The L-Proline Residue as a 'Break-point' in the Co-ordination of Metal–Peptide Systems

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The L-proline residue in oligopeptides has been shown to act as a 'break-point' in their co-ordination to copper(II) ions leading to the formation of large chelate rings with the peptide locked in the β-conformation.

Study of molecular models of oligopeptides demonstrates clearly that an L-proline (Pro) and sarcosine (Sar) residue inserted in a peptide sequence may act as a 'break-point' in the co-ordination of donor centres of the peptide chain to metal ions which are able to deprotonate peptide nitrogens (*e.g.* Cu^{2+}). As a result the sections of peptide chain on either side of the inserted residue will tend to co-ordinate independently to the metal ion. On the other hand, L-Pro, when it is the second residue of the chain induces a bent or folded structure for the oligopeptide, and when it is the third residue an unfolded structure is favoured.[1] If the chain length is short (*e.g.* a tetrapeptide) a proline in the second position will bring the donor atoms of the C and N terminals close to each other. In this conformation there is the possibility of the formation of a stable but abnormally large chelate ring. Hence the metal ion could stabilise the folded (bent) structure of a small peptide molecule, a conformation which is often of critical importance in many biological processes.[2]

To test the above predictions and to provide a quantitative measure of the magnitude of the effect we have synthesised the series of tetrapeptides (HL): X-Gly-Gly-Gly, Gly-X-Gly-Gly, Gly-Gly-X-Gly, and Gly-Gly-Gly-X, where X = L-Pro and Sar and studied their complexes with Cu^{2+} using spectroscopic (c.d., absorption, and e.s.r.) and potentiometric techniques. From the spectroscopic studies we were able to identify the bonding centres used (*e.g.* one, two, or three N) as a function of pH. Complex formation constants for each species were calculated from the potentiometric studies and species distribution curves were calculated. Excellent agreement was found between these curves and the pH ranges for each species identified in the spectroscopic studies.

The results are shown diagramatically in Figure 1, together with suggested structures for the $CuH_{-3}L$ species. Figure 1 shows the pH ranges over which the species identified are the major species in a 1:1 copper:ligand mixture. Values for complexes of tetraglycine (4Gly) are included for comparison. From these it is apparant that tetrapeptides with L-Pro and Sar in the first and fourth positions (Pro^1, Sar^1 and Pro^4, Sar^4) behave very similarly to tetraglycine. The only significant difference is found in the $CuH_{-3}L$ species for the peptides substituted in the fourth position. This species must be an NNNO complex, the last proton ionization being the hydrolysis of a co-ordinated water molecule, rather than an NNNN complex since the C-terminal peptide nitrogen is now the

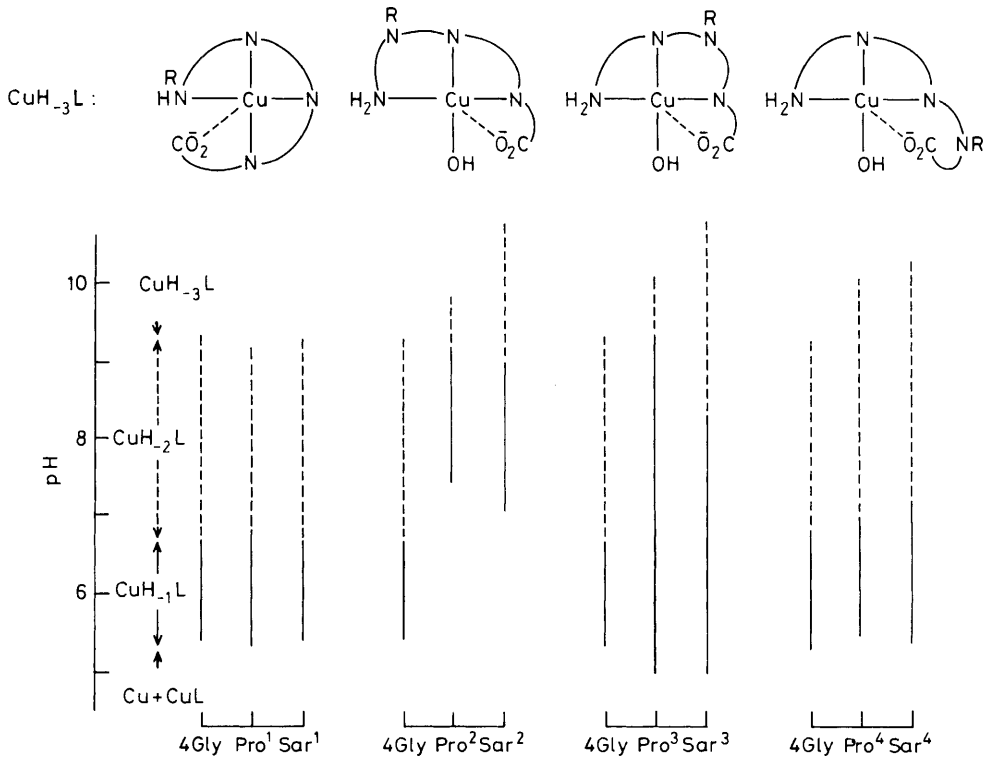

Figure 1. The pH ranges of the major species in 1:1 tetrapeptide:copper solutions [tetrapeptide (HL) = Gly-Gly-Gly-Gly, Pro(Sar)-Gly-Gly-Gly, Gly-Pro(Sar)-Gly-Gly, Gly-Gly-Pro(Sar)-Gly, Gly-Gly-Gly-Pro(Sar)].

'break-point' and *cannot* co-ordinate. Hence its stability is markedly lower than that of the complex with tetraglycine and nearer to that with triglycine.

When one of the 'break-point' residues is in the second *position* (Pro^2, Sar^2), both tetrapeptides form bis complexes [CuL_2, *cf.* amino acid bis complexes]. While these species are of only minor importance in 1 : 1 ligand : copper mixtures, they become major species in the presence of excess ligand. The formation of the $CuH_{-1}L$ species (two N) is delayed until pH >7 but, once formed, the extent of their ranges of existence is comparable to that of the tetraglycine analogue (see Figure 1).

With L-Pro or Sar as the third residue (Pro^3, Sar^3) a comparable situation is found but now the $CuH_{-2}L$ species are destabilized by the presence of the 'break-point.' The $CuH_{-1}L$ species are comparable in stability to the species with tetraglycine but formation of the $CuH_{-2}L$ species is delayed by about two pH units. Once formed the $CuH_{-2}L$ complexes lose a further proton at about the pH expected for the hydrolysis of co-ordinated water to give the $CuH_{-3}L$ species. A difference apparant from Figure 1 is the lower stability of the $CuH_{-3}L$ species of tetrapeptides with sarcosine in the second or third positions compared to the proline analogues.

From these results it is clear that large chelate rings can form around both proline and sarcosine 'break-points' in copper complexes with oligopeptides. The species with these large rings are less stable than normal complexes but, with 'break-points' in the second position of the peptide sequence, they form in the physiologically important pH range of pH 7—8 to stabilize the β-conformation.

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