Model Structures for a Copper(I)–Copper(II) Redox Couple in Copper Proteins: *X*-Ray Powder Structure of Bis(imidazole)copper(I) Perchlorate and Crystal Structure of Bis(imidazole)copper(II) Diacetate

By HANS-ÅKE HENRIKSSON, BO SJÖBERG, and RAGNAR ÖSTERBERG*

(*Department of Medical Biochemistry, University of Göteborg, Fack, S-400 33 Göteborg 33, Sweden*)

Summary Structural models for the co-ordination geometries of a Cu^{I}–Cu^{II} redox couple in copper enzymes have been prepared and characterized; they suggest a mechanism for certain copper enzyme reactions where both electrons and protons are involved.

IT is generally believed that univalent copper is the true electron donor in the redox reactions of copper enzymes and that their reactions involve valency changes between the Cu^{I} and Cu^{II} states; however, very little detailed knowledge is available regarding the nature of the copper binding sites. So far, only the co-ordination structure in the superoxy dismutase of bovine erythrocytes has been determined by *X*-ray crystallography.[1] Therefore, information regarding the particular copper co-ordination involved in the redox reaction of copper enzymes might be provided *via* studies on model structures.[2] Here, we report both a Cu^{I} and a Cu^{II} structure with imidazole ligands; they illustrate the co-ordination geometries required for a Cu^{I}–Cu^{II} redox reaction within a copper protein.

The crystals were grown from butanol solutions in order to simulate a hydrophobic environment similar to that supposed to exist within a protein. The $[Cu^{I}(im)_2]ClO_4$ crystals[3] (im = imidazole) were obtained only in a size suitable for powder diffraction. Powder photographs were taken with a Guinier camera at 21 °C, using $Pb(NO_3)_2$ as an internal standard. Least squares refinement of 31 *d* values yielded the following cell parameters: $a = 11{\cdot}01 \pm 0{\cdot}01$, $b = 18{\cdot}35 \pm 0{\cdot}02$, $c = 5{\cdot}22 \pm 0{\cdot}01$ Å, corresponding to an orthorhombic unit cell. These cell parameters are very

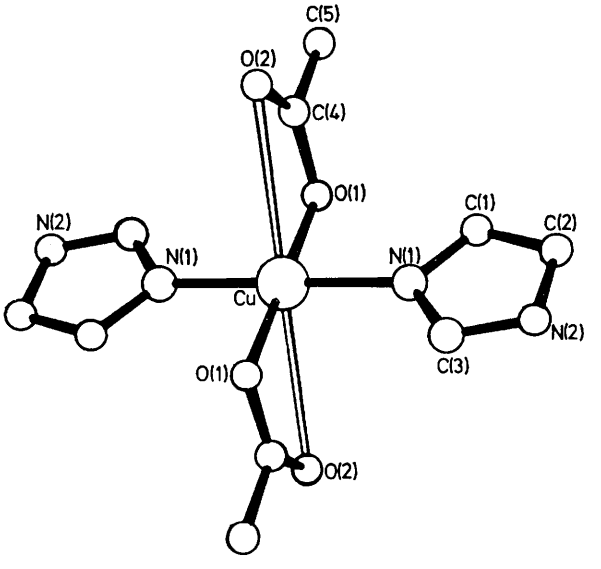

FIGURE. The structure of $[Cu^{II}(im)_2(OAc)_2]$

similar to those reported[4] for the orthorhombic [Ag(imidazole)$_2$]NO_3 crystals (a = 10·96, b = 18·23, c = 4·99 Å). A comparison of the intensity values of the present Cu^I structure and those of the Ag^I structure indicated that these structures are essentially isomorphous. Thus, we suggest that the co-ordination of the copper atom is linear in the bis-imidazole copper(I) perchlorate structure.

The violet crystals of $[Cu^{II}(im)_2](OAc)_2$ are orthorhombic with a = 11·66, b = 12·28, c = 9·37 Å, D_m = 1·55, D_c = 1·57 g cm^{-3}, Z = 4 for $Cu(C_3H_4N_2)_2(C_2H_3O_2)_2$, space group P_{ccn}. Intensity data were recorded using equi-inclination Weissenberg photographs and Cu-K_α radiation; the reflections were estimated visually. At the present stage of refinement, for 497 independent reflections, R is 0·060. The structure is shown in the Figure.

Within the Cu^{II} crystal, each copper atom binds two imidazole molecules *via* pyrrole nitrogen atoms, N(1), and two acetate ions *via* carboxylate oxygen atoms, O(1), in *trans* positions (Figure). The Cu–N(1) distances are 2·01 Å and the Cu–O(1) distances are 1·92 Å. Both the second carboxy-oxygen atoms, O(2), interact weakly with the copper atom at 2·78 Å; and as a result, copper is six-co-ordinate. The discrete $[Cu^{II}(im)_2](OAc)_2$ complexes are connected *via* hydrogen bonds from the pyridine nitrogen atoms, N(2), in the imidazole rings to the O(2) carboxylate oxygen atom of a neighbouring complex forming a three-dimensional network; N–H · · · O is 2·84 Å.

There are important differences between the present Cu^{II}–imidazole structure and those reported previously (for a review, see ref. 5); this structure contains discrete complexes. This is one of the reasons why the present structure, rather than the other Cu^{II}–imidazole structures,[5] may have direct implications for the specific copper binding sites in copper proteins. Another aspect of biological interest is that the present structures indicate an idea regarding the mechanism for certain copper enzyme reactions that involve the transfer of *both* electrons and protons (for a review, see ref. 6). For instance, a co-ordination structure might exist in a copper protein that both donates (or accepts) electrons and protons according to the redox reaction (1)

$$Cu^I(R^1\text{-imidazole})_2 + 2R^2\text{-}CO_2H \rightleftharpoons Cu^{II}(R^1\text{-imidazole})_2\text{-}(R^2\text{-}CO_2)_2 + e^- + 2H^+ \quad (1)$$

where the imidazole and carboxylic groups are assumed to belong to the side chains of the protein amino-acid residues. It is assumed in equation (1) that within the supposed hydrophobic environment the two neighbouring carboxylic groups might remain protonated under reducing conditions[7] and that the Cu^I co-ordination is similar to that of the present Cu^I structure. When Cu^I is oxidized to Cu^{II} the increase in net charge might lead to the dissociation of these carboxylic groups and the simultaneous co-ordination of Cu^{II}, yielding a co-ordination geometry similar to that of the Cu^{II} structure (Figure).

This work was supported by grants from the Swedish Natural Science Research Council, the International Copper Research Association, and the Söderberg's Fund.

(*Received*, 12*th December* 1975; *Com.* 1379.)

[1] J. S. Richardson, K. A. Thomas, B. H. Rubin, and D. C. Richardson, *Proc. Nat. Acad. Sci. USA*, 1975, **72**, 1349.
[2] R. Österberg, *Co-ordination Chem. Rev.*, 1974, **12**, 309.
[3] B. Sjöberg, Doctoral Dissertation, University of Göteborg, 1974.
[4] C.-J. Antti and B. K. S. Lundberg, *Acta Chem. Scand.*, 1971, **25**, 1758.
[5] B. K. S. Lundberg, Doctoral Dissertation, University of Umeå, 1972.
[6] R. Malkin and B. G. Malmström, *Adv. Enzymology*, 1970, **33**, 177.
[7] R. Österberg, *J. Phys. Chem.*, 1969, **73**, 2230.