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Oxidations by copper metalloenzymes and some biomimetic approaches

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Contents

Abstract

This paper illustrates the various aspects of the reactivity of the Cu(II)–Cu(I) system in biological systems, with one example of an enzymatic reaction in which Cu(II) alone is oxidizing enough to carry out the reaction (superoxide dismutase), one example in which a Cu(II)-bound peroxo intermediate is the active species (tyrosinase) and the examples of galactose oxidase and copper amine oxidases in which Cu(II) is associated with a redox active organic cofactor. In some cases, we will show some illustrations of biomimetic approaches developed in our laboratories, aimed at a better understanding of reaction mechanisms and at an original design of new catalysts with potential applications in synthetic chemistry. Some comments are given concerning the respective features of copper and iron.

Keywords: Amine oxidase; Copper enzymes; Galactose oxidase; Models; Oxidizing equivalent; Superoxide dismutase; Tyrosinase

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1. Introduction

One of the simplest elementary acts in homogeneous solution chemistry is the transfer (exchange) of an electron as no bonds need to be formed or broken. Two partners are required, D (donor or reducing agent) and A (acceptor or oxidizing agent), each with at least two accessible oxidation states. When both D and A are metal ions, two electron transfer pathways have been elucidated:

(1) Long range or outer-sphere electron transfer, when the coordination sphere of both donor and acceptor is altered to a minor degree during the reaction. No metal–ligand bond is broken or formed. The theoretical basis for this type of electron transfer was established by Marcus.
(2) Inner-sphere electron transfer when the donor and acceptor form a direct chemical bond: a bridging ligand common to both coordination shells connects the two metal ions providing a series of overlapping orbitals for efficient adiabatic electron transfer. This case is often reserved to catalytic steps in enzymes.

Strict electron transfer (as it occurs in the absence of group transfer) between a metallic and a non-metallic center has been less studied both theoretically and experimentally. This process can be monitored by electrochemistry.

We will focus our attention on catalytic oxidation reactions. Reductions are strictly the reverse, but the reaction is not necessarily reversible. Of course, the oxidation of a substrate implies the reduction of the oxidizing agent; the terminology used herein refers to the function of the enzyme. Usually, the equation of the catalyzed reaction reveals a multi-electron transfer, but in several cases, the mechanism involves successive mono-electronic transfers, mediated by a redox active metal ion such as Fe^{3+} or Cu^{2+}. In some cases, non-metallic redox centers may be used by metalloenzymes.

Iron and copper are the two most familiar redox metals serving biological functions. At first sight, it seems that they are able to catalyze similar reactions and either iron or copper sites are found in oxygen carriers, mono-oxygenases, di-oxygenases or oxidases [1]. As pointed out by Williams [2], Ochiai [3] and by Kaim [4], copper can be regarded as a ''modern bioelement'' as it became a biological element after iron. Prior to the photosynthetic generation of an oxidizing environment, water insoluble copper(I) prevailed and was, thus, not biologically available. Soluble ferrous iron was present in large amounts in the oceans and was used for biological functions. The production of O_2 by photosynthetic organisms resulted in the oxidation of Cu(I) to soluble Cu(II) and of soluble Fe(II) to insoluble Fe(III). This is suggested to be the reason why living organisms selected copper then instead of iron. Copper and iron display distinct features, in terms of their coordination chemistry and their redox properties. One of the major difference resides in their distinct electrochemical properties, which explain most of their specific reactivity. As a matter of fact, the reduction potentials of the Fe(III)–Fe(II) system can be finely tuned by small modifications in the metal coordination sphere so that iron sites can encompass the entire biologically significant redox potential range: from about -0.5 V to about 0.6 V. In contrast, a relatively high redox potential for the Cu(II)–Cu(I) system is found in copper enzymes, most of them working between

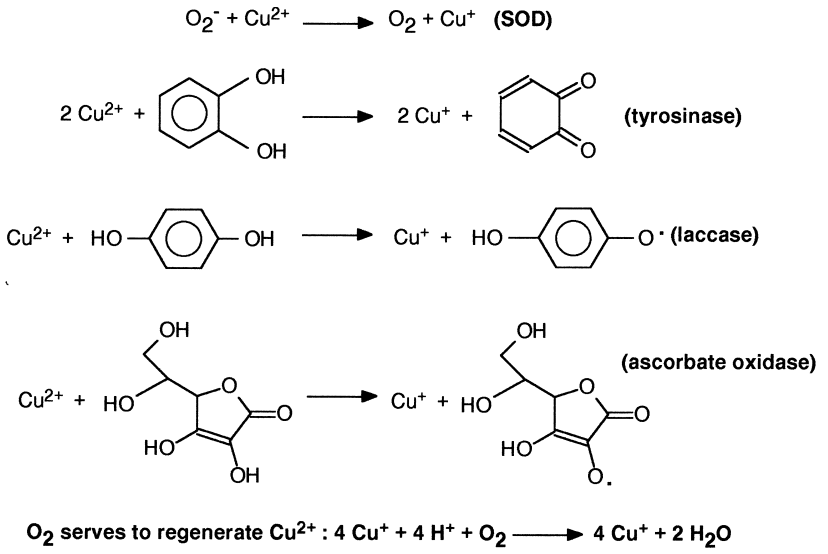


Fig. 1. Direct oxidations by copper enzymes.

+0.25 and +0.75 V. This high potential can be utilized for a direct oxidation of certain substrates, easy to oxidize, such as superoxide (in superoxide dismutase) or ascorbate (in ascorbate oxidase) or catechols (in tyrosinase or in laccases) (Fig. 1).

As copper is more difficult to oxidize than iron, the Cu(III) redox state might not be biologically relevant. On the contrary, there is now convincing evidence that a number of iron-dependent mono-oxygenases generate high-valent Fe(IV) or Fe(V) reactive intermediates during their catalytic cycle (Fig. 2).

The most such well-known examples are the cytochrome P-450 mono-oxygenases, which use an iron porphyrin, and methane mono-oxygenase, which uses a dinuclear non-heme iron center, for oxygen activation and hydrocarbon oxidation. In both cases, it has been unambiguously demonstrated, through the exquisite application of various spectroscopic methods and rapid kinetics experiments, that a reduced

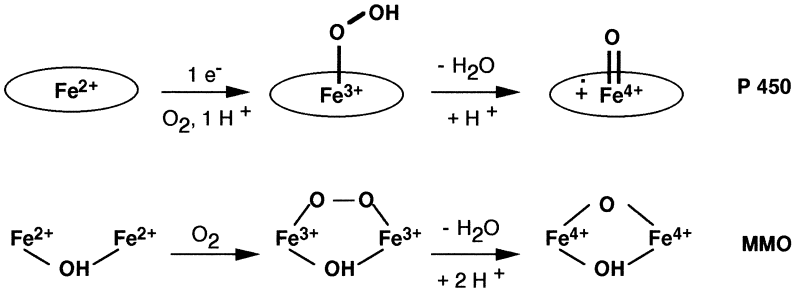


Fig. 2. High valent iron species.

center binds oxygen to generate an iron-peroxo intermediate [5]. The latter is not active during oxidation until it is transformed into a high valent complex by cleavage of the O–O bond.

High valent iron complexes are amongst the most powerful oxidants and they are capable of alkane (including methane) hydroxylation. In general, nature has selected Fe and not Cu for oxidation of resistant substrates such as hydrocarbons. One fascinating exception is an ill-defined particulate Cu-dependent methane monooxygenase [6].

In the case of copper centers, the impossibility to reach a highly oxidizing Cu(III) state has, thus, limited the range of substrates to oxidize. During the few C–H bond oxidations by copper enzymes such as those catalyzed by dopamine β-hydroxylase [7], containing a mononuclear active Cu site (a second site serves for electron transfer), or by tyrosinase, containing a dinuclear Cu site, an intermediate copper peroxo Cu–OOH or $Cu(O_2)Cu$ complex, in which the bound peroxide is highly activated has been suggested to be the oxidizing reagent (see Section 3). There is actually no evidence that the O–O bond is broken prior to oxidation of the substrate (Fig. 3).

More recently, some Cu enzymes have revealed another molecular strategy for designing efficient catalysts of oxidation reactions. These new sites are based on the association of a Cu(II) center with a non-metallic redox cofactor. The most fascinating enzymes of that class are galactose oxidase in which the Cu(II) site is coupled to an unusually substituted tyrosyl radical and copper amine oxidases in which Cu(II) is associated with a quinone, the so-called TPQ cofactor. Therefore, an original bioinorganic chemistry field is emerging in terms of new structures of copper active sites, enzyme mechanisms and model chemical complexes [7].

This paper will illustrate the various aspects of the reactivity of the Cu(II)–Cu(I) system in biological systems, with one example of an enzymatic reaction in which Cu(II) alone is oxidizing enough to carry out the reaction (superoxide dismutase), one example in which a Cu(II)-bound peroxo intermediate is the active species (tyrosinase) and the examples of galactose oxidase and copper amine oxidases. In some cases, we will show some illustrations of biomimetic approaches developed in our laboratories, aimed at a better understanding of reaction mechanisms and at an original design of new catalysts with potential applications in synthetic chemistry. For simplicity, detailed structural and spectroscopic data have been omitted in this

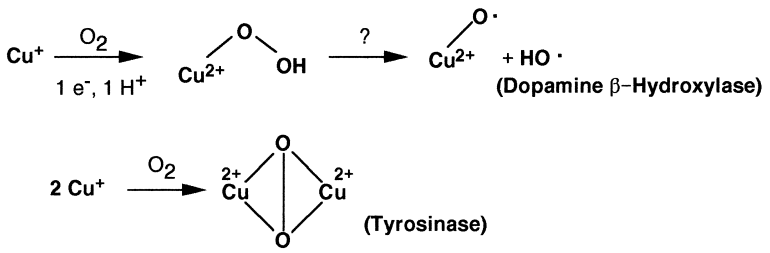


Fig. 3. Intermediate in dopamine β-hydroxylase and tyrosinase.

paper, but the reader can refer to recent and excellent reviews in which these aspects are discussed [1,7,8].

2. Superoxide dismutase: only one metal center is used; no bond is broken in the substrate for a one-electron transfer

Copper–zinc superoxide dismutase (SOD) is composed of two identical subunits each of them containing in its active site an imidazolate-bridged bimetallic center with one copper(II) and one zinc(II) ion [9]. It has been found in the cytosol of eukaryotic cells and it is believed to protect cells from the toxic effects of superoxide ions. It catalyzes the dismutation of superoxide via the two diffusion-controlled one-electron redox processes (''ping-pong'' mechanism):

$$O_2^- + [Cu(II)] \rightarrow O_2 + [Cu(I)],$$

$$O_2^- + [Cu(I)] + 2H^+ \rightarrow H_2O_2 + [Cu(II)].$$

The metal-binding site is depicted on Fig. 4. The Cu ion is coordinated by four histidines in a distorted square planar coordination sphere and by a fifth axial water ligand. The tetrahedral zinc ion is coordinated to three histidines, one of them bridging to the copper and by a carboxylate group.

The superoxide ion is electrostatically guided by a deep ($\approx$13 Å) and narrow ($\approx$4 Å) channel lined by positively charged amino-acid residues. The probable role of the zinc ion is to confer stability to the protein which is remarkably stable to heat and is active from pH 4.5 to 10. The catalytic mechanism is depicted in Fig. 5. The oxidation step (i) consists of an inner sphere electron transfer between the coordinated superoxide and the copper(II) center. The concomitant breaking of the copper–imidazolate bond is assisted by protonation of the imidazolate by the solvent and oxygen is released.

Many low molecular weight copper chelates are known to exhibit, in vitro, superoxide dismutase-like activity. Most of them lose their activity in the presence of bovine serum albumine (BSA) which is able to mobilize the copper(II) ions from these complexes [BSA is a plasmatic protein which is one of the strongest biological chelators of cupric ions ($\log K = 16.2$)]. A biomimetic model has been described by one of us [10] which catalyzes the dismutation of superoxide at biological pH and the activity survives in the presence of BSA. Moreover, this model shares many of the spectroscopic properties of the enzyme. It is an imidazolate-bridged heterodi-

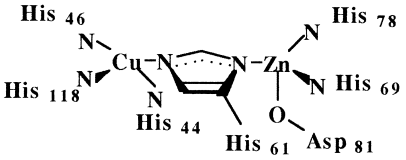


Fig. 4. The active site in Cu–Zn SOD.

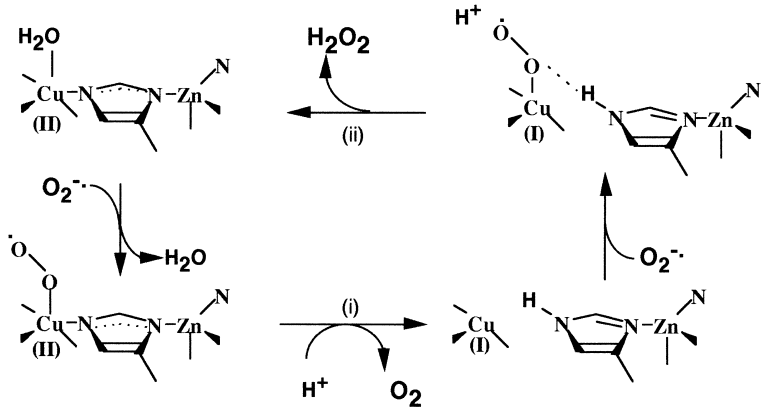


Fig. 5. Possible mechanism for Cu–Zn SOD (from Ref. [9]).

nuclear copper(II)–zinc(II) complex of a macrobicyclic ligand (Fig. 6). The ligand is able to accommodate the two Cu(II) and Cu(I) redox states without large conformational changes, owing to the flexibility of the tris-(2-aminoethyl) amine moiety. The macrobicyclic structure provides a very stable environment for the $[Cu\text{-}Im\text{-}Zn]^{3+}$ moiety. The closed bicyclic structure of the ligand is more selective than monocyclic or open-chain ligands: only small ligands (e.g. O_2^-) can get access to the copper center and the bicyclic structure is expected to play the role of the protein channel of SOD.

X-ray diffraction studies have shown a Cu–Zn distance of 5.93 Å (6.3 Å for the enzyme). ESR and electronic spectral parameters are close to those of the protein. Electrochemical studies have evidenced a quasi-reversible process (in DMA) in the first step of copper reduction ($E_{1/2} = -0.29$ and 0.31 V/SCE in water and DMA, respectively), as it is the case for the protein. This potential is lower than for the enzyme (0.075 V/SCE for the human enzyme in water), but in the good range for

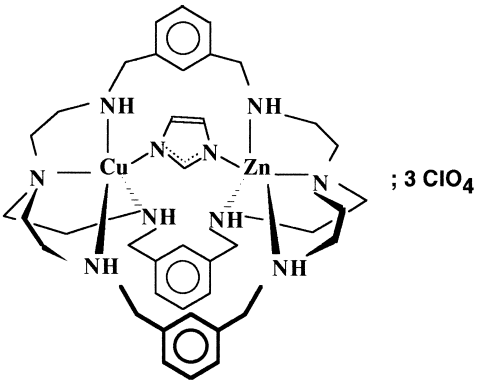
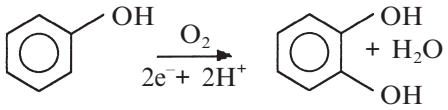


Fig. 6. A biomimetic model for the active site in Cu–Zn SOD (from Ref. [10]).

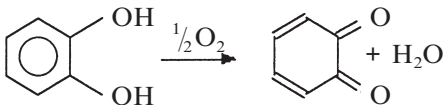
superoxide dismutation. Also remarkable was the large pH range stability of the imidazolate bridge in solution (from pH 6 to 10.5). This stability is significantly better than that of other abiotic models described in the literature and is close to that of the enzyme (from pH 4.5 to 11).

3. Tyrosinase: two metal centers for a two-electron oxidation

Tyrosinase (Tyr) is a type III protein containing a dinuclear copper active site in which the copper(II) centers are electronically coupled [11]. Tyr is a monooxygenase utilizing dioxygen for the ortho hydroxylation of monophenols (monophenolase activity):



Tyrosinase also possesses oxidase activities, catalyzing the oxidation of *o*-diphenols to *o*-quinones (catecholase activity):



As the diphenol product can serve as the two-electron source required for phenol oxidation and thus as a cosubstrate, tyrosinase is classified as an internal monooxygenase. Tyrosinase is widely distributed in nature; it is found in bacteria, plants, insects and mammals. Tyrosinase is used, for instance, for the formation of melanin pigments and is responsible of the browning reaction observed in fruits. The structure of tyrosinase is not known, but strong similarities exist between tyrosinase and hemocyanin, which performs the function of oxygen transport in mollusks and arthropods. Three imidazole groups are coordinated to each copper center and the copper–copper separation is 3.7 Å. The stable oxy form (oxy-Tyr) can be generated either from $Cu(I)_2$ plus O_2 or from $Cu(II)_2$ plus H_2O_2. The catalytic cycle for the phenolase activity is depicted in Fig. 7.

The crucial mono-oxygenation step (i) may be described with three possible scenarios (Fig. 8) [1]. Mechanism A may be regarded as an electrophilic substitution on the aromatic ring; the O–O bond cleavage of the μ-η^2:η^2 peroxo intermediate is concerted with the attack of the ring and no change occurs in the redox state of the copper centers. In mechanism B, an aryl peroxide species is formed resulting from two one-electron transfers to Cu(II). A third scenario (C) is suggested by Solomon et al. [8] in which the O–O bond is cleaved prior to the attack on the ring, yielding a binuclear Cu(III) bis μ-oxo species. Some comments may be added concerning the electron transfer steps. In mechanism A, the two-electron oxidation of the phenolic substrate is formally achieved by the copper-bound peroxide, without a

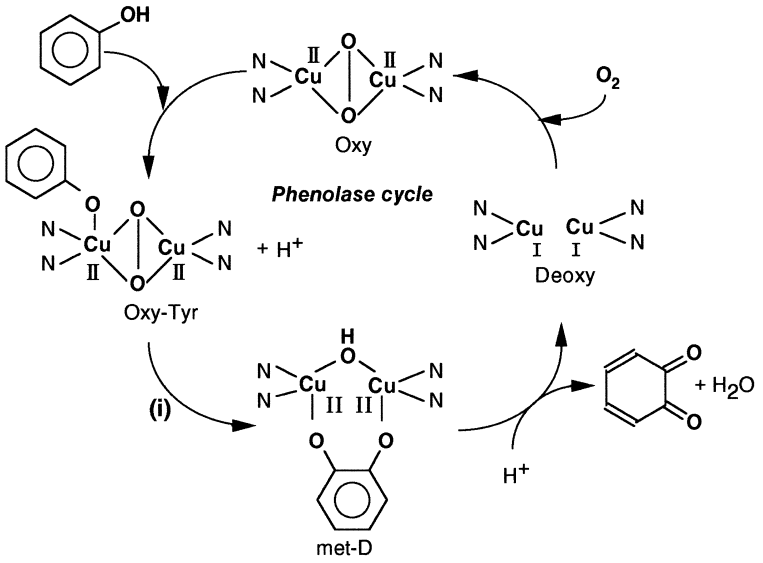


Fig. 7. Proposed mechanism for phenolase activity of tyrosinase (adapted from [8]).

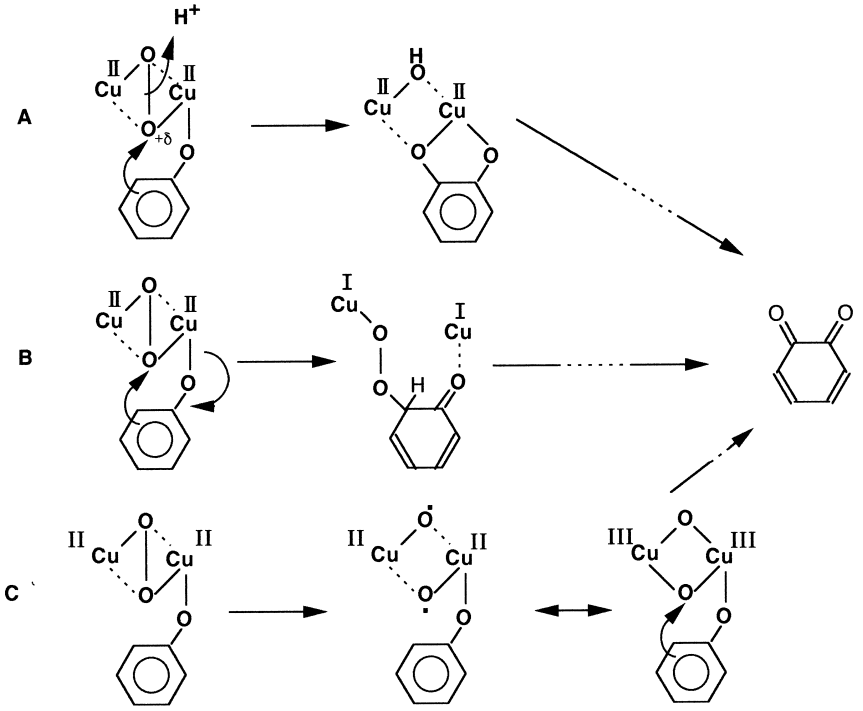


Fig. 8. Possible mechanistic pathways for step (i) (see Fig. 7).

change of the oxidation state of the copper ions. In mechanism B, the copper centers are involved in the electron transfer: each copper provides one oxidizing equivalent and is converted from Cu(II) to Cu(I). In mechanism C, the two copper centers have been formally one electron oxidized by each one of the oxygen atoms which are reduced to the −II state. Oxidation of the substrate proceeds by transfer of one oxygen atom and reduction of the copper centers.

A number of biomimetic models for the chemistry of tyrosinase have been described [8,12,13]. Among them, μ-η^2:η^2 peroxo-coordinated dioxygen complexes has been shown to be able to activate C–H bonds [14,15].

4. Galactose oxidase: one metal center and one organic free radical on a specific amino-acid residue for a two-electron transfer

Galactose oxidase (GOase) is an extracellular type II copper protein (68 kDa) of fungal origin [16]. GOase catalyzes the oxidation of several primary alcohols to aldehydes with the concomitant reduction of molecular oxygen to hydrogen peroxide, involving a two-electron transfer reaction. The crystal structure (1.7 Å resolution) of GOase [17] reveals a unique mononuclear copper site with two histidine imidazoles, two tyrosines (one axial) and an exogenous water or acetate, in a distorded square-pyramidal coordination. The equatorial tyrosine ligand is covalently linked to a cysteine residue by a C–S bond at the position ortho to the hydroxyl group (Fig. 9).

In GOase, the number of metal ions involved in the reaction does not match the number of electrons transferred. This paradox has been solved with the identification of a tyrosyl free radical incorporated into the redox unit during the catalytic cycle. The enzyme exists in three well-defined and stable oxidation levels: the active oxidized form is EPR-silent indicating that the cupric ion is antiferromagnetically coupled to a free radical, the intermediate form shows a cupric EPR signal (type II copper

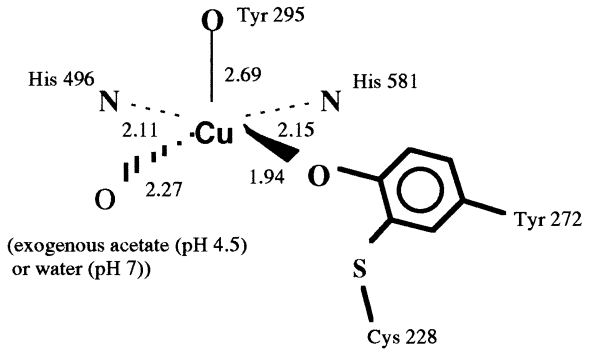


Fig. 9. The active site in GOase.

protein) and the reduced form contains a cuprous center:

$$Cu^{2+}-Tyr^{\cdot} \overset{+e^-}{\rightleftharpoons} Cu^{2+}-Tyr \overset{e^-}{\rightleftharpoons} Cu^{+}-Tyr.$$

The enzyme could easily be interconverted between the active and the inactive forms in a redox titration using ferri/ferrocyanide solution [16]. Whittaker has shown that the tyrosyl free radical is located on the equatorial cysteine-substituted residue and is coordinated to the metal ion. As a matter of fact, the active enzyme has a diamagnetic ($S=0$) ground state. SQUID measurements gave evidence for the thermal population of a paramagnetic excited state at higher temperature with $-2J \geq 200\ cm^{-1}$. The electronic spectrum of the active form displays a band at 445 nm which has been shown by resonance Raman spectroscopy to be contributed by the tyrosyl radical-to-metal charge transfer transition: the tyrosyl radical is ligated to the copper(II) center [16]. The redox potential for activation is O.41 V/NHE. The thioether substitution could stabilize the radical by several hundred millivolts.

The specificity of the enzyme for primary alcohols is low, ranging from small molecules (e.g. propanediol or dihydroxyacetone) to polysaccharides. Nevertheless, GOase is stereospecific removing the pro-S methylene hydrogen of the C-6 alcohol in galactose. Specificity for oxidants is also broad: in the absence of O_2, several one-electron redox agents can act as electron acceptors.

The radical active form of GOase is ESR silent, but the ESR signal of the tyrosyl radical can be generated from the apoenzyme [16]. When the metal-free GOase was exposed to mild oxidizing conditions (e.g. ferricyanide) a sharp signal was detected in the ESR spectrum (fitting simulated with experimental ESR spectrum gave $g_1=2.0017$ and $g_2=2.0073$). The radical of the metal-free protein is stable; it is unreactive towards galactose; it may be reduced by hydroxyurea. Labelling experiments demonstrated that the radical was formed on the thioether substituted tyrosine.

The mechanistic scheme for the catalytic cycle of GOase is depicted in Fig. 10 (adapted with minor changes from Ref. [16]). Two electrons are transferred from the substrate, one to the free radical and one to the cupric ion; then oxygen restores the active form by oxidation of the cuprous center and the modified tyrosine. In the proposed mechanism, the aldehyde release precedes dioxygen reduction. Under anaerobic conditions, galactose is oxidized into aldehyde and the copper(I) state is stable. The biological function of GOase is probably to serve as a generator of hydrogen peroxide (this accounts for the unusually low specificity of GOase for organic substrates) supporting lignin peroxidase reactions rather than as a source of aldehydes.

5. Biomimetic models for GOase

Some copper(II) complexes containing one or two phenolic and benzimidazole or pyridine ligands have been described as models for the structure of the active site in GOase, two of them involving a phenoxy radical associated to a copper(II) center

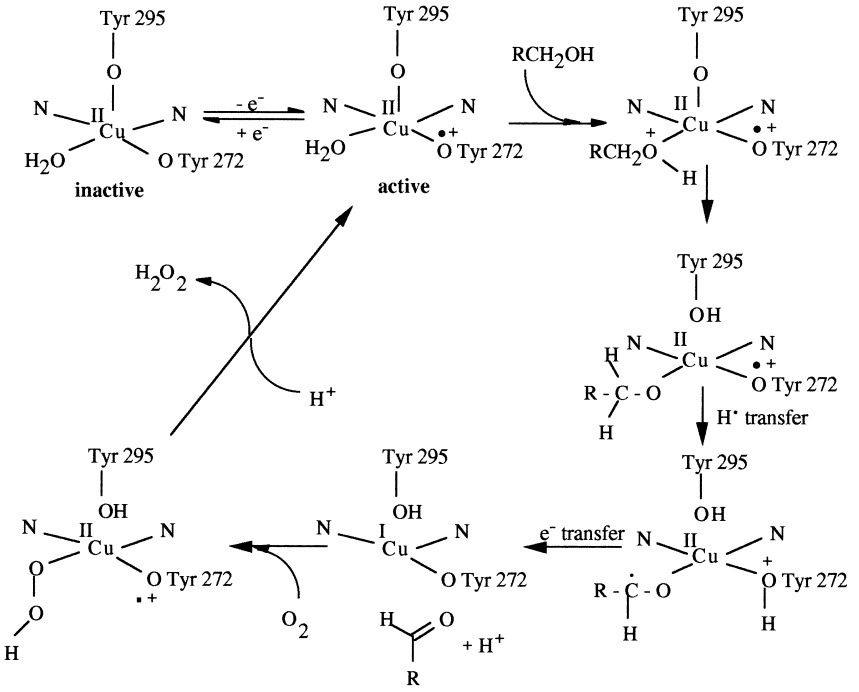


Fig. 10. The possible catalytic cycle of GOase (adapted from Ref. [16]).

[18,19]. Pierre et al. [18] have evidenced in acetonitrile the formation of an axial phenoxy radical upon electrochemical one-electron oxidation of a mononuclear copper(II) complex derived from bis (3′-t-butyl-2′-hydroxybenzyl)(2-pyridylmethyl)amine (Fig. 11). This radical–copper(II) complex has a N_2O_2 coordination sphere of the same type as the enzyme and reproduces several features of the enzymatic system (electronic and Raman spectroscopy, electrochemistry), but it does not reproduce the radical control observed in GOase (the axial instead of equatorial phenoxy radical is not antiferromagnetically coupled to the copper). On another hand, the species obtained in methanol which may be easily protonated on the axial

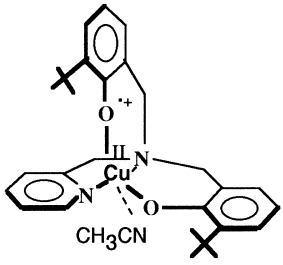


Fig. 11. A model for the active form of GOase (from Ref. [18]).

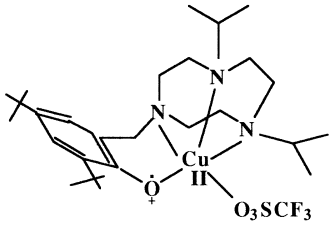


Fig. 12. Tolman model for GOase (from Ref. [19]).

phenolic moiety may be considered as a good model for a substrate adduct [20,21].

Tolman [19] has used a triazacyclononane derivative bearing one phenolic arm as a N_3O pivotal ligand. He has obtained upon one-electron electrochemical oxidation in acetonitrile of the trifluoromethane sulfonate copper(II) complex, an antiferromagnetically coupled equatorial phenoxy radical–copper(II) complex (Fig. 12).

An exogenous alcoholate ligand (from benzylic alcohol) instead of trifluoromethane sulfonate, leads to a copper(II) complex which is destroyed upon irreversible one-electron oxidation giving benzaldehyde and concommitant oxidative breaking of the C–N bond between the triazacyclononane and its phenolic arm.

Further studies are needed to realize the catalytic oxidation of primary alcohols to aldehydes by a phenoxy radical–copper(II) complex. Nevertheless, the first studies presented here are very promising. Attention has to be drawn to the functional competence of the adduct of copper(II) salts with TEMPO to oxidize alcohols [22].

6. Copper amine oxidases use a quinone (topaquinone) cofactor for a two-electron redox process

Copper-containing amine oxidases catalyze the oxidative deamination of primary amines by dioxygen in a two-step aminotransferase reaction to produce hydrogen peroxide, ammonia and aldehydes [23,24]. These enzymes are ubiquitous, occuring in both prokaryotes, in which they allow the microorganism to utilize various amine substrates as sources of carbon and nitrogen, and eukaryotes, in which they are suggested to participate in detoxification processes, cell signaling, lignification (in plants) and defense.

The active site is known with some details, since two crystal structures are now available, both from *Escherichia coli* and pea seedling enzymes [25,26]. It contains a single Cu(II) ion with three histidine ligands and two water ligands, one equatorial and one axial, in a distorted square pyramidal geometry. More remarkable is the presence of a new type of protein-bound cofactor, 2,4,5-trihydroxy-phenylalanine quinone (TPQ), located about 6 Å away from the copper atom (Fig. 13). The TPQ cofactor is, thus, not directly coordinated to Cu(II). The active site also contains a conserved aspartate, which has been recently shown, from the crystal structure of a complex between the copper amine oxidase from *E. coli* and a covalently bound

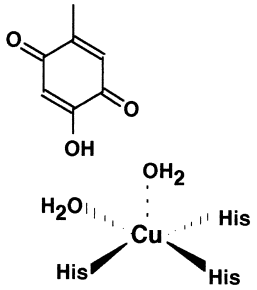


Fig. 13. The active site in PSAO.

inhibitor, 2-hydrazinopyridine and from site-directed mutagenesis studies, to be essential for catalysis [27,28].

There is evidence that the formation of the TPQ cofactor is a self processing reaction that does not require any specific enzyme system [24]. The protein-bound copper plays, thus, a crucial role in the conversion of a specific tyrosine residue, belonging to a highly conserved sequence, T-X-X-N-Y-D/E, into TPQ. As a matter of fact, the ability to form TPQ was totally abolished by mutation of one of the histidine ligand and this was associated with very poor copper-binding capacity of the mutant enzyme. Further, a copper-free and quinone-less inactive form of phenethyl amine oxidase could be transformed into an active form, containing TPQ, just by addition of Cu(II), in the presence of air, suggesting that the activation reaction looks like a Cu-mediated air-dependent autoxidation of the tyrosine side chain. The selectivity of the reaction is due to the highly specific Cu-binding property of the protein.

A likely pathway for the generation of TPQ is depicted in Fig. 14 [24,29]. Binding of Cu(II) provides to the protein a one-electron oxidant which can oxidize the

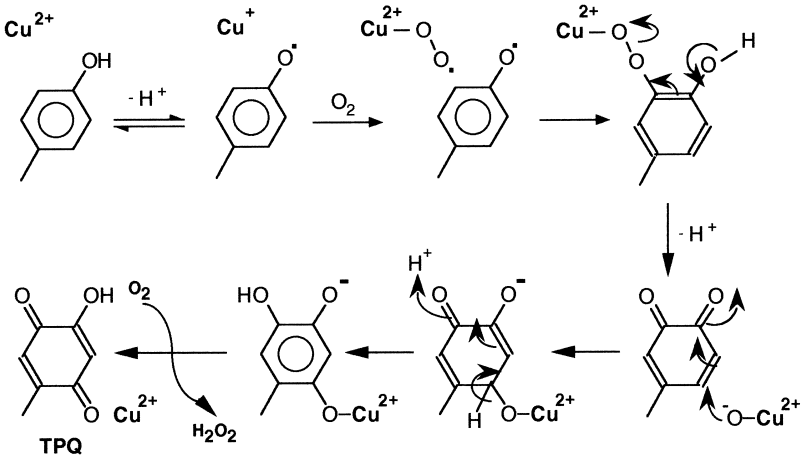


Fig. 14. Proposed mechanism for the generation of TPQ.

target tyrosine into a transient tyrosyl radical. This intermediate is difficult to detect as the equilibrium lies strongly in favor of the precursor, as shown by Cu-binding experiments under anaerobic conditions. Now Cu(I) can bind molecular oxygen and generate a superoxide radical-like species. A coupling reaction with the ortho carbon of the tyrosyl radical generates a copper-bound peroxo intermediate. Coupling between tyrosyl and superoxide radicals has been reported previously both in model reactions or during inactivation of the tyrosyl radical enzyme, ribonucleotide reductase, by superoxide radicals generating systems [30]. The peroxo complex is then decomposed to generate dopaquinone and a copper oxide/hydroxide complex. After rotation of the phenyl ring, the water-exchangeable nucleophilic hydroxide attacks the dopa quinone to yield TPQ. This is in agreement with ^{18}O labeling studies which showed that the oxygen at position 2 derives from solvent water.

The TPQ cofactor is essential for oxidation of primary amines. It, thus, provides the two-electron oxidizing power of the enzyme. As a consequence, the enzyme mechanism shown in Fig. 15 consists of two half-reactions. The first involves covalent binding of the substrate to TPQ and generation of a Schiff-base complex and was supported by elegant model studies [31]. Deprotonation of that complex by the active site aspartate and hydrolysis of the intermediate imine then yields a reduced aminophenol form of the TPQ cofactor and liberates the aldehyde product. In the second step of the reaction, the reduced cofactor is reoxidized to TPQ by molecular oxygen, thus producing hydrogen peroxide and ammonia. There, the copper center

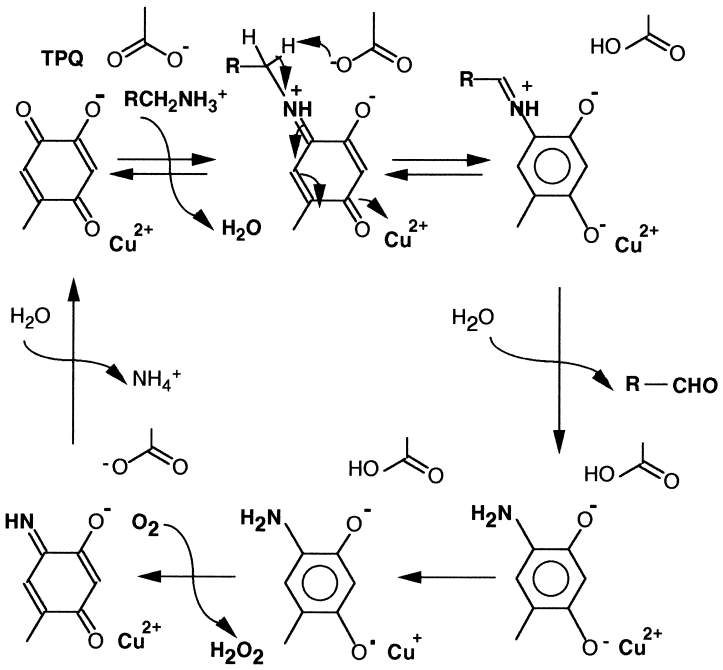


Fig. 15. Proposed reaction scheme for the oxidation of amines by amine oxidases.

serves to catalyze the reaction which probably occurs in two sequential one-electron steps via a Cu(I)–semiquinone intermediate.

7. Conclusion

The common feature of the catalytic systems described in this paper is the oxidation of the substrate. Two situations are encountered with regard to the dioxygen chemistry:

(1) The removing of two protons and two electrons observed in the $CH_2OH \rightarrow CHO$ chemistry (galactose oxidase); no interaction occurs between the substrate and dioxygen and the role of dioxygen is only to transfer two oxidizing equivalents (abstract two electrons) to the reduced form of the enzyme: so, dioxygen is reduced into hydrogen peroxide.

(2) The monooxygenase activity which requires the O–O bond breaking of dioxygen for the transfer of one oxygen atom to the substrate to assume the C-H→C-OH chemistry, the second atom being recovered into a water molecule (tyrosinase). A biomimetic dicopper complex, modeling the O–O bond breaking process has been described in an outstanding paper by Tolman et al. [32]. A very recent account summarizes the last insights from studies of synthetic copper complexes, concerning making and breaking the dioxygen O–O bond [33].

As far as biological oxidations are concerned, the actual challenge is the generation of an active oxidant. In most cases, the solution is a metal-based species. Nevertheless, it is now clear that an alternative is the generation of an organic oxidant. The case of copper enzymes described here illustrates this concept with either free radicals (like the substituted tyrosyl radical in galactose oxidase) or quinones (like TPQ in copper-amine oxidases) being used for substrate oxidation. The copper center may, thus, not directly involved in the reaction but rather in the O_2-dependent formation of the oxidant. This strategy has recently emerged, both in copper and iron-dependent enzymes and it appears that organic oxidants may be as useful as high-valent metal oxo species. This opens a way for new synthetic catalytic systems based on organic radicals. For example, we have described a process of synthetic value for the oxidation of organic substrates (including hydrocarbons) by molecular oxygen, mediated by a phtalimide *N*-oxyl radical [34].

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