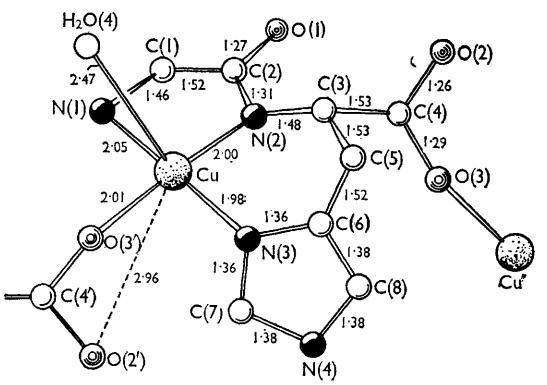
Model Compounds for Metal–Protein Interaction. The Crystal Structure of the Copper(II) Complex of Glycyl-L-histidine

By J. F. BLOUNT, K. A. FRASER, H. C. FREEMAN, J. T. SZYMANSKI, and C.-H. WANG,
(*School of Chemistry, University of Sydney, Sydney, Australia*)
and F. R. N. GURD
(*Indiana University Medical Center, Indianapolis, Indiana, U.S.A.*)

CRYSTALS of (glycyl-L-histidinato)copper(II) sesquihydrate are tetragonal, space-group $P4_32_12$, with $a = 11{\cdot}27$, $c = 17{\cdot}92$ Å, $D_m = 1{\cdot}72\ (\pm 0{\cdot}02)$ g.cm.$^{-3}$, $Z = 8$, $D_x = 1{\cdot}755$ for $C_8H_{10}O_3N_4Cu,1{\cdot}5\ H_2O$. To prepare the complex, freshly precipitated and washed copper(II) hydroxide (1·5 equivalents) was added to a solution of glycyl-L-histidine hydrochloride in slightly more than one equivalent of 0·2M-sodium hydroxide. The resultant violet solution was filtered and carefully titrated with 0·15M-hydrochloric acid until the colour was blue (pH 6·5). Blue crystals separated overnight.

The structure was solved by a single heavy-atom Fourier synthesis in space-group $P4_12_12$ but comparison with the known configuration of L-histidine[1] showed that the enantiomorphous space-group $P4_32_12$ was correct. After three cycles of isotropic and three cycles of anisotropic full-matrix least-squares refinement, the reliability factor R is 0·086 for the 1340 observed independent reflexions. The structure is shown in the Figure.



FIGURE

In the crystal, each dipeptide binds one copper atom at its amino-, peptide-, and imidazole-nitrogen atoms N(1), N(2), and N(3), and a second copper atom at the carboxyl oxygen atom O(3). The copper atom is therefore surrounded by a square of four donor atoms belonging to two different peptide molecules related by a two-fold screw-axis. These four donor atoms are not truly co-planar but deviate from their plane of best fit by −0·13, 0·13, −0·12, and 0·12 Å, respectively. As in several other copper(II)–peptide complexes,[2,3] they thus lie at the corners of a considerably flattened tetrahedron. With respect to the same plane, the copper atom is displaced 0·17 Å in the direction of the oxygen atom O(4) of a water molecule, which is the fifth ligand in the square-pyramidal co-ordination. The oxygen atom O(2′) of the co-ordinated carboxyl group also interacts weakly with the copper atom, the line Cu · · · O(2′) lying 50° from the direction expected for the sixth bond in an octahedral complex. A second water molecule lies on a two-fold axis; it forms only two hydrogen bonds with carbonyl oxygen atoms.

The peptide group C(1)C(2)O(1)N(2)C(3), the carboxyl group C(3)C(4)O(2)O(3) and the imidazole group C(5)C(6)N(3)C(7)N(4)C(8) are all planar. The copper atom lies 0·07, 0·04, and 0·46 Å out of their respective planes. Not only is the N(3)–Cu bond thus bent about 14° out of the plane of the imidazole ring, but the ring itself is also rotated about N(3)–Cu until its normal makes an angle of 33° with the normal to the co-ordination square.

There are important differences between the present copper(II) complex of glycyl-L-histidine and that of β-alanyl-L-histidine (carnosine).[3] The copper atom is bound at the 1-nitrogen and not, as in the latter, at the 3-nitrogen of the imidazole ring. The imidazole group is, and the carboxyl group is not, involved in chelate-ring formation. The present complex is not a dimer; and—in contrast with that of the copper(II)–carnosine complex—it has a crystal structure which is in complete agreement with the structure predicted from potentiometric titrations of the peptide in the presence and absence of copper(II) ions.[4,5] In the case of glycyl-L-histidine, the structure analysis supports the conclusions drawn from the solution data: that the initial metal-binding site is the 1-nitrogen of the imidazole ring; that at pH 6—8 the predominant species in solution has copper(II) co-ordinated with three nitrogen atoms belonging to the imidazole, peptide, and amino-groups, respectively; and that at this pH the imidazole proton is not dissociated

[since N(4) participates in a hydrogen bond to a carbonyl oxygen in which it must be the hydrogen donor]. The Cu–carboxyl interaction is presumably replaced in solution by a bond to a water molecule.

Finally, the structure of (glycyl-L-histidinato)-copper(II) hydrate explains why imidazole side-chains in peptides apparently form chelate rings with peptide groups only on the NH_2-terminal side.[5] If the peptide chain in the present complex were continued on the CO_2^--terminal side, then the next peptide nitrogen would occupy a position similar to that of O(3). Neither a rotation about C(3)–C(4) nor any reasonable distortion could bring this atom into a chelating position with respect to the central copper atom, since the peptide group characteristically remains planar [*i.e.*, N(2) has a trigonal and not a tetrahedral configuration] in metal complexes.[6] A peptide group on the CO_2^--terminal side of a histidine residue can therefore participate in chelation only if the imidazole ring binds a *different* copper atom. That the imidazole group can behave in this way has already been established from the structure of the copper(II)–carnosine complex.[3]

(*Received, December 6th*, 1965; *Com.* 756.)

[1] W. Langenbeck, *Ber.*, 1925, **58**, 227.
[2] H. C. Freeman, G. Robinson, and J. C. Schoone, *Acta Cryst.*, 1964, **17**, 719; H. C. Freeman and M. R. Taylor, *Acta Cryst.*, 1965, **18**, 939.
[3] H. C. Freeman and J. T. Szymanski, *Chem. Comm.*, 1965, 598.
[4] R. B. Martin and J. T. Edsall, *J. Amer. Chem. Soc.*, 1960, **82**, 1107.
[5] G. F. Bryce, R. W. Roeske, and F. R. N. Gurd, *J. Biol. Chem.*, 1965, **240**, 3837.
[6] H. C. Freeman, in "The Biochemistry of Copper", Proceedings of an International Symposium held at Harriman, N.Y. on Sept. 8—10, 1965, Academic Press, N.Y., 1966, in the press.