The bioinorganic chemistry of copper

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Many enzymes and proteins have copper at their active sites, which plays a key role in biology. An important goal of bioinorganic chemistry is the development of small inorganic coordination complexes that reproduce structural, spectroscopic features and functional aspects in a manner similar to their natural counterparts. To provide an overview of the activities in this field, some results on synthetic modelling of a selected number of copper proteins/enzymes are described in this article.

Copper is one of the transition elements frequently found at the active site of proteins. The copper-containing enzymes and proteins constitute an important class of biologically active compounds. The biological functions of copper proteins/enzymes include electron transfer, dioxygen transport, oxygenation, oxidation, reduction and disproportionation.

In nature, a variety of copper proteins are essential constituents of aerobic organisms, including hemocyanins (arthropodal and molluscan O2 carriers) and enzymes that “activate” O2, promoting oxygen atom incorporation into biological substrates. The latter include tyrosinase (a monoxygenase, incorporating one oxygen atom to the substrate and reducing the other to water) and dopamine β-hydroxylase (a monoxygenase). “Blue” multicopper oxidases (e.g., laccase (phenol and diamine oxidation), ascorbate oxidase (oxidation of L-ascorbate) and ceruloplasmin) promote substrate one-electron oxidation while reducing O2 to water. Ceruloplasmin may be involved in copper metabolism. Cytochrome c oxidase transduce energy from the same 4e-/4H+ reduction of O2 occurring at a heme-Cu binuclear centre, and couple this to membrane proton translocation, utilized in ATP synthesis. Amine oxidases and galactose oxidase effect amine → aldehyde oxidative deaminations and alcohol → aldehyde oxidative dehydrogenations, respectively. Copper ion reactions with reduced dioxygen derivatives (e.g., superoxide (O2), hydrogen peroxide) are essential in Cu-Zn superoxide dismutase, and may be involved in copper-mediated oxidative damage in biological media, including possibly in Alzheimer’s disease.

Correlated with the enzymatic activity, the copper proteins exhibit unique spectroscopic properties and, accordingly, the proteins are divided in mainly three types.

Type I copper proteins (also called “blue” copper proteins) are known to have one copper ion in the active site. This copper ion shows some remarkable spectroscopic features: an intense absorption around 600 nm, with an extinction coefficient of about 3000 M⁻¹cm⁻¹. Another characteristic feature of the Type I copper proteins is the extremely small hyperfine splitting in the EPR spectra (ΔHpp= 40-90 x 10⁻⁴ cm⁻¹).

Type II copper proteins have no distinct unique properties. The spectroscopic data of these proteins are comparable to those of “normal” copper compounds.

Type III copper proteins contain antiferromagnetically coupled copper dimers. These proteins are diamagnetic and therefore are EPR silent. In some proteins, all three types of copper sites are present. Such proteins were proposed to classify as Type IV. In ascorbate oxidase one of the copper ions is found in a distorted tetrahedral (trigonal pyramidal) coordination with two histidines, a methionine and a cysteine. This resembles the active site of the blue copper protein plastocyanin. Also, a trinuclear copper site was found consisting of a Type III copper pair and a “normal” Type II copper ion.

Reactions that copper proteins carry out have long interested inorganic chemists. Copper is an important element in oxidation catalysts for laboratory and industrial use. Interest in the copper-dioxygen complexes stems from the diverse occurrence of copper proteins which function as highly efficient biooxidation catalysts. Copper-dioxygen adducts are
suggested as key reaction intermediates in these enzymatic reactions. The differentiation in the function of these proteins is attributed primarily to the coordination structure of the copper-dioxygen intermediate formed in the protein matrices, depending on the ligand donors, the geometry, and the coordination mode of the dioxygen. However, the correlation between these structural factors and the function/catalysis of the enzymes remains to be elucidated.

Importance of inorganic model chemistry

Investigations of metallobiomolecules have increased markedly during the last two decades. High-resolution X-ray crystallographic results, in particular, have facilitated detailed considerations of structural, electronic and reactivity properties at the molecular level. These metallobiomolecules are highly elaborated coordination complexes whose metal-containing sites (coordination units), termed as “active sites”, comprising one or more metal ions and their ligands, are usually the loci of electron transfer, binding of exogenous molecules and catalysis. The demonstrated or potential relation between the properties of these sites and those of synthetic coordination complexes has contributed significantly to the emergence of the interdisciplinary field of bioinorganic chemistry. The complexity of biological systems renders a detailed study of their mechanism very difficult. An increasingly popular method of elucidating structures and mechanisms is the use of a simple chemical compound or system.

Interest in elucidating or mimicking the physico-chemical properties of metalloproteins has led to spurring activity in the synthesis of numerous interesting coordination complexes. However, recently there has been an increased emphasis upon functional modelling of proteins. While the structural and spectroscopic modelling of metalloprotein active sites is an important and ongoing endeavour, the realization that coordination chemists can and should make significant contributions to reactivity studies and mechanism has become apparent. The value of models for metalloproteins will always be relative. One of the difficulties encountered in simulating a biosite is that, as time passes, the objective may change with advancing knowledge. If the structure of the metal ion environment in the metalloprotein is unknown, the objective may be to reproduce some property of the system in a similar model coordination compound. However, when the structure of the biosite is known, then the complex that it reproduces has, as far as possible, the known structure. A different emphasis is obtained when the action of the metal in the protein is reproduced by a model compound and the mechanism of a particular reaction is elucidated or partially explained.

The purpose of models is not necessarily to duplicate natural properties but to sharpen or focus certain questions. The goal is to elucidate fundamental aspects of structure, spectroscopy, magnetic and electronic structure, reactivity and chemical mechanism. A synergistic approach to the study of metalloenzymes can and has yielded crucial information because synthetic analogues can be used to investigate the effects of systematic variations in coordination chemistry, ligation, local environment and other factors, often providing insights that cannot be easily attained from protein studies (Fig. 1). Reproducing complex biological reactivity within a simple synthetic molecule is a challenging endeavour with both intellectual and aesthetic goals.

Several researchers have endeavoured to understand the structure and function of copper proteins involved in copper(1)/O₂ interactions by studying inorganic models, i.e., synthetically derived copper(I) complexes, and their O₂ reactivity. Such biomimetic approaches can lead to fundamental insights into the copper-based chemistry. One might also envision the development of reagents or catalysts for use in practical oxidation processes.

It is the purpose of this article to highlight recent advances in bioinorganic model (structurally characterized) studies on some selected copper proteins/enzymes, including some results from author’s laboratory.

Protein(s) and synthetic models

**Blue copper proteins: Type I copper**

“Blue” copper proteins (azurin, plastocyanin),

![Fig. 1—The synergistic relationship between studies involving metalloprotein biochemistry and inorganic modelling.](image-url)
which function as electron transport agents in a number of biochemical systems, gain their colour from an intense electronic absorption band that arises from a charge transfer transition to the Cu$^{2+}$ ion at the active site from the cysteine thiolate ligand. The unusually low energy of the transition results from the coordination geometry about the Cu$^{2+}$, which involves a nearly trigonal arrangement of two imidazole N atoms and the thiolate S atom, as shown in Fig. 2. The methionine S atom is found along the trigonal axis at a long distance, 2.6-3.1 Å, reflecting a weak bonding interaction; sometimes a peptide carbonyl oxygen atom is located on the other side of the trigonal plane, at an even longer distance. This bonding arrangement, together with a high degree of covalency associated with the short Cu-S (thiolate) bond, leads to reduced values of the A$^{II}$Cu hyperfine coupling constant. The coordination geometry stabilizes the copper(I) oxidation state and the redox potentials are unusually high in relation to ordinary copper complexes.

The synthesis and structural characterization of a thiolate-copper(II) complex which closely mimics the spectroscopic characteristics of blue copper proteins have been longtime goals in bioinorganic chemistry. The main difficulty in synthesizing an accurate model for Type I copper comes from the instability of the copper(II)-thiolate bond [2Cu$^{II}$-SR → 2Cu$^{I}$ + RSSR$^-$]. Kitajima and co-workers were successful in providing structural proof of a copper(II) complex (trigonal pyramidal) with C$_6$H$_5$S· coordination (Fig. 2). The Cu(II)-S(thiolate) distance (2.18 Å) is distinctly shorter than those of the reported complexes. Spectroscopic features of this complex are comparable to those of “blue” copper proteins. The X-ray analysis (poor data set) of a closely similar complex with Ph$_3$S as the thiolate ligand was also achieved.

Superoxide dismutase: Type II copper

The reduction of dioxygen probably proceeds, in every instance, by a series of one-electron transfer reactions. Therefore, unless the intermediate reduction products are retained within the active site of an enzyme or coordinated to a metal complex, there is every likelihood that most oxidation reactions will generate superoxide as the initial reduction product. As superoxide ion is toxic to cells, a defense mechanism must have been initiated by nature. We now know that essentially all organisms, which use dioxygen, and many that have to survive an oxygenated environment, contain at least one superoxide dismutase. The one which is pertinent to this article is a Cu-Zn protein found in cells that contain a nucleus (eukaryotic cells). Its function is to catalyze the disproportionation of superoxide ion ($O_2^-$), i.e., it is a “superoxide dismutase”$^{10}$.

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

Copper-zinc superoxide dismutase (SOD) contains an imidazolate-bridged Cu(II)-Zn(II) heterodinuclear metal centre in its active site (Fig. 3). The copper ion is in a distorted square-pyramidal geometry, while the zinc ion located at a distance of 6.2 Å from the copper ion is in a distorted tetrahedral structure. The catalytic cycle (Fig. 4) starts with the replacement of a water molecule at the axial position by superoxide ion and reduces the copper to Cu(I). Concomitantly the bond from Cu to imidazolate is broken and O$_2$ is released.
The Cu-facing nitrogen of histidine becomes protonated and a second $O_2^-$ becomes bound. An electron is transferred from Cu(I), coupled with a proton transfer from histidine. After addition of a second proton from an active-site water, the uncharged hydrogen peroxide is released.

In the copper ions in the imidazolate-bridged Cu(II)-Zn(II) heterodinuclear complex (Fig. 3) synthesized by Fukuzumi and co-workers\textsuperscript{10}, the coordination site occupied by a solvent can be susceptible to ligand substitution, thus providing a binding site for substrate superoxide. The Cu(II)-Zn(II) distance of 6.197(2) Å agrees well with that of native enzyme. The complex catalyzed the dismutation of superoxide at biological pH.

**Nitrite reductase: Type I and Type II copper**

Denitrification, the dissimilatory transformation of $NO_3^-$ and $NO_2^-$ to gaseous N$_2$O or N$_2$, is a central process in the biological nitrogen cycle (Fig. 5) responsible for depletion of nitrogen, necessary for plant growth, from soil\textsuperscript{11}. The copper-containing nitrite reductases isolated from bacteria and fungi, comprise an important subclass of the set of denitrification enzymes. These enzymes catalyze the reduction of $NO_3^-$ to NO, although N$_2$O generation has been induced under some conditions. The active site (Fig. 5) of this enzyme contains a pair of copper ions, one of which has been assigned as a “green” Type I, electron transfer site. The other site is an unusual, distorted pseudotetrahedral Type II site.

![Diagram of Cu-Zn-SOD and model complex](image-url)

**Fig. 3**—(a) The metal-binding region of Cu-Zn-SOD; (b) The model complex.

![Diagram of catalytic cycle of superoxide dismutase](image-url)

**Fig. 4**—The catalytic cycle of superoxide dismutase.
Proposed mechanism for reactions of copper-containing nitrite reductases is presented in Fig. 5.

Tolman and co-workers\textsuperscript{12} provided examples of a number of substrate-bound copper(I) and copper(II) complexes (Fig. 6). In an elegant manner they modelled N\(_2\)O generation by copper proteins through reductive disproportionation of Cu-NO species. To prove the reaction sequence they isolated a nitrite-bound complex.

**Hemocyanins: Type III copper**

Hemocyanin\textsuperscript{*} is a ubiquitous dioxygen carrier for invertebrates, containing a dinuclear copper site to which dioxygen is bound as peroxide (Fig. 7); the two copper ions are divalent in the dioxygen binding state (so-called oxyhemocyanin, oxy-Hc). Oxy-Hc is EPR silent and in fact, diamagnetic at room temperature due to a very strong antiferromagnetic exchange coupling (2\(J > 600 \text{ cm}^{-1}\) between the two Cu(II) ions. Furthermore, instead of \(d-d\) bands normally observed at 600-700 nm for Cu(II) complexes, Oxy-Hc exhibits two intense bands at ca. 350 nm (\(-20000/2\text{Cu}\)) and ca. 580 (\(-10000\)), both attributable to \(\text{O}_2^2- \rightarrow \text{Cu(II)} \) LMCT transitions.

While studying modelling copper-dioxygen chemistry Kitajima \textit{et al.} reported\textsuperscript{13} the synthesis of a \(\mu\)-peroxo dinuclear complex with 3,5-dimethylsubstituted tris(pyrazolyl)borate ligand, which showed remarkable physicochemical similarities to oxy-Hc and oxy-Tyr\textsuperscript{6}. Using 3,5-di-isopropyl-substituted terminal ligand they provided the first structural proof (Fig. 7) of the existence of \(\mu\)-\(\eta^1\)-\(\eta^2\) peroxodicopper(II) core (copper geometry: distorted square pyramidal; Cu-Cu: 3.560 \(\text{Å}\)) and reported detailed characterization properties, which eventually led to the structural characterization of oxy-Hc\textsuperscript{14}.

Tolman and co-workers discovered\textsuperscript{15} a novel phenomenon that when copper(I) complex of 1,4,7-trisopropyl-triazacyclononane oxygenated at \(-78°C\), there exists an equilibrium between the two oxygenated species \([\text{Cu}^\text{II}_2(\mu-\eta^1\eta^2\text{-O}_2)]^{2+}\) [side-on peroxodicopper(II)] and \([\text{Cu}^\text{II}_2(\mu-\text{O}_2)]^{2+}\) [bis(\(\mu\)-oxo)dicopper(III)] depending on the solvent chosen, with CH\(_2\)Cl\(_2\) favouring the former species and THF favouring the latter.
Tyrosinase: Type III copper

It is known that when potatoes, apples, bananas, sweet potatoes or mushrooms are injured they turn brown. This is due to the conversion of tyrosine to the pigment melanin, by the sequence of reactions shown in Fig. 8. The same process causes skin tanning, following exposure to ultraviolet radiation. The enzymatic reactions are catalyzed by tyrosinase\(^5\). The enzyme is present in the interior of the plant material and since the reaction requires molecular oxygen, the pigmentation does not occur until the interior is exposed. Tyrosinase catalyzes (i) the \(\alpha\)-hydroxylation of monophenols to \(\alpha\)-diphenols (cresolase activity) and the further oxidation of these to \(\alpha\)-diquinones (catecholase activity). These quinones undergo further enzymatic and nonenzymatic reactions that lead to polymeric pigmented material. Thus, tyrosinases possess both monooxygenase and oxidase activity. In animals, these reactions give skin, eyes and hair their distinctive pigmentation. In order to deduce the
structures and mechanism of action of the protein-active sites, a major focus of research has utilized the biomimetic approach.

Comparisons of chemical and spectroscopic properties of tyrosinase and its derivatives with those of hemocyanin, establish a close similarity of the active sites structures in these two proteins. The active site of tyrosinase apparently has greater accessibility to exogenous ligands, including substrate molecules, by comparison to the active site in hemocyanin. The similarity of the oxy-states of hemocyanin and tyrosinase points to the probable close relationship between the binding of dioxygen and the ability to activate it for incorporation into organic substrates.

A considerable number of ligand oxidations has been reported, where aerobic treatment of a copper(I) complex, mostly dinuclear, yields a dinuclear copper(II) complex with an oxidized ligand, which can be isolated. Using tailor-made binucleating N-donor ligands having \( m-\text{CH}_2\text{C}_6\text{H}_4\text{CH}_2 \) spacers between the coordination units, Karlin and co-workers reported the first model (Fig. 8) consisting of a ligand that provides two tridentate bis[2-(2-pyridylethyl)amine] donor units to each copper ion. A mechanism was proposed which involves an electrophilic attack of a bent \( \mu\eta^1:\eta^1 \)-peroxide to the CH bond of the aromatic ring. Interestingly, when 1-pyrazolyl or 2-imidazolyl donor groups fully or
partially replace the 2-pyridyl ligands hydroxylation does not occur. However, when Schiff base ligands providing three or even only two nitrogen donors are used, hydroxylation takes place. We demonstrated\(^4\) the synthesis of the first \(\text{m-CH}_2\text{C}_6\text{H}_4\text{CH}_2\) hydroxylation ligand system, within the non-Schiff base family, providing only two nitrogen coordinations to each copper centre (Fig. 8). While the \(\mu\)-peroxo intermediate could not be identified spectroscopically owing to its instability, on the basis of the closely related ligand structure to our ligand, it was demonstrated that the reaction proceeds via a \(\mu\)-peroxo intermediate.

**Cathechol oxidase: Type III copper**

The ubiquitous plant enzyme cathechol oxidases\(^{17}\), in contrast to tyrosinases, catalyze exclusively the oxidation of catechols to the corresponding \(\alpha\)-quinone by molecular oxygen without acting on monophenols. Thus, cathechol oxidase lacks hydroxylase activity. The resulting highly reactive quinones auto-polymerize to form brown polyphenolic catechol melanins, a process thought to protect the damaged plant from pathogens or insects. The enzyme contains an antiferromagnetically coupled (EPR silent) dicopper centre. Three-dimensional X-ray crystal structural analysis of cathechol oxidase, from sweet potato, in the resting Cu(II)-Cu(II) state, the reduced Cu(I)-Cu(I) form, in complex with the inhibitor have been achieved. Both copper centres have three histidine ligands. In the oxidized cathechol oxidase structure the two Cu(II) ions are 2.9 Å apart. In
addition to the six histidine ligands, a bridging hydroxide ion completes the four-coordinate trigonal pyramidal coordination sphere for each Cu(II) ion. Mechanism of cresolase and catecholase activity of tyrosinase and/or catechol oxidase is presented in Fig. 9.

Recently we have shown\textsuperscript{18} that the phenoxo/hydroxo-bridged dicopper(II) complex [Fig. 8(c)] acts as an efficient catalyst for catechol oxidase-like activity (Fig. 9).

Galactose oxidase: Type II copper

Galactose oxidase\textsuperscript{19} is a fungal enzyme that catalyzes the oxidation of galactose and a number of other primary alcohols to the corresponding aldehyde, a reaction in which dioxygen is reduced to hydrogen peroxide (Fig. 10). The active site structure is presented schematically in Fig. 10. A unique feature of this active site embodies the modification of the tyrosinate residue located in the equatorial plane by a covalent linkage to the sulphur atom of a nearby cysteine residue.

Stack et al. were able to synthesize\textsuperscript{20} nonplanar copper(II) complexes (Fig. 10) from which they could obtain the corresponding copper(I) complexes and relatively stable phenoxy-radical copper(II) complexes by reduction and oxidation, respectively. The complex can act as a catalyst or as a precursor for a catalyst in the reaction of benzylic and allylic alcohols with molecular oxygen at room temperature, yielding the respective aldehyde and hydrogen peroxide. Turnover numbers as high as 1300 are reported for the catalytic cycles. Most noteworthy, the catalytic oxidation seems to proceed by the same mechanism as the enzyme-catalyzed reaction (Fig. 11).

"Blue" multicropper oxidase -- ascorbate oxidase: Type IV copper

Ascorbate oxidase\textsuperscript{21} catalyzes the oxidation of L-ascorbate with concomitant reduction of O\textsubscript{2} to water. The trinuclear Cu site is shown in Fig. 12. Stack and co-workers reported\textsuperscript{22} an unusual 3:1 (copper:O\textsubscript{2} stoichiometry) reaction between a mononuclear copper(I) complex of a N-permethylated (IR, 2R) cyclohexanediamine ligand with dioxygen. The end product of this reaction, stable at only low-temperatures (X-ray structure at -40°C), is a discrete mixed-valence trinuclear copper cluster (Fig. 12), with two terminal Cu(II) and a central Cu(III) centre (Cu-Cu: 2.641 and 2.704 Å). The relevance of this synthetic complex to the reduction of O\textsubscript{2} at the trinuclear active sites of multicropper oxidases was discussed (three copper(I) centres produce 4e\textsuperscript{-} to reduce O\textsubscript{2} to H\textsubscript{2}O).

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