The L-Proline Residue as a 'Break-point' in Metal–Peptide Systems

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Results are reported of a potentiometric and spectrophotometric study of the H^+ and Cu^{2+} complexes of the tetrapeptides X-Gly-Gly-Gly, Gly-X-Gly-Gly, Gly-Gly-X-Gly, and Gly-Gly-Gly-X where X is the proline (Pro) and sarcosine (Sar) residue (Gly = glycine). All the tetrapeptides (HL) form the series of complexes [CuL], [$CuH_{-1}L$], [$CuH_{-2}L$], and [$CuH_{-3}L$] (charges omitted). The ligands Gly-X-Gly-Gly also form the bis-complex, [CuL_2]. When inserted in a peptide chain the Pro and Sar residues cannot co-ordinate to Cu^{2+} through their peptide nitrogens since they do not possess ionizable protons. In addition the Pro residue tends to force the peptide chain to form a 'β-turn' and so adopt a 'bent' conformation. These studies demonstrate the formation of a large chelate ring when tetrapeptides containing Pro (and, to a smaller extent, Sar) in the second or third positions co-ordinate to Cu^{2+}. This ring spans the terminal residues of the peptide chain and locks the peptide into a 'bent' or 'horse-shoe' shaped conformation. Cu^{2+} could therefore play an important role in activating oligopeptides (*e.g.* neuropeptides) containing proline.

Copper(II) forms stable complexes with simple oligopeptides (HL). Around pH 5, co-ordination is generally through the terminal amine N-donor and the carbonyl oxygen of the first peptide linkage to give an NO-bonded complex $[CuL]^+$. Between pH 5 and 6 and in the presence of Cu^{2+} the hydrogen on the peptide nitrogen atom ionizes with the formation of a Cu–N bond, making the resulting complex (generally formulated as [$CuH_{-1}L$]) a NN-bonded species. As the pH is raised this is followed by successive ionizations of peptide hydrogens and Cu–N co-ordination until, with a tetrapeptide or higher, the $[CuH_{-3}L]^{2-}$ species (NNNN-bonded complex) is formed. With a tripeptide (*e.g.* triglycine) the final species will be a NNN-bonded complex, the fourth co-ordination position being occupied by the carboxylate group which may be displaced at higher pH by a hydroxyl ion formed by hydrolysis of co-ordinated water.[1] Stepwise co-ordination by triglycine is shown in Figure 1(*d*)—(*f*).

Among the common constituents of naturally occurring proteins and peptides, proline (Pro) is unique in having a secondary nitrogen. This can co-ordinate normally when in the N-terminal position but when inserted in the peptide chain it does not possess an ionizable hydrogen, hence it cannot form a typical Cu–N peptide bond. As a result the sections of peptide chain on either side of the inserted Pro will tend to co-ordinate independently to the metal ions. Hence the Pro residue may be described as acting as a 'break-point' in the co-ordination of metal ions able to deprotonate peptide nitrogens (*e.g.* Cu^{2+}).

The L-Pro residue in a peptide chain also influences the conformation of that chain. In a protein the 'β-turns' (regions of protein involving four consecutive residues where the peptide chain folds back on itself by up to 180°) give the globular structure necessary for its activity and Pro is among the most frequently occurring bent residues, particularly when in the second position.[2] The importance of the L-Pro residue in the second position to the formation of bent or folded conformations of oligopeptides (*e.g.* tetrapeptides) has recently been confirmed by ^{13}C n.m.r. studies of tetrapeptides containing L-Pro and L-alanine.[3] As a result, L-Pro in the second position will tend to bring the donor atoms of the N- and C-terminal positions of a tetrapeptide close to each other. In this conformation there is the possibility of the formation of a stable but abnormally large chelate ring bridging the two terminal positions, particularly since the metal ion is unable to co-ordinate to the peptide N atom of the second residue of the chain. Hence the Cu^{2+} ion can stabilise the folded (bent) structure of a small peptide molecule containing L-Pro in the second position. Since this conformation appears to be of critical importance in many biological processes it is likely that Cu^{2+} could have a role in biologically activating many small peptides.

Recently 30 or more peptides have been identified as neurotransmitters, many with opiate activity.[4] Many of these peptides contain one or more Pro residues, *e.g.* 'substance P' (L-Pro in positions 2 and 4 while the antagonist contains D-Pro in position 2), thyrotropin releasing hormone (L-Pro-NH_2 in position 3), and β-casomorphin (L-Pro in positions 2, 4, and 6). Copper is widely distributed throughout the body at low concentrations, the highest concentrations being found in the liver gall-bladder area and the brain, although no role has been reliably attributed to this brain copper. Labile Cu^{II} is generally present in the body as amino-acid or peptide complexes, hence any labile Cu^{II} present would tend to co-ordinate neuropeptide molecules and, possibly, influence their conformations and so their neurological activity.

To investigate the chemical basis for these suggestions we have synthesised the series of tetrapeptides: X-Gly-Gly-Gly, Gly-X-Gly-Gly, Gly-Gly-X-Gly, and Gly-Gly-Gly-X [Gly = glycine, X = L-Pro or sarcosine (Sar) residues]. Sarcosine, $NH(Me)CH_2COOH$ was selected because, like proline, it contains a secondary nitrogen and hence cannot bond to Cu^{II} when inserted into a peptide chain; it should therefore also act as a break-point to co-ordination. However, it does not contain a chiral centre and may have a different effect on the conformation of the peptide chain. The copper complexes of these tetrapeptides have been studied by potentiometric and spectrophotometric techniques and the results have been compared with those for Phe-Pro-Gly-Gly and Gly-Pro-Phe-Gly (Phe = L-phenylalanine).[5]

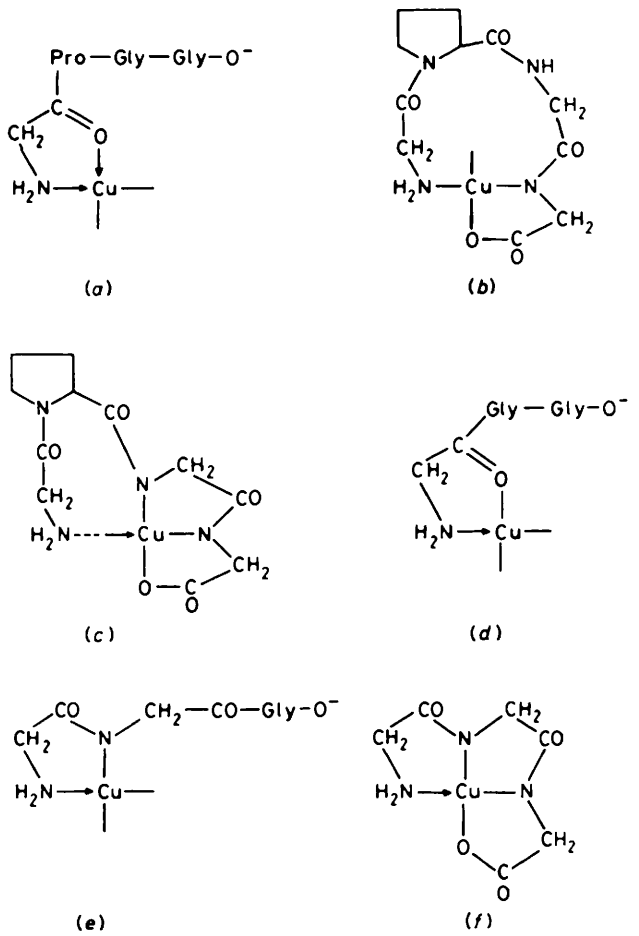
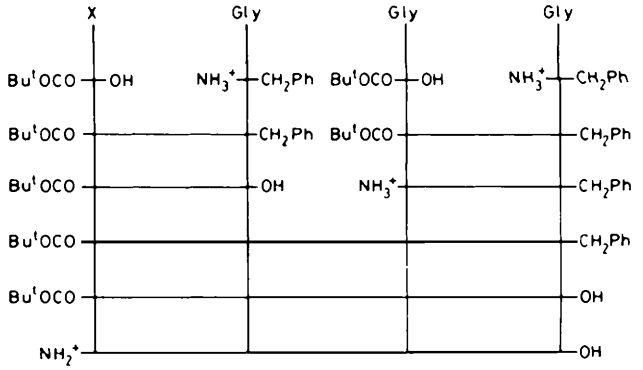


Figure 1. Complexes of Cu^{II} with (*i*) Gly-Pro-Gly-Gly: (*a*) [CuL] species, (*b*) [$CuH_{-1}L$] (note large chelate ring which would be either *cis* or *trans* on the copper ion), (*c*) [$CuH_{-2}L$] (bonding through the amine N is now much less significant); (*ii*) triglycine; (*d*) [CuL] species, (*e*) [$CuH_{-1}L$], (*f*) [$CuH_{-2}L$]



Scheme. The tetrapeptide X-Gly-Gly-Gly; Gly-X-Gly-Gly, Gly-Gly-X-Gly, and Gly-Gly-Gly-X are similar, but with X in the second, third, and fourth positions respectively, starting with $PhCH_2XNH{\cdot}HCl$.

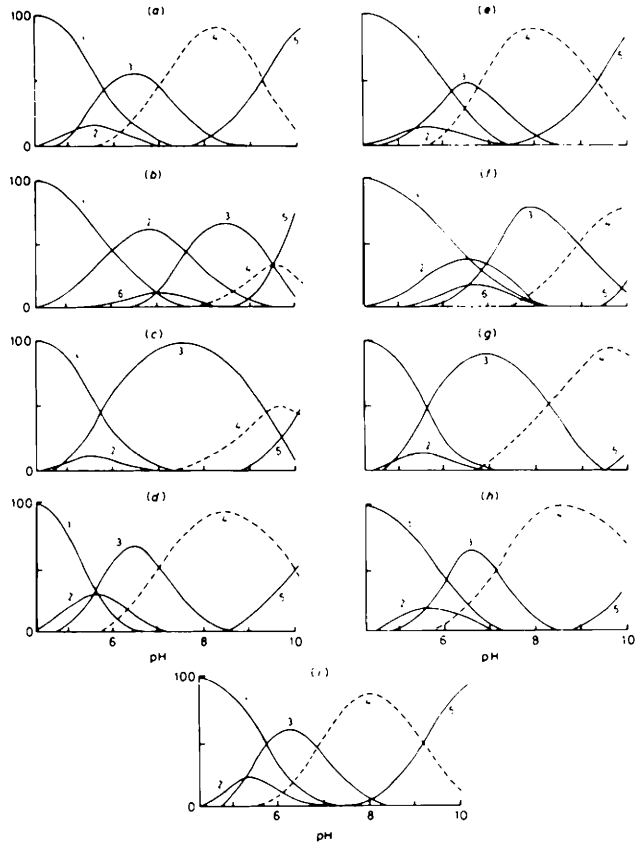


Figure 2. Species distribution curves (%) for the Cu^{2+} complexes of the tetrapeptides in 1:1 solutions of 0.001 mol dm^{-3}: 1, Cu^{2+}; 2, [CuL]; 3, [$CuH_{-1}L$]; 4, [$CuH_{-2}L$] (- - - -); 5, [$CuH_{-3}L$]; 6, [CuL_2]. (*a*) Pro-Gly-Gly-Gly, (*b*) Gly-Pro-Gly-Gly, (*c*) Gly-Gly-Pro-Gly, (*d*) Gly-Gly-Gly-Pro, (*e*) Sar-Gly-Gly-Gly, (*f*) Gly-Sar-Gly-Gly, (*g*) Gly-Gly-Sar-Gly, (*h*) Gly-Gly-Gly-Sar, (*i*) tetraglycine

Experimental

Organic Syntheses.—The tetrapeptides were synthesised by standard liquid-phase methods. The starting materials were Bu'OCO-Gly, Bu'OCO-Sar, Bu'OCO-L-Pro, Gly-CH_2Ph·HCl, L-Pro-CH_2Ph·HCl, and Sar-CH_2Ph·HCl.

The syntheses are outlined in the Scheme (X = L-Pro or Sar). C-Protected derivatives were neutralized with triethylamine before coupling. The coupling reagents were dicyclohexylcarbodi-imide (dcci, Merck) and 1-hydroxybenzotriazole (Aldrich). Benzyl groups were removed by hydrogenolysis using 10% Pd on charcoal as catalyst. The Bu'OCO groups were cleaved using HCl (4 mol dm^{-3}) in dioxane. Tetrapeptides were purified by gel filtration (Sephadex G-15, eluant water) and lyophilised.

Product purity was checked by paper chromatography [Whatman no. 3, eluant (% v/v) water (30), pyridine (35), and butanol (35)]. Percentages of amino-acid residues (Pro:Gly and Pro:Sar = 1:3) were confirmed by amino-acid analyses.

Potentiometric Studies.—Formation constants for complexes with H^+ and Cu^{II} were calculated from titration curves carried out at 25 °C using total volumes of 1.5—2.0 cm^3. Alkali was added from a 0.1-cm^3 Hamilton micrometer syringe which had been calibrated by both weight titrations and by titration of standardized materials. Changes in pH were followed using electrodes calibrated in hydrogen ion concentrations with $HClO_4$. All solutions were of ionic strength 0.10 mol dm^{-3} (KNO_3) with tetrapeptide concentrations of 0.003 mol dm^{-3}. Calculations were made with the aid of the SUPERQUAD computer program. This is a development of the MINIQUAD

Table 1. Proton and Cu^{2+} complex formation constants at 25 °C and I = 0.10 mol dm^{-3} (KNO_3), with estimated standard deviations in parentheses

(*a*) H^+ complexes

Peptide (HL)	log K_{HL}	log β_{H_2L}	log K_{H_2L}	Ref.
Pro-Gly-Gly-Gly	8.67(1)	11.94(1)	3.27	*a*
Gly-Pro-Gly-Gly	8.25(1)	11.36(1)	3.11	*a*
Gly-Gly-Pro-Gly	8.06(1)	11.23(1)	3.17	*a*
Gly-Gly-Gly-Pro	8.04(1)	10.95(1)	2.91	*a*
Pro-Gly	8.98	12.13	3.15	*b*
Gly-Pro	8.55	11.34	2.79	*b*
Sar-Gly-Gly-Gly	8.39(1)	11.721(5)	3.34	*a*
Gly-Sar-Gly-Gly	8.28(1)	11.63(1)	3.35	*a*
Gly-Gly-Sar-Gly	7.97(1)	11.311(4)	3.34	*a*
Gly-Gly-Gly-Sar	7.92(1)	10.967(2)	3.04	*a*
Sar-Gly	8.54	11.62	3.08	*b*
Gly-Sar	8.59	11.37	2.78	*b*
Gly-Sar-Gly	8.23	11.41	3.18	*c*
Gly-Gly-Sar	8.3	11.5	3.2	*c*
Triglycine	7.96	11.09	3.20	1
Tetraglycine	7.97	11.05	3.18	1

(*b*) Cu^{2+} complexes

	log β values for species:					
Peptide (HL)	[CuL]	[CuL_2]	[$CuH_{-1}L$]	[$CuH_{-2}L$]	[$CuH_{-3}L$]	Ref.
Pro-Gly-Gly-Gly	5.59(4)		0.17(1)	−6.78(2)	−16.10(2)	*a*
Gly-Pro-Gly-Gly	5.46(2)	9.61(4)	−2.46(5)	−11.86(5)	−21.34(3)	*a*
Gly-Gly-Pro-Gly	4.95(9)		−0.06(1)	−9.46(3)	−19.47(2)	*a*
Gly-Gly-Gly-Pro	5.16(4)		−0.48(1)	−7.50(2)	−17.58(3)	*a*
Pro-Gly	6.42		2.66			*b*
Gly-Pro	6.50	11.63				*b*
Sar-Gly-Gly-Gly	5.00(5)		−0.54(1)	−7.43(2)	−16.86(3)	*a*
Gly-Sar-Gly-Gly	5.18(9)	9.92(9)	−1.78(6)	−11.02(6)	−21.9(1)	*a*
Gly-Gly-Sar-Gly	5.13(9)		0.20(2)	−9.06(3)	−20.39(6)	*a*
Gly-Gly-Gly-Sar	5.28(2)		−0.22(1)	−7.34(1)	−17.74(2)	*a*
Sar-Gly	5.32		1.36			*b*
Gly-Sar	6.34	11.48				*b*
Gly-Sar-Gly	5.65	10.23				*c*
Gly-Gly-Sar	7.0		1.9	−7.3	−19	*c*
Triglycine	5.24		−0.02	−6.58	−18.5	1
Tetraglycine	5.08		−0.42	−7.31	−16.60	1

(*c*) Stepwise protonation constants of complexes

Tetrapeptide	log K_1'	log K_2'	log K_3'	Tetrapeptide	log K_1'	log K_2'	log K_3'
Pro-Gly-Gly-Gly	5.42	6.95	9.32	Sar-Gly-Gly-Gly	5.54	6.89	9.43
Gly-Pro-Gly-Gly	7.92	9.40	9.48	Gly-Sar-Gly-Gly	6.96	9.24	10.9
Gly-Gly-Pro-Gly	5.01	9.40	10.01	Gly-Gly-Sar-Gly	4.93	9.26	11.33
Gly-Gly-Gly-Pro	5.64	7.02	10.08	Gly-Gly-Gly-Sar	5.50	7.12	10.40
Triglycine	5.22	6.60	11.9				
Tetraglycine	5.50	6.89	9.29				

$K_1' = [CuL]/[CuH_{-1}L][H]$, $K_2' = [CuH_{-1}L]/[CuH_{-2}L][H]$, $K_3' = [CuH_{-2}L]/[CuH_{-3}L][H]$

[a] This work. [b] H. Sigel, *Inorg. Chem.*, 1975, **14**, 1535. [c] W. L. Koltun, R. H. Roth, and F. R. N. Gurd, *J. Biol. Chem.*, 1963, **238**, 124.

program[6] and allows the inclusion of a second ligand and the refinement of the total ligand concentrations. Hence it was possible to confirm the chemical purity of the ligands apart from associated solvent and, in particular, the absence of acetate (a frequent impurity in peptide samples) or other co-ordinating ions. In all cases duplicate or triplicate titrations were performed at Cu:L ratios of 1:1 and 1:2.

Spectroscopic Studies.—Solutions were prepared with the same concentrations as were used in the potentiometric studies. Absorption spectra were recorded on a Beckman UV 5240 spectrophotometer and circular dichroism (c.d.) spectra were measured on an automatic recording spectropolarimeter JASCO-J-20. All c.d. results are expressed in terms of $\Delta\varepsilon$ ($\varepsilon_l - \varepsilon_r$). E.s.r. spectra (at 9.12 GHz) were obtained on a JEOL JES-ME-3X spectrometer at liquid nitrogen temperature.

Results and Discussion

Calculated protonation constants and Cu^{II} complex formation constants are given in Table 1, together with literature values for related complexes. Calculated species distribution curves for 1:1 Cu:L mixtures (0.001 mol dm^{-3}) are shown in Figure 2. Spectroscopic data for the complexes are given in Table 2.

Protonation constants are close to those expected from values for comparable peptides. In particular, log K_{HL} (amine protonation) is high when Pro or Sar is in the first position of the peptide chain as a result of inductive effects, and drops towards the value for tetraglycine as the substituent is moved along the chain. All of the tetrapeptides form the series of complexes [CuL], [$CuH_{-1}L$], [$CuH_{-2}L$], and [$CuH_{-3}L$] (charges omitted). In addition, the tetrapeptides with Pro or Sar in the second position form a bis-complex, [CuL_2].

Table 2. Spectroscopic characterization of Cu^{II} complexes of the tetrapeptides

Tetrapeptide	Species	Visible λ/nm (ε/dm³ mol⁻¹ cm⁻¹)	C.d. λ/nm (Δε/dm³ mol⁻ cm⁻¹)	Assignment[a]	E.s.r. $A_{\|}$/G	E.s.r. $g_{\|}$
Pro-Gly-Gly-Gly	NN, $[CuH_{-1}L]$	655 (50)	655 (+0.18)	*B*	174	2.242
			520 (−0.01)	*E*		
			310 (+0.04)	N^-–Cu		
	NNN, $[CuH_{-2}L]$	585 (115)	585 (+0.20)	*B* + *E*	201	2.203[b]
			310 (+0.06)	N^-–Cu		
	NNNN, $[CuH_{-3}L]$	515 (180)	620 (+0.07)	*A*	211	2.182
			497 (+0.37)	*B* + *E*		
			295 (+0.06)	N^-–Cu		
Gly-Pro-Gly-Gly	N, [CuL]		725 (−0.01)	*B* + *E*	160	2.317
	NN, $[CuL_2]$ or $[CuH_{-1}L]$	655 (45)	715 (+0.01)	*A*	172	2.274
			620 (−0.03)	*B* + *E*		
	NNN, $[CuH_{-2}L]$	560 (80)	590 (+0.17)	*B* + *E*	197	2.210[b]
			290 (+0.18)	N^-–Cu		
Gly-Gly-Pro-Gly	N, [CuL]				155	2.322
	NN, $[CuH_{-1}L]$	640 (95)	665 (−0.17)	*B* + *E*	175	2.270[c]
					170	2.240
	NNN, $[CuH_{-2}L]$	565 (70)	595 (−0.04)	*B* + *E*	190	2.210
			320 (−0.003)	N^-–Cu		
Gly-Gly-Gly-Pro	N, [CuL]				160	2.326
	NN, $[CuH_{-1}L]$	655 (55)			175	2.247
	NNN, $[CuH_{-2}L]$	570 (140)	670 (+0.01)	*A*	194	2.212[b]
			550 (−0.02)	*B* + *E*		
Sar-Gly-Gly-Gly	NN, $[CuH_{-1}L]$	650 (70)			172	2.247
	NNN, $[CuH_{-2}L]$	585 (120)			205	2.211[b]
	NNNN, $[CuH_{-3}L]$	525 (160)			203	2.196[d]
Gly-Sar-Gly-Gly	N, [CuL]				158	2.320
	NN, $[CuL_2]$ or $[CuH_{-1}L]$	670 (45)			176	2.273
	NNN, $[CuH_{-2}L]$	580 (90)			186	2.220[b]
Gly-Gly-Sar-Gly	N, [CuL]				156	2.320
	NN, $[CuH_{-1}L]$	640 (80)			176	2.275[c]
					170	2.240
	NNN, $[CuH_{-2}L]$	570 (100)			195	2.210
Gly-Gly-Gly-Sar	N, [CuL]				159	2.325
	NN, $[CuH_{-1}L]$	660 (55)			166	2.252
	NNN, $[CuH_{-2}L]$	570 (130)			195	2.215[b]

[a] N^-–Cu indicates a charge-transfer band for N→Cu (or N←Cu). [b] Seven lines of superhyperfine structure. [c] Two different e.s.r. spectra are observed for the NN complex, one at pH 5—6, the other at pH 7—9. [d] Nine lines of superhyperfine structure.

Pro-Gly-Gly-Gly *and* Sar-Gly-Gly-Gly.—As would be expected the complexes formed are similar in both stability and range of existence to those with tetraglycine,[7] complexes with Pro-Gly-Gly-Gly being rather more stable as a result of the inductive effect of the methylene groups of the prolyl ring. The similarity is apparent from the values for the stepwise protonation constants given in Table 1, and it can be assumed that co-ordination centres and geometries are the same as those for tetraglycine [*cf.* Figure 1(*d*)—(*f*) for triglycine], *i.e.* –NH and C=O (NO) in [CuL], –NH and peptide N (NN) in $[CuH_{-2}L]$ through to NNNN in $[CuH_{-3}L]$. Absorption, c.d. and e.s.r. spectroscopy identified three different complex species with NN, NNN, and NNNN co-ordination (see Table 2).[8-10] The *d*–*d* and charge-transfer bands in the c.d. spectra were rather weak due to the presence of only one asymmetric carbon in the molecule, *i.e.* in the Pro residue.

Gly-Pro-Gly-Gly *and* Gly-Sar-Gly-Gly.—Complexes formed by these tetrapeptides demonstrate clearly the assumptions which prompted this work. Co-ordination in the [CuL] complexes involves NO-donor centres as in Pro-Gly-Gly-Gly or tetraglycine. Since neither Pro nor Sar in position 2 possesses ionizable peptide protons, complexes similar to the $[CuH_{-1}L]$ species with tetraglycine cannot be formed. However, two alternative co-ordination schemes are possible: (*i*) formation of a bis-complex, $[CuL_2]$, particularly in the presence of excess ligand (*cf.* amino-acid complexes) and (*ii*) formation of a $[CuH_{-1}L]$ species involving ionization and Cu–N bond formation to a peptide nitrogen further along the chain giving a NN complex. This, of necessity, forms an unusually large chelate ring; hence formation of this species is delayed to a significantly higher pH. This gives the [CuL] species, which is normally only a minor species in Cu^{II}-peptide equilibria, a wide pH range of existence as demonstrated in Figure 2. The thermodynamic magnitude of this effect is apparent from a comparison of the values for the first stepwise constant, log K_1' (see Table 1). Once formed this $[CuH_{-1}L]$ species soon loses further protons to form $[CuH_{-2}L]$ and $[CuH_{-3}L]$ complexes. The co-ordination scheme proposed is shown in Figure 1 (*a*)—(*c*), with the scheme for co-ordination to triglycine for comparison, Figure 1(*d*)—(*f*).

Absorption and c.d. spectra show that above pH 6 and with an excess of ligand a NN complex is formed (λ = 655 nm). The low value for Δε observed for this NN species supports the formation of a $[CuL_2]$ with co-ordination through the terminal NH_2 and CO groups with both donors well removed from the asymmetric carbon atoms of the Pro residues.[5,11] The spectroscopic studies confirm that the $[CuH_{-1}L]$ species are NN complexes. However, the peptide nitrogen involved could be from either Gly (2) or the terminal Gly (3). Space filling molecular models clearly favour the nitrogen of Gly (3), resulting in a very large chelate ring of 11 atoms as shown in

Figure 1 (*b*). This structure will be further stabilized by carboxylate co-ordination. Formation of a large chelate ring spanning the two terminal residues of the tetrapeptide is supported by a study of the c.d. spectra of Phe-Pro-Gly-Gly, Gly-Pro-Phe-Gly, and Gly-Pro-Gly-Phe[5] and analogues containing the tyrosyl residue.[11] The magnitude of $\Delta\varepsilon$ ($\varepsilon_l - \varepsilon_r$) for the bands resulting from Cu–N interaction gives an indication whether the Cu atom is bonded to a nitrogen attached to a chiral centre (*e.g.* in Phe) or not as in Gly.[12] The spectra for the above tetrapeptides support the co-ordination scheme shown in Figure 1 (*b*) and (*c*), *e.g.* $\Delta\varepsilon$ is small for the $[CuH_{-1}L]$ complex of Gly-Pro-Phe-Gly suggesting co-ordination through the Gly nitrogens only, not the Phe nitrogen.

Spectroscopic studies suggest that the $[CuH_{-2}L]$ complex is a NNN species (see Table 2). The high-energy *d–d* band (λ = 560 nm) and $A_{\parallel}$ and $g_{\parallel}$ parameters are typical of NNN Cu^{II} complexes.[8–10] In this complex the Cu^{II} ion is bound to NH_2, N^- [Gly (2)], N^- [Gly (3)], and COO^- donor centres. The formation of a macro-chelate which includes the Pro residue would cause the distinct increase in the value for $\Delta\varepsilon$ (+0.17 at 590 nm). From the potentiometric data for Gly-Pro-Gly-Gly the stability of the $[CuH_{-2}L]$ species relative to that of the $[CuH_{-3}]$ species (as measured by $\log K_3' - \log K_2'$) is less than expected, $[CuH_{-2}L]$ being only a comparatively minor species in the equilibrium (see Figure 2). Space-filling models demonstrate the steric problems in forming a NNN complex as a result of the influence of the prolyl ring. When Pro is replaced by Sar these steric constraints are much smaller, making NNN co-ordination more stable (as demonstrated by the larger value for $\log K_3' - \log K_2'$).

Co-ordination in the $[CuH_{-3}L]$ complexes is presumably similar to that found in the $[CuH_{-2}L]$ species with the addition of an hydroxyl ion formed by hydrolysis of a co-ordinated water molecule (*cf.* triglycine).

Gly-Gly-Pro-Gly *and* Gly-Gly-Sar-Gly.—The trends found in tetrapeptides substituted in the second position are also found with these ligands but now the different behaviour is found in the $[CuH_{-2}L]$ species. The [CuL] and $[CuH_{-1}L]$ complexes resemble those of other peptides being NO- and NN-bonded respectively [Figure 1(*d*) and (*e*)]. Now, however, a $[CuH_{-2}L]$ complex cannot be formed without introducing a large chelate ring spanning the Pro or Sar residue. Hence the range of existence of the $[CuH_{-1}L]$ complex is extended considerably (see Figure 2) and the value for $\log K_2' - \log K_1'$ is abnormally large (see Table 1). Again steric constraints in Gly-Gly-Pro-Gly hinder formation of a NNN complex as demonstrated by the small size of $\log K_3' - \log K_2'$. These constraints are again much less in the Sar analogue. The $[CuH_{-3}L]$ complex will again be similar to the $[CuH_{-2}L]$ species with the addition of a co-ordinated hydroxyl ion.

Gly-Gly-Gly-Pro *and* Gly-Gly-Gly-Sar.—Complexes formed by these tetrapeptides resemble closely those formed by triglycine, rather than tetraglycine since they only possess two ionizable peptide protons. Hence the complexes [CuL], $[CuH_{-1}L]$, and $[CuH_{-2}L]$ form with expected stabilities. Spectroscopic studies confirm that these are NO-, NN-, and NNN-bonded species respectively. The final proton to ionize to form $[CuH_{-3}L]$ cannot be from the tetrapeptide molecules and must be from a co-ordinated water molecule. The resulting complex will therefore be a NNN complex also. This is confirmed by the spectroscopic results and by the small values for $\log K_3'$. These are significantly smaller than those for tetraglycine (which has an additional ionizable peptide proton) and closer to that for triglycine.

Conclusions

The results presented confirm the ability of the Pro residue to act as a break-point to Cu^{II} co-ordination when inserted into the second or third position of an oligopeptide chain. As a result, chelated complexes are formed which have unexpectedly large chelate rings and which bridge the two ends of a tetrapeptide chain to lock the peptide into a 'bent' or 'horse-shoe' shaped conformation. Complexation with Cu^{II} will therefore clearly influence the biological activity of oligopeptides containing the proline residue. In some ways the sarcosine residue behaves similarly but its influence on the conformation of the chain is much less significant. Hence the overall effect is less dramatic.

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