Model Compounds for Metal–Protein Interaction: The Crystal Structure of the Copper(II) Complex of β-Alanyl-L-histidine (Carnosine)

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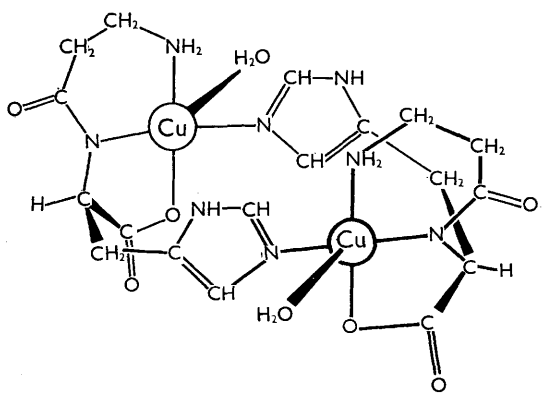
(β-ALANYL-L-HISTIDINATO)COPPER(II) DIHYDRATE was prepared by adding an excess of freshly precipitated and washed copper(II) hydroxide to a solution of peptide (0·1M) containing sufficient alkali so that the final pH was 10—11. After filtration, deep blue crystals separated on slow evaporation.

The crystals are trigonal, space group $P3_12$, with $a = 8{\cdot}641 \pm 0{\cdot}009$, $c = 30{\cdot}576 \pm 0{\cdot}015$ Å, $D_m = 1{\cdot}65$, $D_x = 1{\cdot}63$ g.cm^{-3}, $Z = 6$ for $C_9H_{12}O_3N_4Cu,2H_2O$. The structure was solved using three-dimensional Patterson, heavy-atom Fourier, and Mathieson[1] "product" syntheses. For the 1319 independent reflexions used in the anisotropic full-matrix least-squares refinement, the final reliability factor R was 0·10. The structure is shown in the Figure.



FIGURE

The complex is a dimer, in which each copper atom has co-ordination number 5. The four closest ligand atoms are the terminal amino-nitrogen, peptide nitrogen, and carboxyl oxygen of one peptide molecule, and the 3-nitrogen of the imidazole ring of the second peptide molecule of the dimer. It follows that each dipeptide is bonded to two different copper atoms. The fifth ligand on each copper atom, completing a square-pyramidal environment, is a water molecule. The dimers are linked by a hydrogen-bond network involving a second water molecule per formula unit.

The atoms of the –N–C–C–CO– β-alanyl chelate ring are disordered in the crystal. They all have abnormally high thermal vibrational parameters perpendicular to the ring, showing that the positional co-ordinates found from the least-squares refinement merely represent the averages of several energetically equivalent conformations. This disorder reduces the precision with which the rest of the structure may be determined.

The copper–ligand bond lengths are: Cu–NH_2, 1·96; Cu–N(peptide), 1·95; Cu–O(carboxyl), 1·93; Cu–N(imidazole), 2·01; Cu–OH_2, 2·48 Å. The four closest ligands deviate by average distances of 0·23 Å from their plane of best fit, so that the co-ordination square is distorted in the sense of a tetrahedron. The copper atom is displaced 0·13 Å from the same plane in the direction of the co-ordinated water molecule. The copper atom also lies 0·19 Å out of the plane of the imidazole ring.

The chemical significance of this structure analysis is that:

(1) The imidazole ring of the histidine side-chain in the peptide binds copper(II) at N-3 and not at N-1 [in contrast with the binding of cobalt(II), nickel(II), zinc(II), and cadmium(II) by N-1 in histidine itself[2]];

[1] A. McL. Mathieson, *Acta Cryst.*, 1962, **15**, 1065.
[2] K. A. Fraser, H. A. Long, R. Candlin, and M. M. Harding, *Chem. Comm.*, 1965, 344.

(2) no dissociation of the imidazole proton on N-1 has taken place under the conditions of complex formation, N-1 being joined to a neighbouring carbonyl oxygen atom by a hydrogen bond in which it must be the hydrogen donor;

(3) the imidazole ring is bonded to a copper atom to which the rest of its peptide molecule is *not* chelated, establishing a significant point of analogy with the copper-binding site in myoglobin;[3,4]

(4) the carboxyl group of carnosine is a metal-binding site, which was not predicted from pH-titration studies of solutions[5,6] and supposedly disproved by the infrared spectrum of the solid complex;[7] and

(5) the crystallisation of the complex as a dimer suggests, especially in conjunction with the similar behaviour of the copper(II)–glycylglycylglycine complex,[8] that the interaction of one peptide molecule with two copper atoms in solution may be more common than has been realised.

The solution behaviour of carnosine is similar to that of glycyl-L-histidine, whose copper(II) complex has been crystallised in this laboratory.[9] The blue crystals are tetragonal, space-group $P4_12_12$, $a = 11{\cdot}21$, $c = 17{\cdot}82$ Å, $D_m = 1{\cdot}73$, $D_x = 1{\cdot}73$ g.cm.$^{-3}$, $Z = 8$ for $C_8H_{10}O_3N_4Cu,H_2O$. Three-dimensional data have been recorded and a structure analysis is in progress.

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[6] R. B. Martin and J. T. Edsall, *J. Amer. Chem. Soc.*, 1960, **82**, 1107; R. B. Martin, *ibid.*, p. 6053.
[7] A. Lukton and A. Sisti, *J. Org. Chem.*, 1961, **26**, 617.
[8] H. C. Freeman, J. C. Schoone, and J. G. Sime, *Acta Cryst.*, 1965, **18**, 381.
[9] K. A. Fraser, H. C. Freeman, J. T. Szymanski, and C.-h. Wang, unpublished work.