A COMPARISON OF COPPER(I) AND COPPER(II) BOUND TO THIOETHER LIGANDS

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GILLIAN E. NORRIS

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Abstract

The structures of $Cu(II)(DTH)_2(BF_4)_2$ (where DTH = 2,5-dithiahexane) and $Cu(I)(DTO)_2BF_4$ (where DTO = 3,6-dithiaoctane) have been investigated by single crystal X-ray diffraction techniques. After full matrix least squares refinement of the structures, with anisotropic temperature factors for all non hydrogen atoms in the Cu(II) structure, and for all atoms larger than fluorine in the Cu(I) structure, the conventional R factor converged to a final value of 0.057 for the Cu(II) structure, and 0.082 for the Cu(I) complex.

The dark red crystals of the Cu(II) complex belong to the centrosymmetric monoclinic space group $P2_1/c$. with a = 8.082(3)Å, b = 10.282(3)Å, c = 11.893(4)Å and β = 115.3 degrees. Two dithiahexane ligands and two BF_4^- ions were found to co-ordinate to the Cu(II) ion to form a tetragonally distorted octahedron, with four Cu(II)-S bonds averaging 2.317Å in length, and two longer Cu(II)-F bonds averaging 2.576Å. The four sulphur atoms are part of two five membered Cu(II)-S-C-C-S rings in which both carbons are on the same side of the plane containing the copper and sulphur atoms.

The colourless crystals of the Cu(I) complex were obtained in the non-centric orthorhombic space group $Pna2_1$, with a = 14.581(2)Å, b = 13.421(2)Å and c = 10.781(2)Å. The molecules exist as discrete monomeric species, with no co-ordination of the BF_4^- ion to the metal ion. The two ligand molecules co-ordinate to the Cu(I) ion to form a distorted tetrahedron, with the S-Cu(I)-S angles varying between 94.0 and 121.1 degrees. The four Cu(I)-S bonds average 2.307Å in length, and hence are approximately equal to the Cu(II)-S bonds (within experimental error). The two five membered Cu(I)-S-C-C-S rings are both in a gauche conformation, with one carbon below the plane containing the Cu(I) and S atoms, and the other above. The BF_4^- ion was disordered and was refined using rigid group restrictions.

Cu-S co-ordination is thought to occur in some copper containing oxidation-reduction proteins. The observation of similar Cu-S bond distances when Cu(I) and Cu(II) are co-ordinated to thioether ligands (resembling the side

chain of the amino acid methionine) may therefore be of direct relevance to the copper co-ordination in such proteins.

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CHAPTER I

INTRODUCTION

In biological systems metal ions are often found bound to proteins. Some of these proteins contain copper ions, and in many of these the copper is essential for their function. Most copper proteins are either involved in electron transfer (e.g. azurin, laccase), or the transfer of oxygen (e.g. haemocyanin). In the light of these functions, it is probable that the copper oscillates between its two oxidation states, viz. Cu(I) and Cu(II). In order to understand any structural changes associated with such oxidation-reduction processes, it is important to know and understand the co-ordination requirements of both Cu(I) and Cu(II). A similar knowledge of the co-ordination and steric requirements of iron, gained from the study of inorganic complexes, was very useful in synthesizing suitable model complexes to elucidate the oxygen binding mechanism in haemoglobin (1). Although extensive studies have been made on many copper proteins, no general conclusions have yet been reached about the nature of the ligands responsible for binding the copper, their stereochemistries, or indeed, the state of the copper itself. Hence, structural models for copper-protein interaction rely to a great extent on the co-ordination chemistry of low molecular weight complexes, since only one complete X-ray structural analysis of a copper protein has been achieved(49).

1. Probable Ligands for Copper Ions in Proteins

In proteins, the donor atoms available to act as ligands to copper ions are oxygen, nitrogen and sulphur. On the basis of the "hard/soft" classification given by Pearson (5,12,13), the type of ligands likely to bind to copper can be predicted. Thus Cu(I) preferentially binds to "soft" bases such as R_2S, RS^-, CN^- and CO because it is a "soft" acid. Cu(II), which is near the middle of the Pearson scale, preferentially binds to such bases as $-COO^-$, H_2O, OH^- and NH_3, but may also bind to "softer" ligands such as RS^-(5,13). In all metalloproteins whose structures have so far been determined, the metal ions are bound by the functional groups on amino acid side chains. Hence potential copper ligands will probably be

confined to those amino acids with suitable side chains. These can then be divided into those whose side chains are expected to prefer to bind to Cu(II), those that should prefer Cu(I), or those that may bind to Cu(II) and Cu(I)(5). To the first group belong serine and threonine, lysine, arginine, glutamic and aspartic acids, and histidine, having as their functional groups hydroxyl, amino, guanidino, carboxyl and imidazole groups respectively. The second, a much less investigated, group contains methionine and cysteine, which have thioether and sulphydryl groups as potential binding sites. From these two groups, the ligands most likely to bind to both Cu(I) and Cu(II) are histidine, methionine and cysteine (5,14). As electron transfer to and from copper in any "valence specific" environment would not be feasible under physiological conditions, suitable ligands in redox active copper co-ordination spheres are most likely to be imidazole, thioether and sulphydryl residues, these being ligands acceptable to both Cu(I) and Cu(II). It must be remembered, however, that in a protein a metal can be forced to accept a ligand it would not usually prefer under inorganic conditions, because a prepared site, depending on the protein structure, may be offered to it.

2. The State of Copper in Proteins

Copper atoms in proteins have been classified into three main types by Malkin and Malmström(2). These are, two paramagnetic forms, and one form which cannot be detected by electron paramagnetic resonance (E.P.R.).

Type I Copper

A characteristic property of this type of copper is the intense blue colour it imparts to proteins in which it is present. Spectrally, this is seen as an intense band in the visible region at about 600nm, with an extinction coefficient approximately one hundred times greater than that found for any low molecular weight copper complex. Since some proteins contain more than one atom of copper per molecule (e.g. ascorbate oxidase, which contains 8 atoms of copper per molecule), early theories predicted that such unusual spectra may be due to some sort of copper-copper interaction(3). It was then discovered that similar

spectra could be obtained from proteins with only one atom of copper per molecule (e.g. stellacyanin). It is now thought that such spectra may be caused by highly distorted ligand fields around the metal ion, which are forced on it by the protein(2,4,5). Unusual E.P.R. parameters also indicate that in blue proteins at least, the copper ion or ions are in an environment of low symmetry(2), which may be important for their function.

It has been suggested that the intense absorption for this type of copper, must be due to a very low energy charge transfer band(6), although it is difficult to decide whether the ground state is Cu(I)X or Cu(II)X^-. The energy of the transfer band means that the group X^- is strongly reducing relative to Cu(II), and the low energy of the d-d absorption bands leads to the conclusion that the site is of unusual geometry. Thus, the Cu(II) of "blue" proteins, may have an extremely reducing ligand such as -N= or $-S^-$. This has now been largely confirmed by N.M.R. and sequence studies which indicate that $-S^-$ is the most likely ligand(6).

Further evidence that the type I copper ligands include sulphur is presented by McMillin et al in two recent papers (7,8). These describe how the Co(II) derivatives of the "blue" proteins stellacyanin, plastocyanin and azurin were prepared and their spectra analyzed. As there was conclusive evidence that the Co(II) and Cu(II) metalloproteins were isomorphous, the spectra of the copper derivatives could be analyzed by reference to those of the cobalt derivatives, in which the bands were more easily interpreted. The results of such analyses strongly suggested that a sulphur ligand was bound to the metal, (Cu and Co), and that this ligand was probably of the type RS^- (i.e. from cysteine). Further evidence has recently been obtained from X-ray photo electron experiments which have established directly that sulphur is bound to copper in plastocyanin, and that the sulphur is most probably contributed by a cysteine residue in the protein(42).

Several explanations have been given for the high oxidation-reduction potentials found in proteins containing type I copper atoms, among which is the suggestion that

sulphur ligands are involved at the active site (2), and that at such sites, the Cu(I) state is favoured. As this state is favoured in non aqueous environments(4), an additional explanation has come from Österberg, who proposed that the "blue" copper sites must be situated in hydrophobic cavities in the proteins.

Type II Copper

This is referred to as "non blue" copper as it has spectroscopic and E.P.R. parameters similar to those found in low molecular weight Cu(II) complexes. It is often found in conjunction with "blue" copper in proteins which have oxidase activity. This may be because "non blue" Cu(II) has an unusual anion affinity, and hence may stabilize intermediates such as peroxide, which may be formed during the reduction of oxygen. It is usually thought to be bound to nitrogen ligands, as in the protein superoxide dismutase, where an X-ray structure determination has shown the copper to be bound to four histidine residues(49).

Type III Copper

In the non paramagnetic form either a cuprous ion(9) or a diamagnetic Cu(II)-Cu(II) pair, acting as a two electron accepting unit(2) is thought to be involved. The suspicion that sulphur ligands were involved in the binding of this type of copper in proteins dates from the investigations of Klotz et al on haemocyanin(10) in 1952. In 1958 they reported the species

$$\left[(Cu(I))_2(RS)_2 \longleftrightarrow (Cu(II)\ RS)_2\right]^{2+}$$

which was reinvestigated by Hemmerich in 1966(5). It was suggested that such a species might be a reasonable approximation to that found in natural systems. Beinert also suggested in 1966 that in cytochrome c oxidase, where there are two different types of copper, one E.P.R. detectable and one not, the latter type probably functions as an electron acceptor and may involve a disulphide system(11). Hemmerich then reported that the E.P.R. inactive copper of this protein became E.P.R. active in the presence of mercoptide blocking mercurials. Thus whether copper is present with a valency of one or two or some state intermediate between them an electron trans-

fer must be occurring within the protein, and binding to a disulphide group could provide a mechanism for this(5).

3. Copper Ion Interaction with Sulphur Ligand Atoms

As described above, spectroscopic studies strongly suggest that some of the copper atoms found in copper proteins have one or more sulphur ligands. Potential sulphur ligands present in proteins (thiols, thioethers, and disulphides) are already known to interact with iron(II) and iron(III). This has been shown by X-ray diffraction studies on single crystals of iron containing proteins and low molecular weight iron complexes(4,19). For example, the X-ray crystallographic structures of some ferrodoxins have shown that their active sites involve sulphur ligands from the thiol group of cysteine(20). Models of these oxidation-reduction proteins have been made by wrapping synthetic peptides of the type Cys-X-X-Cys (where X is an amino acid) around iron-sulphur cluster compounds of the type Fe_4S_4. In these complexes, the sulphur from the cysteine residues is bound to the iron atoms of the Fe-S clusters(4,5). Such complexes are found to have very similar spectra and redox potentials to the native proteins. The co-ordination of thioether groups is seen in the electron transfer protein cytochrome c. In this structure a thioether sulphur atom from methionine occupies the sixth co-ordination position of the iron atom(50). In both the iron proteins mentioned above the metal atom or cluster compound at the centre of the active site is bound to the apo-protein through at least one sulphur atom. Thus, it could be possible that the ligands which bind the metal to the protein play some role in its function of accepting or losing electrons. As the function of some copper proteins is very similar to that of these iron proteins (i.e. electron transfer or oxidation-reduction) the ligands offered to the copper ions by the proteins may be similar to those found in iron proteins, and hence involve some sulphur.

4. Small Molecule Studies of Copper Binding Sites

The structures of very few metalloproteins have so far been determined by X-ray crystallography. Even for those which have been, the accuracy of the data on the metal

co-ordination is limited in most cases by the relatively low resolution of the protein structure analysis. The study of small inorganic molecules, therefore, provides a means of obtaining very accurate data which may yield important information on aspects of the geometry of metal binding sites in metal-protein complexes. The studies of copper-peptide complexes by Freeman (15,16) have shown a variety of possible modes of co-ordination between copper atoms and amino acid ligands. However, these are mainly concerned with Cu(II) ions and oxygen and nitrogen ligands (e.g. amino and carboxyl groups) some of which would be involved in peptide bond formation in proteins and thus would probably not be available as ligands to metal ions.

Blue Cu(II) complexes in which the ligands are either four ammonia or four imidazole molecules have been reported (82) but although they have the colour characteristic of the "blue" copper proteins, its intensity is much less. A large number of crystal structures of Cu(II) complexes with nitrogen and oxygen ligands have been determined. In these complexes the ligand is usually found to be bound to the metal ion by a single covalent bond, the most common stereochemistries being square planar and octahedral (distorted)(4,79). Although simple complexes of Cu(II) and these ligands are known, chelated ones are generally found to be more stable, and hence are predominant(83). A number of compounds in which Cu(II) is bound to combinations of sulphur and nitrogen ligands (e.g. thiosemi-carbazides) have also been investigated(17,18) and although few of the ligands are closely comparable with those available in proteins, they can give valuable information about the type of bonding possible between Cu(II), and sulphur and nitrogen.

Most of the Cu(I) crystal structures investigated so far contain ligands such as acetonitrile, chloride, bromide and iodide, which are far removed from the sort of ligands available in proteins. However, Österberg has reported some Cu(I)-imidazole structures(4) which can be compared with very similar Cu(II) complexes. X-ray diffraction studies have been carried out on Cu(I)-thiourea chloride and other similar complexes(78), but only a single Cu(II) structure of this kind is known, viz. that of Cu(II)-

tetramethylthiourea chloride (34). Other structures which may have biological significance are those of the Cu(I) thiocarbamates, which form cluster compounds(17). These can be compared with similar Cu(II) clusters, and are interesting because in some copper proteins (e.g. ceruloplasmin), there is more than one copper atom per molecule and clusters could occur. Complexes in which both sulphur and nitrogen are co-ordinated to Cu(I) ions have been reviewed, together with the similar co-ordination complexes of Cu(II) ions (17,18). The former complexes are invariably tetrahedral, although the tetrahedra may be distorted(71), and may involve some $p\pi$ or $d\pi$ bonding between the Cu(I) ion and the ligands(79).

In general, however, ligands containing sulphur as a donor atom to copper ions have been much less studied than those containing oxygen and nitrogen. In particular, few complexes have been prepared involving sulphur ligands similar to the potential ligands available in proteins. Thioether ligands such as dithiahexane (DTH), and dithiaoctane (DTO), appear to offer excellent opportunities to study the interaction of copper ions with a sulphur ligand closely resembling one of those likely to bind copper in proteins. This is because DTH and DTO are very similar to the side chain of the amino acid methionine.

Methionine $CH_3-S-CH_2-CH_2-\underset{\displaystyle H}{\overset{\displaystyle NH_2}{C}}-COOH$

DTH $CH_3-S-CH_2-CH_2-S-CH_3$

DTO $CH_3-CH_2-S-CH_2-CH_2-S-CH_2-CH_3$

In addition, it was shown by Bergen(21) that both Cu(I) and Cu(II) complexes with DTH ligands could be prepared. It was the aim of this project, therefore, to determine the structures of a Cu(I) and a Cu(II) complex with the same ligand system, in order to compare differences between them. As copper proteins are involved in redox processes, a binding site for the copper ion which does not change ligand geometry drastically during the uptake or loss of one electron would be expected(14). Changes in bond length, or in the type of bonding, or small changes in geometry, may however be crucially important in the function of such

proteins. Although it is unlikely that the geometry around the metal ion in these proteins could be simulated, such a study may be of considerable help in further understanding about bond lengths and bond types between copper ions and proteins.