DALTON COMMUNICATION

Specific binding of Cu^{2+} ions by a pentapeptide fragment present in the cysteine-rich region of amyloid precursor protein

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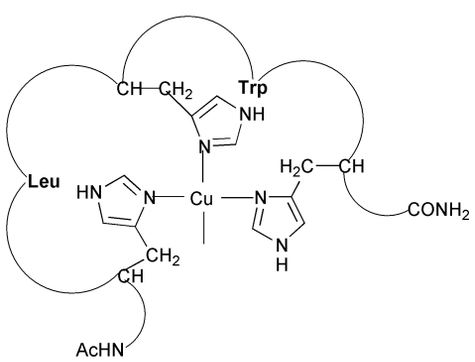
The βA4 amyloid precursor protein fragment situated in the cysteine-rich region is a very effective binding site for Cu^{2+} ions due to the presence of three His residues in the His–Xaa–His–Yaa–His sequence.

The βA4 amyloid precursor protein (APP), a multifunctional glycoprotein, is a source of the characteristic βA4 amyloid deposits found in Alzheimer's disease.[1] APP is known to bind to Zn^{2+} ions, which may modulate its interactions with heparin.[2] Studies by Multhaup *et al.*[3–6] have shown that the βA4 amyloid precursor protein binds very effectively to Cu^{2+} ions and then reduces them to Cu^{+}, producing hydrogen peroxide. The copper-binding also results in a site-specific fragmentation of APP, which could be an important process during Alzheimer pathology.[5] The main cause of the specific Cu^{2+} ion binding seems to be the presence of His residues in the cysteine-rich region of APP, while redox reactions are induced by two cysteine residues at positions 144 and 158.[5,6] The –His–Xaa–His– sequence, present in the SOD1 copper-binding centre for example, could be a major factor for metal ion binding by APP.[3] In this work we have tested the specificity of a three His residue site, –His–Leu–His–Trp–His–, which is present in a cysteine-rich region of APP, using potentiometric and spectroscopic techniques (absorption, EPR and CD spectra). To model the protein binding site we have used a pentapeptide fragment protected at the N- and C-termini (see Scheme 1).



Scheme 1 Structure hypothesis for the CuL complex.

The synthesis of the peptide fragment Ac–His–Leu–His–Trp–His–NH_2 was performed by a solid-phase method using Fmoc (Fmoc = 9-fluorenylmethoxycarbonyl) and continuous-flow methodology (9050 Plus Millipore Peptide Synthesizer) on the CLEAR-Amide Resin (Peptides International, Inc.).[7,8] The purity of the peptide was assessed by RP-HPLC using a C_8 Kromasil column (4.6 × 250 mm, 5 μm) and a linear gradient of 0–80% acetonitrile in 0.1% aqueous trifluoroacetic acid as a mobile phase over 60 min and by fast atom bombardment mass spectroscopy (FAB-MS, glycerol matrix). Analytical data for the peptide are: R_t(HPLC) = 23.10 min, $M^+ + 1$ (FAB-MS) = 770.8 (calculated molecular weight M_w = 769.8).

According to potentiometric data Ac–His–Leu–His–Trp–His–NH_2 has three protonation constants with p*K*s 6.97, 6.32 and 5.68 corresponding to three His imidazoles. The results of the calculations based on the potentiometric data, EPR, UV–Vis and CD spectra indicate the formation of six complex species above pH 3 (see Table 1 and Fig. 1). The CuL complex

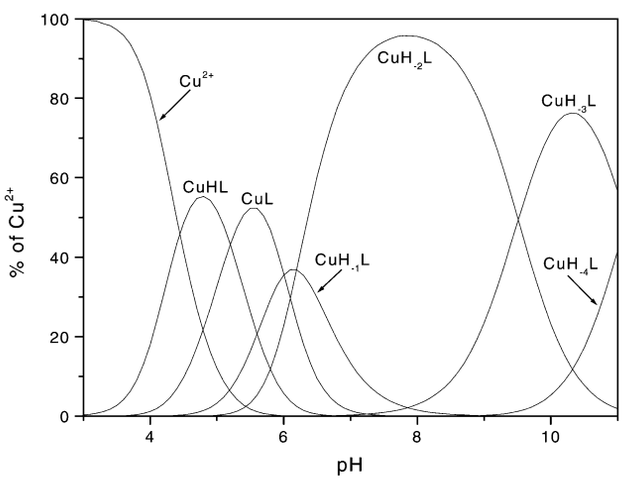


Fig. 1 Distribution diagram of complexed species formed as a function of pH in the system Cu^{2+}–Ac–HLHWH–NH_2. $[Cu^{2+}] = 1 \times 10^{-3}$ M; metal : ligand ratio = 1 : 1.2.

with three imidazoles bound to the Cu^{2+} ion predominates at around pH 5 and its stability (log β = 8.06) is distinctly higher than the respective Cu^{2+} complex with peptide having only two His residues: Ac–His–Gly–His–Gly (log β = 6.49[9]). However, the stability of the CuHL species for APP peptide with two imidazoles bound to the Cu^{2+} ion and one imidazole still protonated is very close to that of the di-histidyl ligand (log β_{CuHL} − log K_{imid} = 6.29). The major complex in the pH range 6–8 is the $CuH_{-2}L$ complex involving two deprotonated amide nitrogens. Its stability is also distinctly higher for the three-histidyl peptide than for the respective species of Ac–His–Gly–His–Gly (Table 1). The competition plot (Fig. 2) clearly indicates that the Ac–His–Leu–His–Trp–His–NH_2 sequence is a much more effective Cu^{2+} binding site than the two histidyl analogue.

The spectroscopic parameters obtained for the $CuH_{-2}L$ species with both peptides are also considerably different from each other (Table 2). The d–d transition for the complex with APP-peptide consists of three d–d transitions at 665, 571 and 494 nm, while in the case of di-histidyl peptide the d–d transition is a single band at 575 nm. This may indicate that the complex formed with the former peptide is of distinctly lower symmetry than that with the latter. The distinct lowering of geometry around a coordinated metal ion may also support the involvement of three imidazoles in the metal ion binding in $CuH_{-2}L$ in the case of APP site resulting in the $\{3 \times N_{imid}, 2N^-\}$ donor set. The coordination of three imidazoles and two amides due to steric reasons would lead to a geometry distinctly different from the tetragonal one usually observed for Cu^{2+}-peptide complexes.[10]

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Table 1 Potentiometric and spectroscopic data for proton and Cu^{2+}–Ac–HLHWH–NH_2 complexes[a]

			UV–Vis		CD		EPR	
	log β	pK	λ/nm	ε/M^{-1} cm^{-1}	λ/nm	$\Delta\varepsilon$/M^{-1} cm^{-1}	A_{II}/G	g_{II}
HL	6.97(1)	pK_{Nim1} = 6.97						
H_2L	13.29(1)	pK_{Nim2} = 6.32						
H_3L	18.97(1)	pK_{Nim3} = 5.68						
CuHL	13.26(1)		662	42	662	0.034	139	2.34
					552	−0.029		
					491	0.032		
					336	−0.162		
					291	0.146		
					253	0.597		
CuL	8.06(1)	5.20	587	89	656	0.091	160	2.31
					550	−0.128		
					488	0.157		
					337	−0.870		
					283 sh	2.095		
					237	7.525		
$CuH_{-1}L$	2.06(1)	6.00						
$CuH_{-2}L$	−4.14(1)	6.19	547	152	665	0.153	187	2.20
					571	−0.320		
					494	0.390		
					380	0.172		
					332	−1.076		
					281 sh	3.399		
$CuH_{-3}L$	−13.65(1)	9.51	548	153	665	0.161	197	2.18
					570	−0.342		
					495	0.366		
					379	0.212		
					332	−1.054		
					280	3.645		
					237	9.191		
$CuH_{-4}L$	−24.78(2)	11.13	544	151	654	0.292	197	2.18
					559	−0.329		
					496	0.034		
					377	0.264		
					329	−0.798		
					231	8.629		

[a] Titration involved an ionic background of 0.1 M KNO_3, a ligand concentration of 1.2×10^{-3} M and metal to ligand ratios of 1 : 1.2. Stability constants for the complexes of H^+ and Cu^{2+} were calculated from titrations carried out using total volumes of 2 cm^3. The pH-metric titrations were performed at 25 °C using a MOLSPIN automatic titration system with a Russel CMAV 711 microcombined electrode, calibrated on hydrogen ion concentration using HNO_3.[14] Titrations were performed in triplicate and the SUPERQUAD computer program was used for stability constants calculations.[15] CD, EPR and UV–Vis spectra were recorded on the Jasco J 715, Bruker ESP 300 E and Beckman DU 650, respectively. The solutions have the same metal and ligand concentrations as those used in potentiometric titrations.

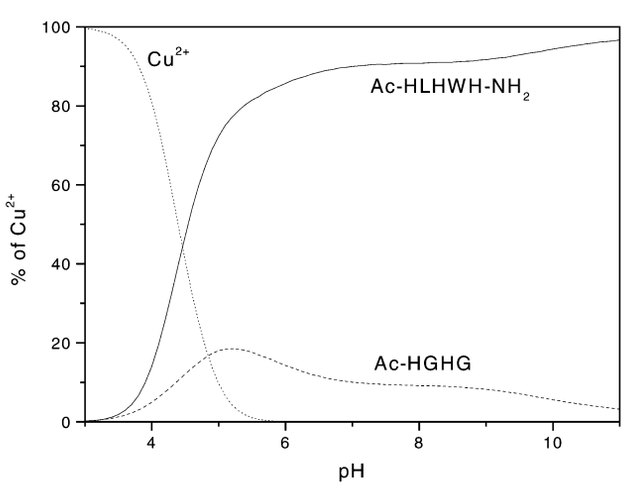

Fig. 2 Distribution profiles of free and complexed fractions of Cu^{2+} ions in the presence of both Ac–HLHWH–NH_2 (solid line) and Ac–HGHG–OH (dashed line). $[Cu^{2+}] = 1 \times 10^{-3}$ M; metal : ligand ratio = 1 : 1.

Above pH 8 the $CuH_{-2}L$ species deprotonates one more amide nitrogen and the major complex around pH 10 is the $CuH_{-3}L$ species, which is also more stable than the respective complex found for Ac–His–Gly–His–Gly (Table 2). The spectroscopic parameters change only slightly suggesting that the major binding core is very similar in both $CuH_{-2}L$ and $CuH_{-3}L$ complexes. It may suggest that a third amide nitrogen substitutes one of the imidazole nitrogens in the coordination sphere of Cu^{2+}. The pK value of the reaction $CuH_{-3}L \longrightarrow CuH_{-4}L + H^+$ equal to 11.13 may suggest that the deprotonation occurs on the metal-coordinated imidazole ring.[11]

Table 2 Comparison of log β for the Cu^{2+}–Ac–HLHWH–NH_2 and Cu^{2+}–Ac–HGHG systems

Species	Ac–HLHWH–NH_2	Ac–HGHG[a]
CuHL	13.26	11.04
CuL	8.06	6.49
$CuH_{-1}L$	2.06	0.4
$CuH_{-2}L$	−4.14	−6.13
$CuH_{-3}L$	−13.65	−16.41
$CuH_{-4}L$	−24.78	

[a] Ref. 9.

Careful examination of the EPR spectra and the potentiometric data calculations have not indicated any dimeric or bis-ligand species formation in the solutions studied.

Comparison of the binding abilities of the APP site with the two-His binding sites of SOD-like peptides (Ac–His–Gly–His–Gly), the SPARC copper-binding site[12] (Ac–His–Lys–Leu–His–Leu–NH_2) and the β-amyloid peptide fragment (Ac–Glu–Val–His–His–Gln–Lys–NH_2)[13] indicates that in the physiological pH range the APP site is the most effective, while SPARC coordination is the least effective. The second most powerful

binding site in the systems discussed above remains the SOD sequence.

The amyloid precursor protein binding site situated between Cys-144 and Cys-158 having three His residues in the –His–Xaa–His–Yaa–His– sequence is the most effective binding site for Cu^{2+} ions when compared to the two-histidyl sites like those of SPARC, SOD and β-amyloid peptide. In the physiologically relevant pH range the high ability for metal ion coordination by the APP cysteine-rich fragment is caused by the involvement of three His residue side chains (imidazoles) in Cu^{2+} ion binding. Thus, the specific binding of Cu^{2+} ion by βA4 amyloid precursor protein is in the cysteine-rich region.

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