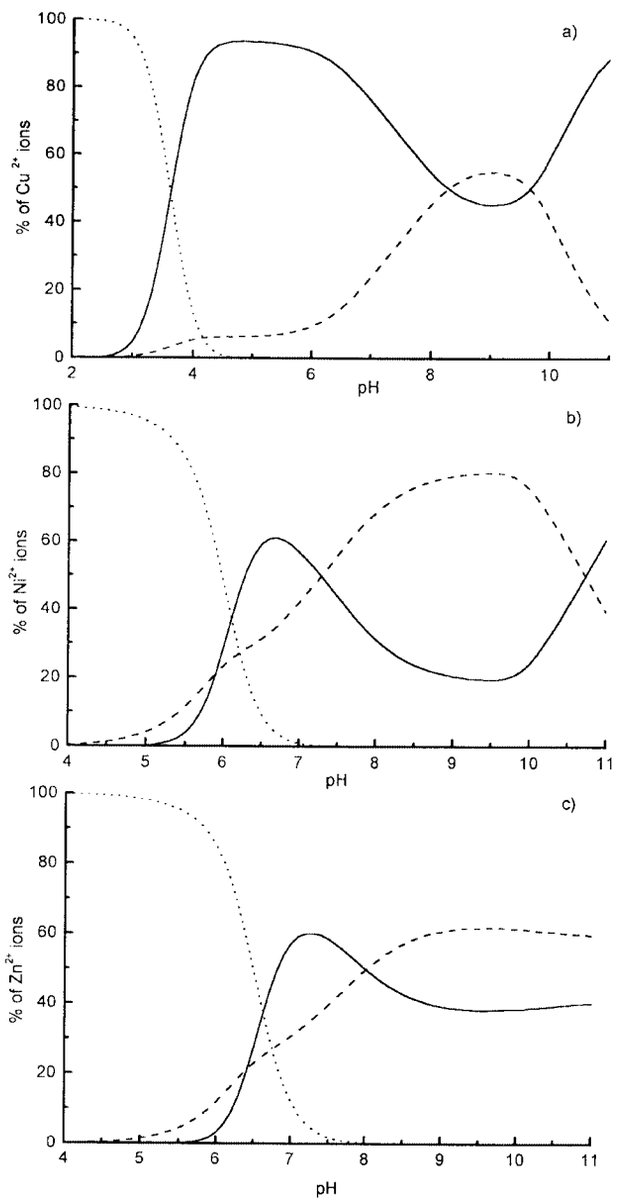

Fig. 6 The distribution of: (a) Cu^{II}, (b) Ni^{II}, (c) Zn^{II} between HmS-His and Gly-His in aqueous solution for a molar ratio of 1:2:2 of metal (dotted line) to HmS-His (solid line), Gly-His (dashed line). Binary stability constants were taken from Table 2.

2). To visualise the chelation ability of HmS-His in comparison with Gly-His a competition plot was made by taking into account the stability constants for the binary M^{II}–HmS-His and M^{II}–Gly-His systems (Fig. 6). The calculations were made assuming a 1:2:2 M^{II}:HmS-His:Gly-His molar ratio. The plot obtained for Cu^{II} clearly indicates that over a broad pH range including physiological pH HmS-His is much more effective in metal ion binding than is Gly-His (Fig. 6a). In the case of Ni^{II} and Zn^{II} the co-ordination abilities of the two dipeptides are similar to each other (Fig. 6b,c).

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