A Study of the Comparative Donor Properties to Cu^{II} of the Terminal Amino and Imidazole Nitrogens in Peptides

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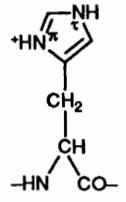
The synthesis of the tetrapeptides Ala-Gly-Gly-His, Boc-Ala-Gly-Gly-His (Boc = t-butoxycarbonyl), Ala-Gly-Gly-His(π-bom) (π-bom = N^{π}-benzoxymethyl), Ala-Gly-Gly-His-OMe, and Ala-Gly-Pro-His is reported, together with the results of a pH-metric and spectroscopic (absorption, c.d., and e.s.r.) study of their complexes with H^+ and Cu^{II}. The work was designed to study the initial site of binding to Cu^{II} in peptides containing both a terminal amino nitrogen and a histidyl residue. Results show that the π-N of the imidazole ring of the histidyl residue is the primary anchoring site for copper(II) co-ordination, and that the next nitrogen to bond can be the terminal amino N, forming a macrocyclic chelate ring.

Metal ions such as Cu^{II} form very stable complexes with peptides not containing side-chain donor groups because, in addition to forming a co-ordinate bond through the terminal amino-nitrogen, they are able to bind to deprotonated nitrogen atoms of the peptide bond to form Cu–N^- bonds. Not only does this additional bonding contribute towards very stable complexes but it also causes the peptide backbone to wrap around the metal ion, forcing a very specific conformation on the chain.[1] In spite of the stability of these Cu–N^- bonds co-ordination of peptides to Cu^{II} cannot be initiated through such interactions. With such peptides the first anchor site is the terminal amino-nitrogen which forms a Cu–NH_2 bond around pH 5. This is then followed by deprotonation of the peptide nitrogen of the neighbouring peptide linkage and bond formation to give a Cu–N^- bond. This is repeated until three successive Cu–N^- bonds have been made to form a planar complex with four nitrogens co-ordinated (a 4N complex). The situation changes, however, when a prolyl residue is inserted in the peptide chain since this contains a secondary nitrogen which is unable to form a Cu–N^- bond.[2,3] This 'break-point' often leads to the formation of macrochelate rings or to the bonding of amino acid side-chain donor centres such as the lysine (Lys) ε-NH_2 or tyrosine (Tyr) phenolate oxygen.[2–5]

Another side-chain donor centre which is extremely important and forms one of the most stable bonds to metal ions is an imidazole nitrogen of the histidine (His) residue, although side chains containing sulphur donors may also be important. In the absence of a terminal amino-nitrogen, the pyridine-like imidazole N is able to initiate co-ordination to Cu^{II}. This has been demonstrated in a number of biologically important peptide derivatives including luteinizing hormone releasing hormone[6] and thyrotropin releasing factor[7] in which the amino group is protected as the pyroglutamyl residue. When co-ordination is initiated at an imidazole N the first peptide N to be deprotonated and bound will be the nitrogen of the His residue to form a six-membered chelate ring. The imidazole N used must be the π-N since chelation including the τ-N and the peptide N is impossible.† Co-ordination from an imidazole N to the peptide N of the next amino acid residue linked through the carbonyl group of the His residue is also energetically unfavourable. Initial co-ordination of the imidazole N donor actually facilitates deprotonation of the



His peptide N allowing the formation of a Cu–N^- bond around pH 4.[8]

Dipeptides containing the His residue in the second position have been studied and shown to form a wide range of complexes, including dimers, with co-ordination probably starting at the imidazole.[9,10] However, in these cases, the complex is stabilised by chelation through the carbonyl group. Complexes with His-His,[11] His-His-Gly and His-Gly-His[12] (Gly = glycine) have also been studied and show the same general behaviour with Cu–N^- bonding well below pH 5 and binuclear species forming at intermediate pH. Cyclic peptides such as *cyclo*-(-His-His-),[13] *cyclo*(-Gly-His-),[14] *cyclo*-(-Gly-His-)$_3$,[15] and *cyclo*-(-Gly-His-Gly-His-Gly-His-Gly-)[16] have also been studied and all show initial co-ordination through the imidazole nitrogens followed, at higher pH, by co-ordination to peptide nitrogens.

In order to study the relative affinities towards Cu^{II} of the terminal amino-N and an imidazole-N donor of the His residue, Ala-Gly-Gly-His (Ala = alanine) has been synthesised, together with derivatives of the tetrapeptide with various donor centres protected Boc-Ala-Gly-Gly-His-, Ala-Gly-Gly-His-OMe, and Ala-Gly-Gly-His(πbom). Gly-Gly-Gly-His was also synthesised as a spectroscopic probe and Ala-Gly-Pro-His (Pro = proline) has been synthesised to investigate the effect of the break-point Pro residue inserted between the two major donor centres. Complexes with Cu^{II} have been studied using potentiometry and absorption, circular dichroism (c.d.), and

† Nitrogen atoms in the imidazole ring are distinguished using the I.U.P.A.C. nomenclature as used by J. R. Bell and J. H. Jones, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2336.

Table 1. Proton chemical shift assignments for the ^1H n.m.r. spectrum of Ala-Gly-Pro-His

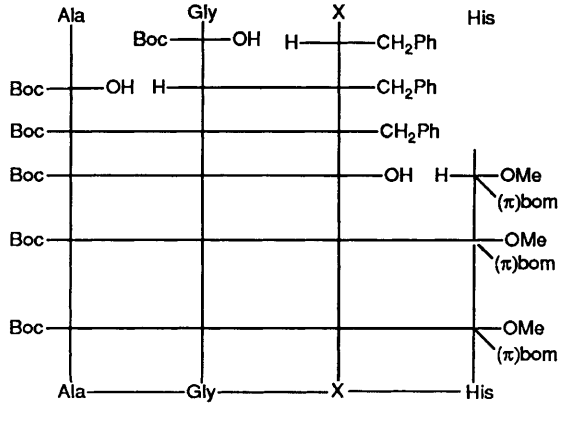
Residue	Proton	δ
Ala	$C_\alpha H$	4.10
	$C_\beta H_3$	1.52
Gly	NH_{amide}	8.72
	$C_\alpha H_2$	4.18, 4.03
Gly′	NH_{amide}	8.60
	$C_\alpha H_2$	3.98, 3.63
Pro	$C_\alpha H$	4.60
	$C_\beta H_2$	2.30, 2.28
	$C_\gamma H_2$	2.04, 1.88
	$C_\delta H_2$	3.58, 3.50
Pro′	$C_\alpha H$	4.50
	$C_\beta H_2$	2.10
	$C_\gamma H_2$	1.98
	$C_\delta H_2$	3.59, 3.69
His	NH_{amide}	8.62
	$C_\alpha H$	4.62
	$C_\beta H_2$	3.13
	CH_{arom}	7.03, 7.05
	NH_{arom}	7.93, 8.00
His′	NH_{amide}	8.29
	$C_\alpha H$	4.50
	$C_\beta H_2$	3.09

e.s.r. spectroscopy. The notation, His(πbom), indicates that the imidazole π-nitrogen on the His residue is protected by benzoxymethyl. In Boc-Ala the N-terminal amino function is protected by the t-butoxycarbonyl function and in the His(OMe) derivative the C-terminal carboxylate is protected.

Experimental

Peptide Syntheses.—Peptides were synthesised by standard liquid-phase methods using Bu^tOCO (Boc) as the amino-N and $C_6H_5CH_2OCH_2$ (πbom) as the imidazole-N protecting groups. The πbom protecting group was selected to ensure that the π-N nitrogen atom of the imidazole ring was protected and that racemisation was suppressed.[17] The absence of racemisation was confirmed by a study of the 400-MHz n.m.r. spectra of the final products.[11,18] The absence of acetate in the products was also confirmed by n.m.r. spectroscopy.

The synthesis of Ala-Gly-Gly-His (X = Gly) and Ala-Gly-Pro-His (X = Pro), typical of all the syntheses, is outlined below. C-Protected derivatives (in chloroform solution) were



neutralised with triethylamine before coupling. Coupling reagents were dicyclohexylcarbodi-imide (Merck) and 1-hydroxybenzotriazole (Aldrich). Benzyl groups were removed by hydrogenolysis using 10% Pd on charcoal as catalyst and t-Boc was cleaved using HCl (4 mol dm^{-3}) in dioxane. The peptides were purified by gel filtration (Sephadex G15, eluant water) and freeze-dried. Percentages of amino acid residues were confirmed by amino acid analysis (*e.g.* Ala-Gly-Gly-His Ala, 1.02; Gly, 1.95; His, 1.00. Ala-Gly-Pro-His Ala, 0.99; Gly, 0.95; Pro, 1.03; His, 1.00).

The purity of Ala-Gly-Pro-His was further checked by a detailed analysis of the 400 MHz ^1H n.m.r. spectrum. This confirmed that the molecule existed as two isomeric forms which differed by *cis-trans* isomerism about the Gly-Pro bond. Characteristic resonances are given in Table 1, and no other resonances were detected.

Potentiometric Studies.—Stability constants for complexes of H^+ and Cu^{2+} were calculated from titration curves carried out using total volumes of 1.5 cm^3. Alkali was added from a 0.1 cm^3 micrometer syringe which had been calibrated by both weight titration and the titration of standardised materials. Experimental details were: peptide concentration, 0.003 mol dm^{-3}; copper concentration, 0.001—0.0028 mol dm^{-3}; ionic strength, 0.10 mol dm^{-3} (KNO_3); pH range for complexation, 4—10.5; method, pH-metric titration using a micro combined glass–calomel electrode (Russel pH), calibrated in concentrations using $HClO_4$;[19] number of titrations, three per ligand (average of 40 data points per titration); temperature, 25 °C; method of calculation, SUPERQUAD.[20]

Standard deviations quoted were computed by SUPERQUAD and refer to random errors only. They give, however, a good indication of the importance of the particular species in the equilibrium.

Spectroscopic Studies.—Solutions were of similar concentrations as those used in the potentiometric studies. Absorption spectra were recorded on a Beckman UV5240 spectrophotometer and c.d. spectra on an automatic recording spectropolarimeter, JASCO-J-20. All c.d. spectra are expressed in terms of $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$. E.p.r. spectra were obtained on a Radiopan SE/X 2543 spectrometer at liquid-nitrogen temperatures and at 9.13 GHz.

Results and Discussion

Stability constants of the complexes of H^+ and Cu^{2+} with the ligands studied are given in Table 2 and spectroscopic data for the complexes in Table 3. Selected species distribution curves are shown in the Figure.

Protonation Constants.—The first protonation constant, log β_{HL}, for the four tetrapeptides with a free N terminal refers to protonation of the amino nitrogen and the values agree well with that for tetra-alanine (log β = 8.13[21]). Three of the tetrapeptides, Ala-Gly-Gly-His, Ala-Gly-Gly-His-OMe, and Ala-Gly-Pro-His, have similar stepwise protonation constants, log K_{H_2L}, which correspond to protonation of the pyridine-like imidazole nitrogen of the His residue and again these values compare well with literature values.[21] The final stepwise protonation, log K_{H_3L}, for Ala-Gly-Gly-His and Ala-Gly-Pro-His is that of the C-terminal carboxylate function.

The value of Log K_{H_2L} obtained for Ala-Gly-Gly-His(πbom) cannot refer to protonation of the π-nitrogen on the imidazole ring as this nitrogen is protected. Instead it must refer to that of the τ-nitrogen in the same ring which will be less basic than normal as a result of the electronic effect of the protecting group. A similar effect has been observed for histidine and some of its protected amino acid derivatives.[22] The butoxycarbonyl-protected tetrapeptide, Boc-Ala-Gly-Gly-His, cannot undergo N-terminal deprotonation and thus log β_{HL} refers to protonation of the τ-imidazole nitrogen. Its acidity is slightly lower than for

Table 2. Stability constants of Complexes of H^+ and Cu^{II} with histidyl tetrapeptides at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

Ligand	log β			
H^+ Complexes	HL	H_2L	H_3L	log K_{Im-NH}[a]
Ala-Gly-Gly-His	8.14(1)	15.03(1)	17.65(1)	6.89
Ala-Gly-Gly-His(πbom)	8.15(1)	14.20(1)	16.77(1)	6.05
Ala-Gly-Pro-His	8.16(1)	14.96(1)	17.59(1)	6.80
Ala-Gly-Gly-His-OMe	8.05(1)	14.67(1)	—	6.62
Boc-Ala-Gly-Gly-His	7.19(1)	10.02(1)		7.19
Ala-Ala-Ala-Ala[b]	8.13	11.65		

Copper(II) complexes	CuHL	CuL	$CuH_{-1}L$	$CuH_{-2}L$	$CuH_{-3}L$
Ala-Gly-Gly-His	12.72(4)	8.43(1)	1.82(2)	−5.36(1)	−15.24(3)
Ala-Gly-Gly-His(πbom)	12.41(4)	8.45(2)	1.56(3)	−6.70(3)	−16.19(3)
Ala-Gly-Pro-His	12.62(2)	8.11(1)	2.82(1)	−7.30(1)	—
Ala-Gly-Gly-His-OMe	—	8.18(1)	1.96(1)	−4.88(1)	—
Boc-Ala-Gly-Gly-His	—	4.97(1)	−1.82(1)	−8.49(1)	−17.42(1)
Ala-Ala-Ala-Ala[b]	—	4.77	−0.45	−8.09	−17.3

Stepwise constants	log K			
	CuHL	CuL	$CuH_{-1}L$	$CuH_{-2}L$
Ala-Gly-Gly-His	4.29	6.61	7.18	9.88
Ala-Gly-Gly-His(πbom)	3.96	6.89	8.26	9.49
Ala-Gly-Pro-His	4.51	5.29	10.12	—
Ala-Gly-Gly-His-OMe	—	6.22	6.84	—
Boc-Ala-Gly-Gly-His	—	6.79	6.67	8.93
Ala-Ala-Ala-Ala	—	5.22	7.64	9.32

Log K_{CuHL} = log K (CuL + H $\rightleftharpoons$ CuHL), log K_{CuL} = log K ($CuH_{-1}L$ + H $\rightleftharpoons$ CuL), log $K_{CuH_{-1}L}$ = log K ($CuH_{-2}L$ + H $\rightleftharpoons$ $CuH_{-1}L$), log $K_{CuH_{-2}L}$ = log K ($CuH_{-3}L$ + H $\rightleftharpoons$ $CuH_{-2}L$)

[a] Log K_{Im-NH} corresponds to log K(HL + H $\rightleftharpoons$ H_2L) for all ligands except Boc-Ala-Gly-Gly-His. [b] Ref. 21.

the other His tetrapeptides since the zwitterionic species will inhibit proton release.

Copper Complexes.—The peptide Ala-Gly-Gly-His-OMe underwent metal-catalysed hydrolysis in the presence of Cu^{II} above pH 7—8 and this badly affected the rate of attainment of equilibrium in the potentiometric titrations. As a result only low pH complexes with Cu^{II} were studied quantitatively.

With the other peptides, computer analysis of the potentiometric data allowed the inclusion of dimeric species in the equilibria. However there was no evidence for such species from the spectroscopic evidence and when dimeric complexes were excluded from the models used to evaluate potentiometric data the 'goodness of fit' was not affected significantly. In the e.p.r. spectra the 'dimeric signal' at $g_{\|}$ about 4 was not observed, nor even the line broadening which would be caused by the relaxation process due to the dipole coupling of two neighbouring copper(II) ions. Instead superhyperfine coupling is seen for the binding nitrogens in the 2N and 4N complexes. The reason for its absence with the 3N species is the superimposition of different spectra with similar $g_{\|}$ (apparent broadening) when several species co-exist in solution. It can be observed clearly, for example, in the species distribution diagrams (Figure) that the 3N complex is never the sole species in solution.

The number of potential co-ordination centres of the N-protected peptides, Boc-Ala-Gly-Gly-His and Ala-Gly-Gly-His(π-bom), are limited when compared to the parent tetrapeptide and hence these should be considered first before interpreting experimental data for all the ligands studied. In both cases only one initial anchoring site is available while still allowing subsequent chelation through peptide deprotonation and co-ordination. The π-N of the imidazole ring is excluded from co-ordination in the πbom protected peptide while this must be the initial co-ordination centre in Boc-Ala-Gly-Gly-His. However, as a result of tautomeric equilibria in the imidazole ring, the τ-N atom of Ala-Gly-Gly-His(πbom) can have pyridine-like character and participate in co-ordination. Subsequent successive deprotonations of amide nitrogens must therefore proceed from these initial co-ordination centres and these can be used as a starting point for a discussion of the experimental results. All the experimental data are in full agreement with these assumptions.

The assignment of charge-transfer (c.t.) transitions for an imidazole N to Cu^{II} is based on the work of Fawcett *et al.*,[23] who have shown that the magnitude and precise energy of the transitions are very sensitive to the position of the ring plane relative to the complex plane, and thus to the possibility of its rotation. As a result the π_1-Cu^{II} transition can be assigned only in one case because it overlaps with the N–Cu^{II} c.t. transition. The magnitudes of some of these transitions in the c.d. spectra are given in Table 3 although some of the minor species may contain contributions from other chiral species present in solution.

1N *Complexes.* Starting at low pH, these would be the first complexed species to form. As shown by potentiometry (Figure) they are minor species for all peptides studied apart from Boc-Ala-Gly-Gly-His and so cannot be characterised clearly by c.d. spectroscopy. However their existence with Ala-Gly-Gly-His and Ala-Gly-Gly-His(πbom) has been confirmed by e.s.r. spectroscopy. The process of co-ordination may be described by the equilibrium (1) (charges omitted for simplicity). The

$$H_xL + Cu \rightleftharpoons CuH_{x-1}L + H \quad (1)$$

equilibrium constant, K', for this reaction may be calculated readily from values given in Table 2 to give the following values for log K': Ala-Gly-Gly-His (x = 2), −2.31; Ala-Gly-Gly-

Table 3. Spectroscopic data for the copper(II) complexes of Ala-Gly-Gly-His tetrapeptides

Ligand	Absorption spectra $\lambda_{max.}$[a]/nm	C.d. spectra $\lambda_{max.}$[b]/nm	E.s.r. spectra $g_{\|}$	E.s.r. spectra $10^4 A_{\|}$/cm^{-1}
1N Complexes				
Ala-Gly-Gly-His(πbom)			2.370	140
Boc-Ala-Gly-Gly-His	740 (27)	—	2.362	142
Ala-Gly-Gly-His	740 (21)	—	2.360	142
Ala-Gly-Pro-His		740 (+0.06)[c] 225 (−1.45)[d]	2.363	141
2N Complexes				
Ala-Gly-Gly-His(πbom)	697 (44)	683 (−0.02)[c] 305 (sh) (+0.03)[e] 290 (+0.04)[f]	2.302	158
Boc-Ala-Gly-Gly-His	—	—	2.312	165
Ala-Gly-Gly-His-OMe	702 (45)	675 (+0.07)[c] 255 (sh) (−0.21)[f] 245 (−0.26)[g]	2.300	150
Gly-Gly-Gly-His	710 (36)	685 (−0.09)[c] 240 (sh) (+1.0)[g]		
Ala-Gly-Gly-His	691 (46)	695 (−0.09)[c] 260 (sh) (−0.13)[f] 252 (−0.17)[g]	2.300	147
Ala-Gly-Pro-His		Too minor to be detected		
3N Complexes				
Ala-Gly-Gly-His(πbom)	594 (79)	610 (−0.17)[c] 300 (+0.15)[e] 286 (+0.17)[f]	2.225	177
Boc-Ala-Gly-Gly-His	598 (83)	640 (−0.03)[h] 530 (+0.05)[i] 325 (+0.25)[i] 300 (sh) (+0.2)[d] 255 (sh) (−0.6)[g] 235 (−1.3)[d,g]	2.236	180
Ala-Gly-Gly-His	585 (76)	658 (−0.06)[h] 520 (+0.05)[i] 306 (+0.21)[e] 250 (sh) (−0.19)[g]	2.250	178
Ala-Gly-Pro-His	624 (102)	628 (−0.39)[c] 298 (+0.23)[e,f] 235 (−1.0)[d,g]	2.234	165
4N Complexes				
Ala-Gly-Gly-His(πbom)	527 (127)	528 (−0.35)[c] 305 (sh) (+0.05)[e] 278 (+0.23)[f]	2.175	223
Ala-Gly-Gly-His	562 (105)	522 (+0.20)[c] 305 (+0.05)[e] 255 (sh) (−0.5)[g]	2.200	202
Gly-Gly-Gly-His	567 (100)	535 (+0.28)[c] 308 (+0.22)[e] 270 (−0.1)[g] 245 (+0.24)[g] 223 (−3.6)[d]		

[a] Approximate absorption coefficient given in parentheses (ε/dm^3 mol^{-1} cm^{-1}). [b] Δε in parentheses (dm^3 mol^{-1} cm^{-1}). [c] *d–d* Transition. [d] Imidazole π–π transition + imidazole–copper(II) sigma transition. [e] $^-$N–Cu Transition. [f] NH_2–Cu Transition. [g] Imidazole π_2–Cu^{II} transition. [h] B-Type transition. [i] E-Type transition.

His(πbom) (x = 2), −1.18; Ala-Gly-Pro-His (x = 2), −2.34; and Boc-Ala-Gly-Gly-His (x = 1), −2.22. These derived constants permit comparisons of processes with ligands having different numbers of dissociable protons such as Ala-Gly-Gly-His and Boc-Ala-Gly-Gly-His. Comparison of the above values for log K with values given in Table 2 suggests that, in all cases, the first complex to form involves co-ordination of an imidazole-N donor centre. This is most probably the π-N for all ligands except Ala-Gly-Gly-His(πbom)) when it would have to be the τ-N, since the π-N is protected (although limited chelation may be possible through the oxygen atom of the protecting benzyloxymethyl group). The facile metal-catalysed hydrolysis of Ala-Gly-Gly-His-OMe noted above also supports the suggestion of initial bonding from the imidazole end of the peptide.

2N *Complexes*. The transition to a 2N species is rapid in complexes with all the ligands except Boc-Ala-Gly-Gly-His. The 2N complexes are the CuL species with all the ligands

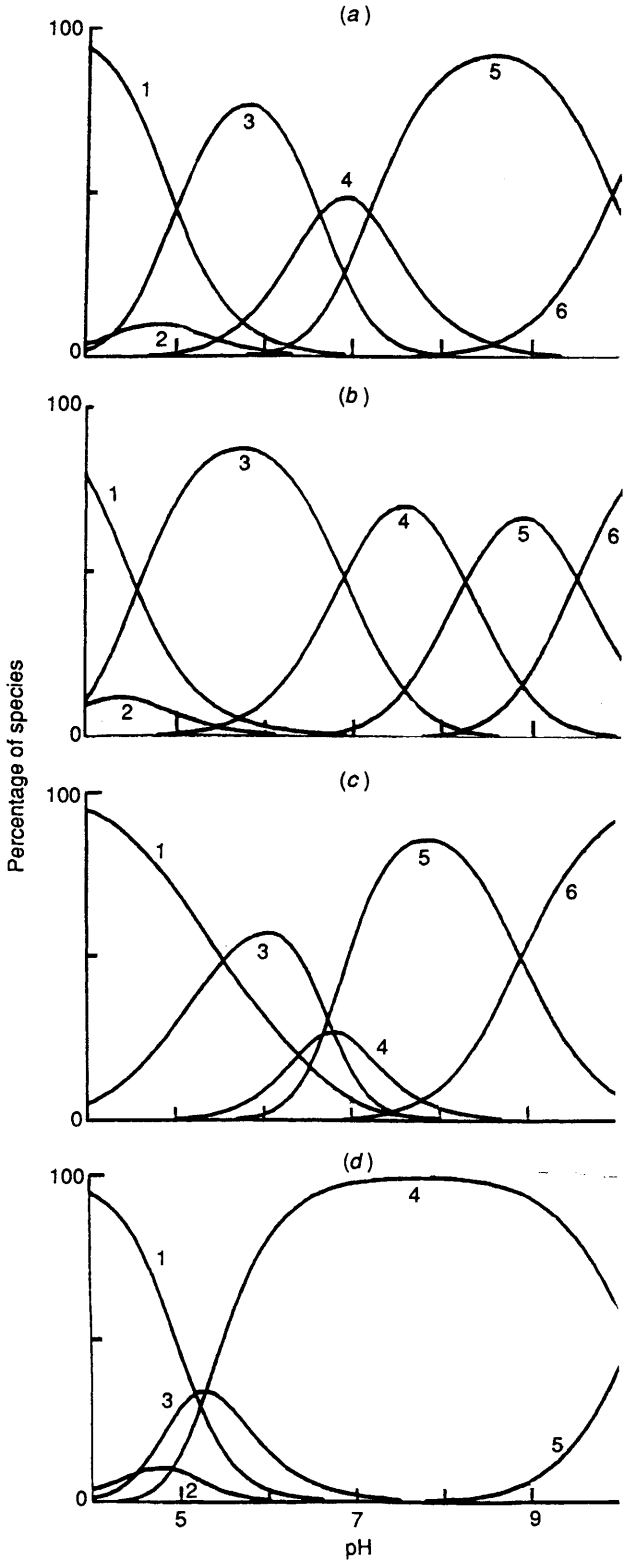


Figure. Species distribution curves for 1:1 mixtures (0.001 mol dm^{-3}) of Cu^{II} with (*a*) Ala-Gly-Gly-His, (*b*) Ala-Gly-Gly-His(πbom), (*c*) Boc-Ala-Gly-Gly-His, and (*d*) Ala-Gly-Pro-His. Species: 1, Cu^{2+}; 2, CuHL; 3, CuL; 4, $CuH_{-1}L$; 5, $CuH_{-2}L$; 6, $CuH_{-3}L$

studied except Boc-Ala-Gly-Gly-His when it is the $CuH_{-1}L$ species. Only three possibilities exist for co-ordination centres in such 2N complexes: N_{im}, N_{amide}; NH_2, N_{amide}, and N_{im}, NH_2. The first possibility can be excluded by examination of the species distribution curves for Boc-Ala-Gly-Gly-His [Figure (*c*)] where the 2N complex, $CuH_{-1}L$, is only a minor species as a result of the significantly lower stability of the required six-membered chelate ring. The other two possibilities differ in that one involves ^{-}N–Cu bonding while the other does not, requiring, instead, the formation of a large, 15-membered, chelate ring. Amide co-ordination can usually be recognised by the ^{-}N–Cu c.t. transition observed in c.d. spectra between 300 and 350 nm. Transitions for 2N complexes in this region were found with Ala-Gly-Gly-His(πbom) only but the absence of such a transition cannot be regarded as a definitive answer, especially because the imidazole N π_1–Cu^{II} transition may occur in the same region and occasionally cancel it by having an opposite sign.

In order to clarify this point, the tetrapeptide Gly-Gly-Gly-His has been synthesised. This peptide possesses only one chiral centre (His) so that binding of Cu^{II} only in the region of this residue will give a measurable Cotton effect in the *d–d* and c.t. regions. As seen from Table 3 the c.d. bands for Ala-Gly-Gly-His and Gly-Gly-Gly-His have similar Δε values in the *d–d* region but they differ distinctly in the c.t. region. This clearly indicates involvement of the His residue in metal-ion binding. The lack of the N–Cu^{II} transition and the fact that there are differences in signs of the Cotton effects at shorter wavelengths, as well as the presence of the NH_2–Cu^{II} c.t. transition at 260 nm in the c.d. spectrum of Gly-Gly-Gly-His, support the conclusion that co-ordination in the 2N species must be through formation of a macrochelate ring spanning the terminal NH_2 and N_{im} donor centres. E.s.r. spectra add additional evidence. The values for $A_{\parallel}$ for 2N copper(II) complexes of Ala-Gly-Gly-His and Ala-Gly-Gly-His-OMe are distinctly lower than for the other species which form typical, small, chelate rings. Such changes in $A_{\parallel}$ may reflect deformation of the complex plane expected when a flexible macrochelate ring is formed. A similar mode of co-ordination for similar complexes was suggested many years ago.[24] The possibility of the formation of polynuclear complexes was considered but this found no support from either the potentiometric or spectroscopic data (see earlier).

It was not possible to detect spectroscopically a 2N complex with Ala-Gly-Pro-His. This was not surprising because the species (CuL) was shown by potentiometry to be only a minor species with a comparatively small range of existence [see Figure (*d*)]. It could not be detected by e.s.r. spectroscopy because of interference from the lines of the $[Cu(H_2O)_4]^{2+}$ and 3N species, always present simultaneously in solution.

3N *and* 4N *complexes.* With Ala-Gly-Gly-His(πbom) the 3N species can exist in two protonation states, $CuH_{-1}L$ and $CuH_{-2}L$, differing in protonation of the non-bonded imidazole τ-N, differences which cannot be detected spectroscopically. The 4N complexes ($CuH_{-3}L$) could generally be detected spectroscopically above pH 9 except with Ala-Gly-Pro-His and Boc-Ala-Gly-Gly-His.

The c.d. spectra for the 3N complexes with both Ala-Gly-Gly-His and Boc-Ala-Gly-Gly-His were very similar, suggesting the same co-ordination. This must involve a rearrangement of the complex with Ala-Gly-Gly-His, with two protons being released from two adjacent amide nitrogens and reprotonation of the amino N, since the reaction takes place about 1 pH unit below the protonation constant for the NH_2 group.

The absence of a 4N complex would be expected when the tetrapeptide contains the Pro residue, with its secondary nitrogen, but is not immediately obvious with Boc-Ala-Gly-Gly-His. With Ala-Gly-Pro-His the bent conformation forced by the Pro residue would induce facile binding of the Ala-Gly peptide N. The resulting structure is comparatively rigid and shows very little conformational strain. In addition, deprotonation of the peptide N adjacent to the imidazole ring is improbable because the resulting six-membered chelate ring

cannot be stabilised by co-ordination of the peptide N of the Pro residue (a 'break-point'[2,3]) as it is with Ala-Gly-Gly-His. With angiotensin–copper(II) complexes there is probably a rearrangement in which the Cu^{II} is transferred from the 3N environment, in which it is anchored to the ^{6}His residue, to co-ordination to the N-terminal amino N in the 4N complex,[25] suggesting that 4N co-ordination starting at the imidazole N is sterically hindered. Such crowding can be readily overcome with Ala-Gly-Gly-His but may be significant with the more bulky Boc-Ala-Gly-Gly-His, so discouraging formation of a 4N complex.

Conclusion

This work has shown that the π-N of the imidazole ring of a His residue is the primary anchoring site for the copper(II) ion in peptides in which it is contained, and that very specific macrochelate rings may be formed between this donor centre and a terminal NH_2 group. In addition, the presence of a Pro residue has a major influence on both the speciation and structures of the complexes formed.

Acknowledgements

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