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COMMUNICATION

Introduction of α-hydroxymethylserine residues in a peptide sequence results in the strongest peptidic, albumin-like, copper(II) chelator known to date

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A tripeptide amide HmS–HmS–His–NH_2 is the strongest peptidic Cu^{II} chelator known to date, due to the steric shielding of the chelate plane as well as electronic effects.

α-Hydroxymethylserine (HmS) is a non-proteinaceous amino acid, found as the N-terminal residue in antibiotic peptides, antrimicin[1] and cirratiomycin.[2] It differs from serine by having another –CH_2OH function at the α carbon. This substitution generates specific constraints on the conformational freedom of a peptide containing HmS, which is the likely reason for its existence. From the co-ordination point of view, the presence of two alcoholic functions in HmS enhances its binding abilities. The direct involvement of alcoholic functions in co-ordination was found for oxovanadium(IV) and copper(II) complexes of the HmS amino acid.[3] Indirect, conformational phenomena also contributed to the stabilisation of particular complex species in di- and tri-peptides containing HmS residues.[4,5]

Peptides containing the N-terminal sequence Xaa–Yaa–His exhibit a particular affinity towards Cu^{II} and Ni^{II}, resulting from the formation of three fused chelate rings and a flat four-nitrogen (4N) co-ordination sphere around the metal ion.[6,7] We have previously shown that the stability of such complexes can be influenced by conformational and electronic effects resulting from substitutions of amino acids Xaa and Yaa. Substitutions of non-bonding Gly with bulkier Val and Ile provided a stability gain of two orders of magnitude, and the introduction of a positive Arg residue in position 1 was even more effective.[8,9] The study presented in this communication was aimed at finding out whether the presence of HmS residues can augment the binding capabilities of Xaa–Yaa–His peptides.

The peptides, H–HmS–HmS–His–OH (**1**) and H–HmS–HmS–His–NH_2 (**2**), were prepared using optimised methodology for incorporation of HmS into the peptide chain.[10,11] Their co-ordination to Cu^{II} was studied by potentiometry and spectroscopy (UV/VIS, CD, EPR) in conditions analogous to those applied previously.[5]

Table 1 contains the stability constants (log β values) and spectroscopic parameters of complexes formed. Fig. 1 presents species distribution diagrams for these complexes, derived from potentiometric and spectroscopic measurements. Table 2

Table 1 Stability constants and spectroscopic characterisation of complexes formed by **1** and **2**

Species	log β[a]	UV/VIS[b] λ	UV/VIS[b] (ε)	CD[b] λ	CD[b] ($\Delta\varepsilon$)	EPR $A_{\parallel}$[c]	EPR $g_{\parallel}$
HmS–HmS–His **1**							
HL	7.140(1)						
H_2L	13.176(1)						
H_3L	15.848(2)						
CuL	7.66(1)						
$CuH_{-1}L$	4.223(3)						
$CuH_{-2}L$	0.064(2)	510	(108)[d]	564	(−0.31)[d]	210	2.17
				487	(+0.73)[d]		
				307	(+0.80)[e]		
$CuH_{-3}L$	−10.41(2)	510	(114)[d]	563	(−0.32)[d]	210	2.17
				487	(+0.74)[d]		
				308	(+0.79)[e]		
HmS–HmS–His–NH_2 **2**							
HL	6.636(3)						
H_2L	12.322(3)						
$CuH_{-1}L$	4.09(4)						
$CuH_{-2}L$	1.271(7)	510	(99)[d]	559	(−0.26)[d]	207	2.18
				484	(+0.69)[d]		
				319	(+0.06)[f]		
				287	(−0.31)[g]		
$CuH_{-3}L$	−10.15(3)	510	(110)[d]	559	(−0.29)[d]	210	2.18
				483	(+0.74)[d]		
				323	(+0.06)[f]		
				288	(+0.39)[g]		

[a] $\beta(CuH_iL) = [CuH_iL]/\{[Cu^{2+}][H^+]^i[L]\}$. Standard errors on the last digits are included in parentheses. Three titrations were performed for each system. [b] UV/VIS and CD units: λ/nm, ε/dm³ mol⁻¹ cm⁻¹, $\Delta\varepsilon$/dm³ mol⁻¹ cm⁻¹. [c] EPR unit: $A_{\parallel}$/G. [d] d–d transition. [e] $N_{im} \longrightarrow Cu^{II}$ and $N^- \longrightarrow Cu^{II}$ CT transitions. [f] $N_{im} \longrightarrow Cu^{II}$ CT transition. [g] $N^- \longrightarrow Cu^{II}$ CT transition.

Table 2 Comparison of log $*K$ values for the 4N complexes of X–X–His peptides with Cu^{II}

Peptide	log $*K^a$
Gly–Gly–His[b]	−16.43
Gly–Gly–His[c]	−16.14
Gly–Gly–His–OMe[c]	−14.97
Gly–Gly–His–Gly–Gly[c]	−14.59
Gly–Gly–hist[d]	−17.14
Arg–Thr–His–Gly–Asn–NH_2[e]	−14.24
Arg–Thr–His–Gly–Asn–(15)[f]	−13.13
HmS–HmS–His[g]	−13.12
HmS–HmS–His–NH_2[g]	−11.04

[a] log $*K$ = log $\beta(CuH_{-2}L)$ − log $\beta(H_2L)$. [b] Ref. 6. [c] Ref. 12, 37 °C. [d] Ref. 14, hist stands for histamine. [e] Ref. 9. [f] Ref. 9, pentadecapeptide. [g] This paper.

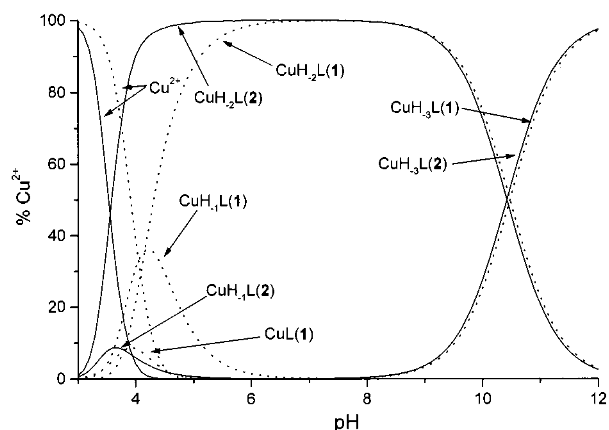

Fig. 1 Superimposed species distributions for Cu^{II} and **1** (···) or **2** (——), calculated for Cu^{II} concentrations of 1×10^{-3} mol dm^{-3} and ligand concentrations of 1.2×10^{-3} mol dm^{-3}.

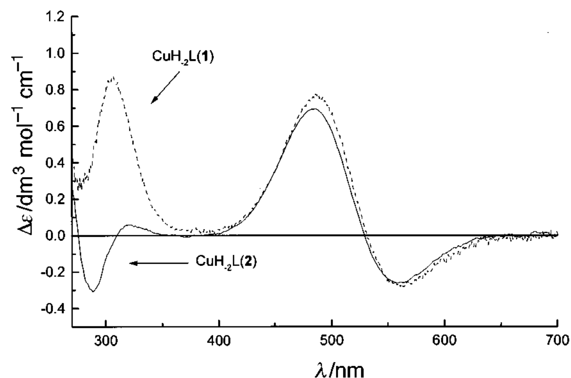

Fig. 2 CD spectra of $CuH_{-2}L$ complexes of **1** (···) or **2** (——), recorded at pH 7.0. Concentrations used were 1.88×10^{-3} mol dm^{-3} (Cu^{II}) and 2.28×10^{-3} mol dm^{-3} (**1**), and 1.75×10^{-3} mol dm^{-3} (Cu^{II}) and 2.1×10^{-3} mol dm^{-3} (**2**).

provides protonation-corrected stability constants for a range of complexes of Xaa–Yaa–His peptides that allow one to compare their metal binding capabilities directly. Spectroscopic parameters indicate that the binding mode in the 4N complex $CuH_{-2}L$, which predominates at pH 4–10 for both ligands, is identical to that of complexes of Gly–Gly–His and other Xaa–Yaa–His peptides. It involves the N-terminal amine (HmS-1), the amide nitrogens of HmS-2 and His-3 and the N-3 nitrogen of His-3 imidazole. There is no evidence for the direct involvement of alcoholic groups of HmS in the binding. The potential C-terminal donors, carboxyl in **1**, or amide in **2**, do not participate in Cu^{II} co-ordination as well.

The dramatic stability gain of 3.3 log units *vs.* Gly–Gly–His, seen for the $CuH_{-2}L$ species (Table 2), is likely to originate from the partial shielding of the Cu^{II} binding site from the bulk of solution both from above and below the co-ordination plane. In this way, the access of water molecules to metal ion-bound amide nitrogens is limited, and the dissociation reaction is slowed. Complexes of Xaa–Yaa–His peptides composed of L-amino acids can provide such shielding only at one side. The NMR-derived solution structure of the Ni^{II} complex of Val–Ile–His–Asn, exhibiting this phenomenon, correlates with the stability gain of 2 log units.[7] Quite surprisingly though, the amidation of the carboxylic function in **2** results in a further hundredfold increase of complex stability (to 5.4 log units *vs.* Gly–Gly–His), making it the strongest peptidic Cu^{II} chelator known to date. There is no reference data for appropriate amides (*e.g.* Gly–Gly–His–NH_2) in the literature, but the stability constants for Gly–Gly–His–OMe and Gly–Gly–His–Gly–Gly indicate that the carboxylate charge neutralisation by means of esterification or peptide chain extension may increase the complex stability by *ca.* one log unit.[12] The much bigger effect seen in our complexes presumably results from more specific interactions. The only major spectroscopic difference between the complexes of **1** and **2** is the sign of the amide nitrogen to Cu^{II} CT, positive with **1**, and negative with **2** (Fig. 2). Complexes of other Xaa–Yaa–His peptides studied previously[7–9] and of human and bovine serum albumins, sharing a similar metal binding site,[13] exhibited a positive CT band. HmS residues are not chiral, so the sign of the CT band is governed by the conformation of the 6-membered chelate ring between the His amide and the imidazole nitrogens. However, any major conformational change in this ring ought to be reflected in the d–d bands, while this region of the spectra is practically identical for both complexes, indicating the essentially unchanged metal ion environment. At this point we conclude that there is a specific interaction in the $CuH_{-2}L$ complex of **2**, which is probably related to its extremely high stability, but the reasons for this effect remain to be elucidated.

Further studies of the complexes presented above and of their nickel counterparts are currently in progress in our laboratory and will be reported soon.

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