Complex Formation by Peptides containing More than One Prolyl Residue; Some Unexpected Trends with Biological Implications

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Pentapeptides of general formula X-Pro-X-Pro-X are unable to co-ordinate to Cu^{II} through any of the peptide-nitrogen atoms while those of formula X-Pro-Pro-X-X form stable, chelated complexes with 2N co-ordination and a large chelate ring; however with X-Pro-Lys-Pro-X (Substance P_{1-5}) and Substance P itself the ε-amino-N of the Lys residue participates in co-ordination, forming a large, stable chelate ring.

Pentapeptides of general formula X-Pro-X-Pro-X and X-Pro-Pro-X-X co-ordinate to Cu^{II} in unexpectedly different ways which could have implications for the co-ordination chemistry of many bio-peptides. The ability of a single prolyl residue to act as a 'break point' to metal–peptide co-ordination has been shown.[1,2] When the prolyl residue is inserted into the peptide chain in positions other than the N-terminal, there is no ionisable proton in the peptide bond since it contains a secondary nitrogen, hence formation of a metal–amide nitrogen bond (*e.g.*, Cu–N^-) is impossible. As well as breaking the co-ordination sequence followed by simple oligopeptides, the prolyl residue encourages the formation of a β-turn structure, thereby stabilising a bent conformation for the peptide chain. This leads to the stabilisation of species co-ordinated through either amide nitrogens of residues beyond the prolyl residue or through side chain donor centres, resulting in the formation of either large chelate rings or dimeric complexes.[3,4]

Many biologically active peptides contain more than one prolyl residue among the first five residues of their sequence. These fall into two main categories, (i) those with the N-terminal sequence X-Pro-X-Pro-X, where the two prolyl residues are in the 2,4 positions, such as substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) and β-casomorphin (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) and (ii) those with the sequence X-Pro-Pro-X-X where the prolyl residues are incorporated into the peptide chain in neighbouring 2,3 positions, *e.g.*, bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg).

In order to study the effect on co-ordination of two prolyl residues in the 2,4 and 2,3 positions we have synthesised seven model pentapeptides containing either the X-Pro-X-Pro or the X-Pro-Pro-X fragment, including Arg-Pro-Lys-Pro-Gln which is Substance P_{1-5} and have studied their complexes with H^+ and Cu^{2+}, together with those formed by Substance P itself. Techniques used were pH-metric titrations and UV-VIS, ESR, and circular dichroism (CD) spectroscopies. A representative selection of the results is shown in Table 1 and species distribution curves in Figure 1. Exciting new binding modes have been identified, including binding of the ε-amino function of the lysyl residue in Substance P_{1-5} and in Substance P, and chelation through the peptide-nitrogen of a glycyl residue in the fourth position when it is preceded by the Pro-Pro sub-unit.

The pentapeptide Gly-Pro-Gly-Pro-Gly does not undergo Cu^{II} promoted ionisation of any peptide nitrogens. Co-ordination is initiated at the amino-terminal nitrogen, supported by chelation through the neighbouring carbonyl oxygen, to give

Table 1. Stability constants for H^+ and Cu^{2+} complexes at 25 °C and I = 0.10 mol dm^{-3} (KNO_3).

	Log β values					
	β_{HL}	β_{H_2L}	β_{CuHL}	β_{CuL}	β_{CuL_2}	$\beta_{CuH_{-1}L}$
Gly-Pro-Gly-Pro-Gly	8.36(1)	11.67(1)	—	5.88(2)	10.60(2)	
Gly-Pro-Lys-Pro-Gly	10.24(1)	18.36(1)	15.45(1)	8.99(1)	—	
Arg-Pro-Lys-Pro-Gln[a]	9.85(1)	17.12(1)	14.30(4)	7.84(2)	—	
Substance P[a]	10.06(1)	17.28(1)	14.35(9)	7.73(3)	—	
Gly-Pro-Pro-Gly-Gly	8.30(1)	11.58(1)	—	5.38(3)	—	−1.32(3)

Spectroscopic data for 2N-copper(II) complexes.

			ESR	
Species	VIS $\lambda_{max.}$/nm[b]	CD $\lambda_{max.}$/nm[c]	$A_{\parallel}$/ $cm^{-1} \times 10^4$	$g_{\perp}$
Gly-Pro-Gly-Pro-Gly(CuL_2)	695(50)	718(−0.104)[d] 260(+0.04)[f]	159	2.277
Gly-Pro-Lys-Pro-Gly(CuL)	662(56)	722(−0.147)[d] 258(+0.414)[f]	165	2.269
Arg-Pro-Lys-Pro-Gln(CuL)		715(−0.14)[d] 270(−0.13)[f]	177	2.270
Gly-Pro-Pro-Gly-Gly($CuH_{-1}L$)	672(55)	645(+0.16)[d] 315(−0.18)[e] 270(+0.20)[f]	157	2.276

[a] Deprotonation of the Arg side chain does not take place under the conditions used. [b] Approximate extinction coefficients in parentheses (ε/mol^{-1} dm^3 cm^{-1}). [c] Δε in parentheses (mol^{-1} dm^3 cm^{-1}). [d] d–d Transition. [e] N–Cu Charge-transfer transition. [f] NH_2–Cu Charge-transfer transition.

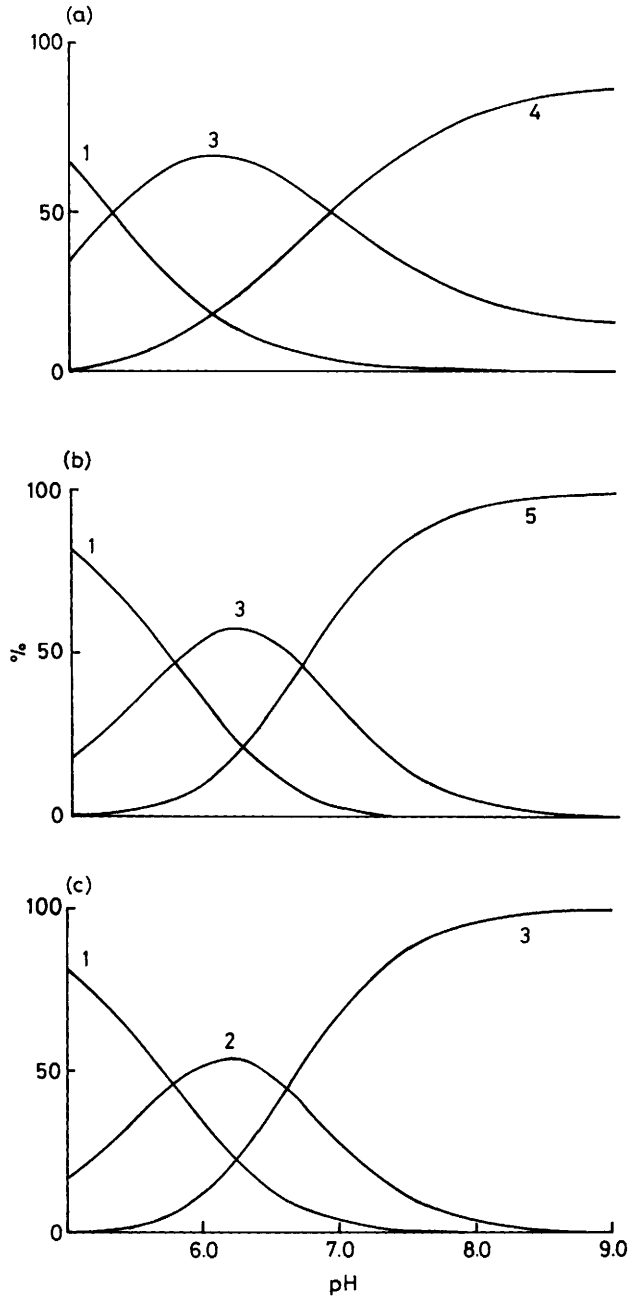

Figure 1. Species distribution curves for complexes of 1 : 2 mixtures of Cu^{II} (0.001 mol dm^{-3}) with (a) Gly-Pro-Gly-Pro-Gly, (b) Gly-Pro-Pro-Gly-Gly, (c) Substance P. 1 = Cu^{2+}, 2 = CuHL, 3 = CuL, 4 = CuL_2, 5 = $CuH_{-1}L$.

[CuL] but the presence of a prolyl residue in the second position places the glycyl residue in the third position in a conformation unfavourable for 2N chelation to copper.[1] The prolyl residue in the fourth position makes chelation to this peptide-N impossible and bidentate co-ordination to the peptide-N of the fifth residue is either conformationally unfavourable or the chelate ring required is too large. Hence only the simple complexes [CuL] and [CuL_2] could be identified. This confirms earlier results which have suggested that 2N chelation with X-Pro-X-X is through the first and fourth nitrogen donors, not the first and third.[1,5]

When the third residue is lysyl (*i.e.*, in pentapeptides containing the Pro-Lys-Pro sub-unit, including Substance P), co-ordination with copper(II) is initiated as before to give the 1N complex [CuHL] with the amino-group protonated. However, on increasing the pH, a [CuL] complex is formed and spectroscopic results confirm that this is a 2N species which has no Cu–peptide-N co-ordination. It must therefore involve the formation of a large chelate ring spanning the α- and ε-amino-N donors. Assuming 2N co-ordination only this chelate ring would be 14 membered but if additional co-ordination through the carbonyl oxygen of the first peptide residue is assumed it would be 13 membered. This complex forms around pH 6 (Figure 1), well below the normal ionisation pH for an ε-amino group of a lysyl residue (about pH 10) and deprotonation and chelate formation must be promoted by the prolyl residue in the second position forcing the peptide into a favourable bent conformation. Species distribution curves for complexes of Cu^{II} with Substance P_{1-5} were virtually identical to those with Substance P. Under normal circumstances the ε-terminal nitrogen of a lysyl residue in a peptide does not co-ordinate to Cu^{II} [7] although there is evidence for such binding in the solid state.[8] As a result of this 2N co-ordination, bis-complexes could not be detected. Additional supporting evidence for co-ordination of the lateral Lys-amino-N comes from the ESR spectra for the 2N complexes which show a small but systematic trend with complexes of ligands containing the Lys^3 residue having slightly larger values for $A_{\|}$ and marginally smaller values for $g_{\|}$ than the others. This suggests a more symmetrical surrounding to the metal ion which could be a result of the high flexibility of the long Lys side chain.

Introduction of the Pro-Pro sub-unit into the second and third positions led to the formation of 2N complexes with charge-transfer CD bands in the 315—325 nm region, characteristic of co-ordination through a peptide-N donor. The peptide nitrogens of the fourth and fifth residues are the only ones which are able to co-ordinate and such chelation would form 10 or 13 membered chelate rings respectively (assuming additional co-ordination through the carbonyl oxygen of the N-terminal glycyl residue). The stability of the resulting large ring is unexpectedly high, being only about an order of magnitude less than that for the [$CuH_{-1}L$] complex with tetraglycine ($\log \beta = -0.4$)[6] which forms a stable 5 membered chelate ring. It is, in turn, more stable than the corresponding complex with Gly-Pro-Gly-Gly ($\log \beta = -2.5$)[1] by an order of magnitude and is, in fact, the major complex above pH 6.5 (Figure 1). Hence the presence of two prolyl residues must hold the peptide chain in a sufficiently bent conformation to encourage formation of a very large, stable chelate ring.

Received, 23rd October 1989; Com. 9/04546E

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