Models of Copper–Protein Interaction: Copper(II) Complexes of Glycyl-L-histidylglycine in Solution and in the Crystalline State

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Summary *X*-Ray structure analysis shows that crystals of copper(II) and glycylhistidylglycine (HA) consist of a three-dimensional network which contains 40% disordered water located in channels limited by rings of six dimers, $(Cu_2H_{-2}A_2)_6$; and, as shown by e.m.f. data and small angle *X*-ray scattering, a very similar species exists in solution.

THE importance of histidyl residues as metal ion binding sites in proteins prompted us to carry out this study on copper–protein interaction. Glycylhistidylglycine (HA) was chosen as a model, since it is the smallest peptide which has a histidyl residue within the chain.

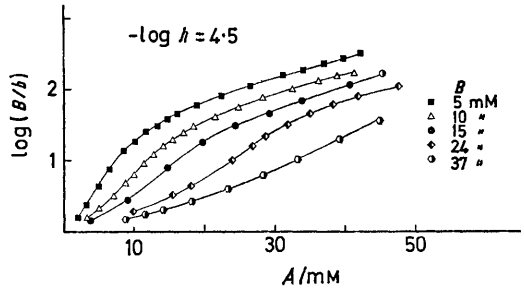

FIGURE 1. *Copper(II) complexes of glycylhistidylglycine in* 3·0M $(Na)ClO_4$ *at* 25°; *data* $(\log B/b,A)_{h,B}$. *The symbols are experimental points and the curves have been calculated assuming the equilibria described in the text.*

High precision pH-static e.m.f. titrations† were carried out on solutions of copper(II) and peptide; an example of the data is shown in Figure 1. By measuring at constant pH, we reduced the usual set of four variables $(B/b, A, B, h)$ to a set of only three variables $(B/b, b, A)_h$. Then for each level of pH it was possible to determine p and r, the number of Cu^{2+} ions and ligand molecules forming the main species, Cu_pL_r, without having to consider the number of protons bound (L is the ligand including all the protonated forms that may enter the complexes).

When the data were analysed by a set of graphical methods (*cf.* ref. 1), the results were found to be consistent in terms of the following species: pH 3·0 CuL; pH 4·50 CuL_2, CuL, Cu_2L, and $Cu_{15}L_{16}$; pH 7·00 CuL_2, Cu_3L_4, and $Cu_{15}L_{16}$; pH 10·0 CuL_2, Cu_3L_4, $Cu_{15}L_{16}$, and $Cu_{15}L_{30}$. In the least squares treatment[2] (by computer) agreement with experimental data was improved when the $Cu_{15}L_{16}$ species of pH 7·0 and the $Cu_{15}L_{30}$ species of pH 10·0 were replaced by the infinite series of species, $Cu_3L_4(Cu_6L_6)_n$ (n = 1, 2, 3, *etc.*), and $CuL_2(CuL_2)_m$, (m = 1, 2, 3, *etc.*), respectively. Finally, from the variation of the constant with the pH it was found that $Cu_{15}L_{16}$, the most important member of the $Cu_3L_4(Cu_6L_6)_n$ series, would correspond to the complex $Cu_3A_4(CuH_{-1}A)_{12}^{2+}$. Here the negative coefficient indicates that one more proton has been removed from the ligand than those which dissociate in the absence of Cu^{II} ions.

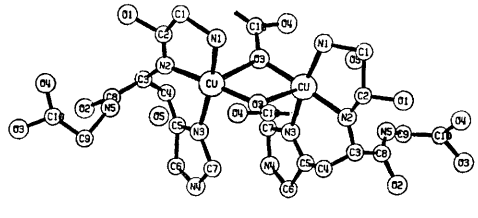

FIGURE 2. *The structure of* $CuH_{-1}A,xH_2O$.

The preparation of solid phases from equilibrium solutions has been initiated, and, at present, two crystal structures have been determined by *X*-ray diffraction.‡

Blue-violet crystals, $CuH_{-1}A,xH_2O$ (x *ca.* 12), were obtained from neutral solutions. The structure is shown in Figure 2. The α-amino, peptide, and imidazole nitrogen atoms of one ligand co-ordinate to the copper atom (*cf.* ref. 3); the Cu–N distances are 2·04, 1·97, and 2·02 Å. Two such chelates form a dimeric unit, held together by two carboxylate oxygen atoms of two other ligands: Cu–O(3) = 2·53 Å, Cu′–O(3) = 2·00 Å. Also, the second carboxy-oxygen, O(4), and a water molecule, O(5), may interact weakly with the copper atom at 3·1 and 3·2 Å. The Cu–Cu distance is 3·5 Å. Each dimeric unit, $Cu_2H_{-2}A_2$, is surrounded tetrahedrally by four other such units, forming $(Cu_2H_{-2}A_2)_5$. This leads to a three-dimensional network. Also, six dimers together form circular structures, $(Cu_2H_{-2}A_2)_6$, and these lead to channels through the crystal. Within these channels there is disordered water; these water molecules constitute about 40% of the total structure, thus markedly reducing the precision with which the rest of the structure may be determined. This structure indicates how a peptide or a protein in an organized biological unit, say a cell membrane, *via* metal complex formation, forms channels through which water and other particles can move.

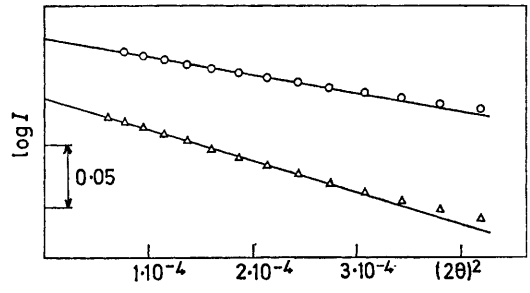

FIGURE 3. *Guinier plots of desmeared scattering data* (Δ, A/B = 1·5; O, A/B = 1·7; B = 90 mM). *The slopes correspond to the radii of gyration* 10·2 *and* 7·8 Å, *respectively.*

The species $Cu_3A_4(Cu_6H_{-6}A_6)_n^{2+}$ (n = 1, 2) formed in solution apparently have their counterparts in the crystalline state; the blue-violet crystals contain very similar fragments $(Cu_2H_{-2}A_2)_m$ (m = 5, 6). Confirmation for the existence of such species in solution was sought by recording small angle *X*-ray scattering data[4] on neutral solutions of peptide and Cu^{II} ions. As shown by Figure 3, the Guinier plots obtained correspond to mean gyration radii of 7·8 and 10·2 Å. For a number of models the radii of gyration have been calculated by using the co-ordinates of the atoms in the blue-violet crystal. It was found that the tetrahedral $(Cu_2H_{-2}A_2)_5$ and circular $(Cu_2H_{-2}A_2)_6$ units have the gyration radii 8 and 11 Å, respectively. This result strongly supports our e.m.f. data: the particles $(Cu_2H_{-2}A_2)_5$ and $(Cu_2H_{-2}A_2)_6$ also exist in solution.

† B, H, and A stand for the total concentrations of copper(II) ions, protons, and ligand; b, h, and a denote the corresponding free concentrations.

‡ One of the structures, that of the violet orthorhombic $(CuH_{-1}A,NaClO_4)$, will be described in a separate communication.

For the copper binding sites in hemocyanin, Gray[5] has recently suggested a model, entirely based on spectral data, which involves a pair of copper ions; he also proposes[5] that when copper is in the bivalent state, carboxylate groups serve to bind the copper pair together. This model is similar to the structure of our blue-violet crystals (Figure 2).

Crystal data: (Glycyl-L-histidyl-glycine)copper(II) hydrate is tetragonal with $a = 14{\cdot}41 \pm 0{\cdot}01$, $c = 26{\cdot}50 \pm 0{\cdot}03$ Å, $D_m = 1{\cdot}4$, $D_c = 1{\cdot}3$ g cm^{-3}, $Z = 8$ for $CuH_{-1}A,xH_2O$, x *ca.* 12; space group $P4_12_12$; the collected data consists of 1223 independent reflections.

The data were recorded by use of equi-inclination Weissenberg photographs and Cu-K_α radiation, the reflections being estimated visually. At the present stage of refinement R is 0·16.

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