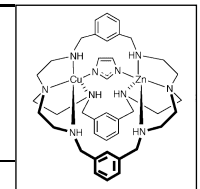
One electron at a time oxidations and enzymatic paradigms: from metallic to non-metallic redox centers



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Biomimetic models of redox metalloenzymes may serve to elucidate structure and mechanisms of these enzymes and, moreover, new catalysts for synthesis can ensue from these models. A number of metalloenzymes utilize free radicals as a catalytic competent cofactor to promote oxidation reactions. Non metallic redox centers (free radicals) may be an advantageous alternative to metallic centers. Several biomimetic approaches are described concerning two selected enzymes, copper–zinc superoxide dismutase and galactose oxidase. The advantages of organic radical instead of metallic centers in abstracting hydrogen atoms are discussed.

Paradigme: il s'agit, à partir d'un objet assez simple de découvrir une structure qui se retrouvera dans un objet plus élevé.

Dictionnaire de Philosophie (Nathan ed. 1990)

1 Introduction: Biomimetic chemistry and redox metalloenzymes: why and what?

The field of biomimetic chemistry covers a large area, quite as large as biochemistry itself. Non-exhaustively, we can emphasize the following topics: (i) abiotic models for the active sites of enzymes, (ii) models for biological processes such as: allostery, photosynthesis, long-range electron transfers, self-assembly and replication, compartmentalization (reactions at liquid–liquid interfaces, microheterogeneous media like micelles, microemulsions, vesicles, membranes), (iii) abiotic models for ion carriers, (iv) organic syntheses inspired by biosyntheses. All the fields of chemistry are concerned: (i) organic and inorganic syntheses, (ii) physical chemistry, electrochemistry, photochemistry and spectroscopy, (iii) reaction mechanisms, (iv) supramolecular chemistry and molecular recognition, (v) macromolecular chemistry.

Abiotic models may serve: (i) to elucidate structures of biomolecules (enzymes) not known or partially known to date, (ii) to elucidate biological mechanisms which remain black boxes, (iii) to prepare authentic reagents (catalysts) usable for chemical syntheses. The applied fields which ensue may be: (i) new catalysts (syntheses), (ii) new drugs (medicinal chemistry), (iii) new nutrients (agrochemistry), (iv) tools for biological studies. It has also to be emphasized that biomimetic chemistry not only serves biology, but may also be a conceptual approach for chemical problems. As an example, organic syntheses may be inspired by known biosynthetic pathways.

Two types of models for the active sites in metalloenzymes can be envisaged: structural models and functional models (of course, the ideal models are relevant to the two types!). *Structural models* for the active sites in metalloenzymes can be used *to help the determination of the active site molecular structure*. The design of these models starts from partial spectroscopic data concerning the enzyme itself and from the ensuing hypotheses for the molecular formula of the active site. The comparison of the spectroscopic data obtained from the model and from the enzyme respectively, confirms or invalidates the hypotheses. So, step by step, the hypotheses for the active site structure are improved. A lot of structures have been established by this approach, which has been further confirmed by crystallographic structural determinations. Structural models may also be functional models. Mimicking the known (crystallographic data) structure of the active site of the enzyme can lead to models which exhibit the catalytic function of the enzyme. These models may be valuable *catalysts* for syntheses. They are also *tools for studies concerning the enzymatic mechanism*: some transient species are often involved for a given enzymatic catalytic cycle, which are more or less characterized. Good models for these reaction intermediates may be key steps for the understanding of the enzymatic process.

The design of *functional models* for redox metalloenzymes can start from the known, partially known or unknown structure of the active site of the enzyme. The stoichiometry of the catalyzed reaction and the products of the reaction are the only properties which have to be known (Scheme 1).

We will place this paper in the field of *biomimetic chemistry of redox enzymes* and we will focus our attention on the use of organic redox species replacing metal centers or synergetically working with metal centers, to perform one electron at a time oxidations.

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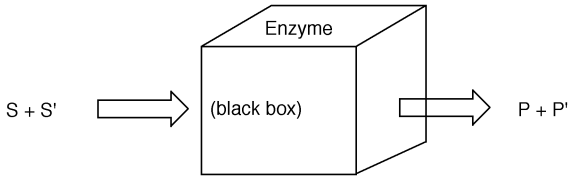


Scheme 1 What is in the black box?

2 One-electron transfer only and superoxide dismutase: one metal center is used; no bond is broken in the substrate

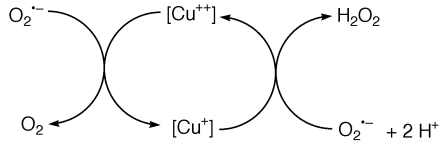
One of the simplest elementary acts in homogeneous solution chemistry is the transfer (exchange) of one electron, as no bond needs to be formed or broken. Two partners are required, D (donor or reducing agent) and A (acceptor or oxidizing agent), each with at least two accessible oxidation states. When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

(i) 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.

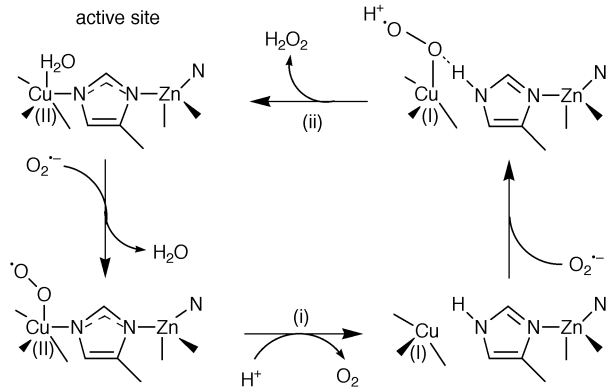
(ii) inner-sphere electron transfer when the donor and acceptor form a direct chemical bond: a bridging ligand common to both coordination shells connects the two metal ions providing a series of overlapping orbitals for efficient adiabatic electron transfer. This case is often reserved for catalytic steps in enzymes.

2.1 Copper–zinc superoxide dismutase

We will quote as an example, the case of superoxide dismutase in which only one metal center is used for a one-electron transfer, and in which no bond is broken in the substrate. Copper–zinc superoxide dismutase (SOD) is composed of two identical subunits each containing in its active site an imidazolate-bridged bimetallic center with one copper(II) and one zinc(II) ion.[1] 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* two diffusion-controlled one-electron redox processes ('ping-pong' mechanism):



The metal-binding site and the catalytic cycle are depicted in Scheme 2. The Cu^{2+} 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 an aspartate and to three histidines, one of them bridging to copper. The superoxide ion is electrostatically guided through a deep (≈13 Å) and narrow (≈4 Å) channel lined by positively charged amino acid residues. The probable role of the zinc ion is to confer stability on the protein which is remarkably stable to heat and is active from pH 4.5 to pH 10. The crystal structure of the azide-inhibited bovine Cu, Zn SOD has been described,[2] evidencing the direct coordination of the azide anion to the Cu(II), at the place of the metal-bound water molecule and, so, mimicking the enzyme–substrate interaction. The oxidation

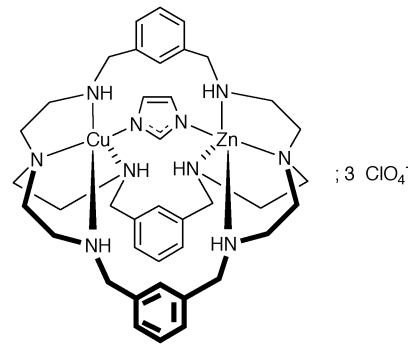


Scheme 2 'Cu–Zn SOD' (from ref. 1).

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 dioxygen is released. The reduction step is an inner sphere electron transfer between a superoxide anion and the copper(I) center,[1] but in a recent study, a structure-based mechanism is described which involves an outer sphere electron transfer for the reduction step.[3]

2.2 Models for superoxide dismutase

Many low molecular weight copper chelates are known to exhibit, *in vitro*, superoxide dismutase-like activity.[4] 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 with log K = 16.2). A biomimetic model has been described by us,[5] 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 heterodinuclear copper(II)–zinc(II) complex of a macrobicyclic ligand (Scheme 3).



Scheme 3 A biomimetic model for the active site in 'Cu–Zn SOD' (from ref. 5).

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–Im–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.

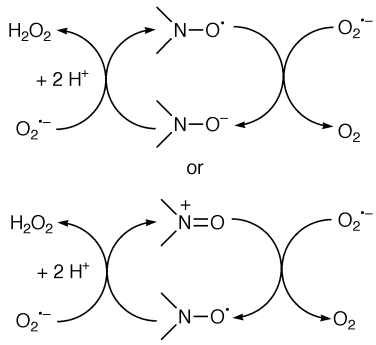
X-Ray diffraction studies have shown a Cu–Zn distance of 5.93 Å (6.3 Å for the enzyme). EPR and electronic spectral parameters are close to those of the protein. Electrochemical

studies have evidenced a quasi-reversible process (in DMA) in the first step of copper reduction ($E_{1/2}$ = −0.29 V/SCE in water), as is the case for the protein. This potential is lower than that of the enzyme (0.075 V/SCE for the human enzyme in water) but in a good range for superoxide dismutation. The pH range for stability of the model (from pH 6 to pH 10.5) is significantly better than that of other abiotic models described in the literature and is close to that of the native enzyme (from pH 4.5 to pH 11). The model described in Scheme 3 is also a functional model showing that a square planar coordination of the Cu(II) center and a high conformational mobility (allowing a reversible geometrical change according to the oxidation state) are not required for catalytic activity.

In theory, the simplest vision of a functional model of SOD is a copper complex [LCu] for which the Cu^{2+}/Cu^{+} redox potential is in accordance with:

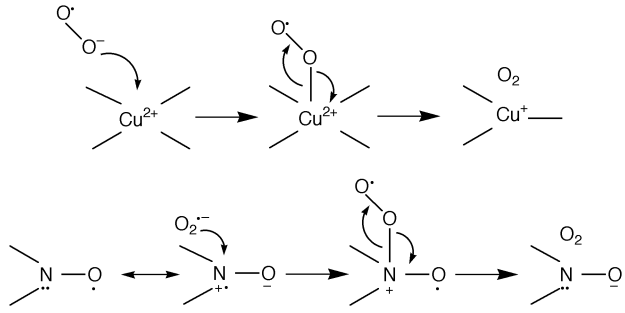
$$E_o O_2^{-}/O_2 < E_o\, LCu^{2+}/LCu^{+} < E_o\, O_2^{-}/O_2^{--}$$

and involving a proton donor species. It is possible to replace the copper atom by another metallic ion exhibiting a well-suited redox potential (Mn, Fe, Ru). It is also possible to replace the metal atom by a *non-metallic redox center* such as an aminoxyl radical[6] (Scheme 4).



Scheme 4 Aminoxyl as a functional mimic of SOD (two mechanistic pathways may be envisaged).

The analogy between the reaction mechanisms can be viewed (for instance for the first step of the former mechanism) in Scheme 5.



Scheme 5 One-electron oxidation of superoxide.

In fact, the two mechanistic pathways of Scheme 4 have been evidenced, the former with oxazolidine aminoxyls and the latter with piperidine aminoxyls.[6] These results demonstrate that organic radicals can be competent to exert a catalytic function which is exhibited by a metal center in the enzyme. Knowledge of the chemical criteria of the enzyme (such as redox potentials) is the only prerequisite for finding solutions other than that utilized by the enzyme.

3 One metal center plus an organic radical for two-electron oxidations

Usually, the equation of the catalyzed reaction reveals a multielectron transfer but, in several cases, the mechanism involves successive monoelectronic transfers, mediated by a redox active metal ion such as Fe^{3+} or Cu^{2+}. Numerous metalloenzymes which catalyse two-electron oxidations involve dinuclear centers (examples: methane monooxygenase, a diiron enzyme which catalyses the oxidation of methane to methanol by molecular oxygen, and tyrosinase, a type III dicopper protein, which catalyses the *o*-hydroxylation of monophenols by molecular oxygen). Biomimetic models have been described for many of them. Recent reviews[7] describe this type of enzyme and chemical models.

It is often claimed that the number of metal ions matches the number of transferred electrons. Nevertheless, this is sometimes not true, due to the *use of non-metallic redox centers by the metalloenzyme*. This point will be developed in the following section of this paper.

Some metalloenzymes involve an organic redox cofactor to provide or abstract electrons and complete the metal-driven electron transfers in the whole catalytic process. For example, cytochrome P-450 and heme peroxidases utilize the combination of an iron center and a porphyrin ligand, which can transiently exist in a rather stable cation radical form. Some metalloenzymes require quinone cofactors, such as topaquinone in copper amine oxidases, which catalyze the oxidative deamination of primary or secondary amines. A fascinating class is that of metalloenzymes which utilize their own polypeptide chain as a cofactor and generate organic free radicals on specific amino acid residues.[8] The amino acids usually involved in such a redox process include tyrosine (ribonucleotide reductase, photosystem II, prostaglandin H synthase), modified tyrosine (galactose oxidase, glyoxal oxidase), tryptophan (cytochrome *c* peroxidase) and glycine (ribonucleotide reductase, pyruvate formate lyase).

We will now present, as a typical case, the copper–tyrosyl enzyme galactose oxidase and structural or functional models of this enzyme.

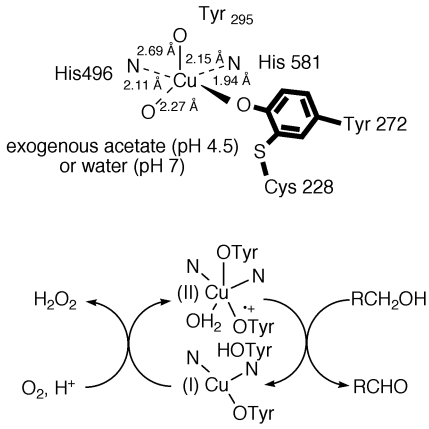
3.1 Galactose oxidase

Galactose oxidase (GOase) is an extracellular type II copper protein (68 kDa) of fungal origin.[9] 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[10] reveals a unique mononuclear copper site with two histidine imidazoles, two tyrosines (one axial) and an exogenous water or acetate, in a distorted 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 hydroxy group (Scheme 6) and is involved in a π-stacking interaction with a neighbouring tryptophan side chain.

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.[9] 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 protein) and the reduced form contains a cuprous center:

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

The enzyme can easily be interconverted between the active and the inactive forms in a redox titration using ferri/ferrocyanide (or other inorganic redox complexes) solution.[9,11] Whittaker has shown that the tyrosyl free radical is located on the equatorial cysteine-substituted residue and is coordinated to the



Scheme 6 The active site in GOase and the catalytic process.

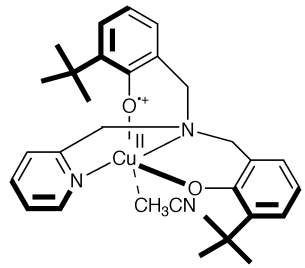
metal ion. The oxidation potential is +0.40 V *vs*. NHE (compared to +0.9 V for a 'normal' tyrosine side-chain). The specificity of the enzyme for primary alcohols is low, ranging from small molecules (*e.g*. propanediol or dihydroxyacetone) to polysaccharides. Specificity for oxidants is also broad: in the absence of O_2, several one-electron redox agents can act as electron acceptors.

3.2 Structural models for GOase

Some copper(II) complexes of tripodal ligands with a pivotal tertiary amine nitrogen atom, containing one phenolic and two pyridine (or benzimidazole) arms leading to a $[N_3O]$ coordination sphere around the copper center, have been described as models for the structure of the active site in the intermediate Cu(II) form of GOase.[12–14] Whittaker *et al.*[15] and we[16] have described models of the same type, but involving two phenolic arms, leading to a $[N_2O_2]$ coordination sphere, which is more relevant to the coordination sphere in the enzyme. Simple ligands with (methylthio) phenolate donors have been used in order to more closely mimic the cysteine-modified tyrosinate in the apoprotein.[17,18]

3.3 Models for the active form of GOase

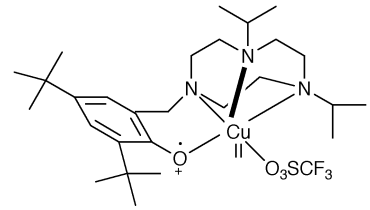
We have described the first model involving a phenoxyl radical associated to a copper(II) center possessing a N_2O_2 coordination sphere of the same type as the enzyme.[19] We have evidenced in acetonitrile the formation of an axial phenoxyl radical upon electrochemical one-electron oxidation of a mononuclear copper(II) complex derived from bis(3′-*tert*-butyl-2′-hydroxybenzyl)(2-pyridylmethyl)amine (Scheme 7). This radical–



Scheme 7 A model for the active form of GOase (from ref. 19).

copper(II) complex 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 phenoxyl radical is not antiferromagnetically coupled to the copper). On the other hand, the species obtained in methanol which may be easily protonated on the axial phenolic moiety may be considered as a good model for a substrate adduct.[20]

Itoh *et al.*[18] have studied the electrochemical oxidation of a tripodal copper(II) complex involving two pyridine arms and only one phenolic arm, plus an external pyridine ligand. Their studies revealed that the methylthio substituent in the phenolic arm shows electronic effects similar to those of the free ligand stabilizing the phenoxyl radical state in the Cu(II) complex. Tolman *et al.*[21] have used a triazacyclononane derivative bearing one phenolic arm as a N_3O pivotal ligand. They have obtained upon one-electron electrochemical oxidation in acetonitrile of the trifluoromethanesulfonate copper(II) complex, an antiferromagnetically coupled equatorial phenoxyl radical–copper(II) complex (Scheme 8). Tolman's model was the first model reproducing the radical control observed in GOase (an equatorial phenoxyl radical antiferromagnetically coupled to the copper).



Scheme 8 Tolman's model for GOase (from ref. 21).

An exogenous alcoholate ligand (from benzyl alcohol) instead of trifluoromethanesulfonate, leads to a copper(II) complex that models the reduced enzyme–substrate adduct. This complex is destroyed upon irreversible oxidation giving benzaldehyde and concomitant oxidative breaking of the C–N bond between the triazacyclononane and its phenolic arm. Although the aldehyde evolution is only stoichiometric, Tolman's model may be considered as the first model mimicking a part of the chemistry of the the galactose oxidase catalytic cycle (Scheme 6): the two-electron oxidation of a primary alcohol into aldehyde by a phenoxyl radical–copper(II) complex. In a subsequent paper, Tolman *et al*.[22] described several models of the same type, one of them involving two phenolic arms linked to the triazacyclononane frame.

The pioneering work concerning well-characterized metal complexes with bonded phenoxyl radicals is that from Wieghardt *et al.* who described, as early as 1993, Fe(III), Ga(III), Mn(III), Mn(IV) and Cr(III) complexes derived from the oxidation of complexes of 1,4,7-tris(phenolato)-1,4,7-triazacyclononanes [(23) and *ref. cit*. therein]. This group also described phenoxyl radical–copper(II) complexes[24] using the same type of ligands, and, in a very elegant further study, correlated the magnetic properties of copper(II)–phenoxyl radical species with the geometry of well-suited synthetic models which were diversely substituted.[25]

Another model of the active form of GOase has been obtained by electrooxidation of the parent phenolate complex by Halcrow *et al.*[26] The resulting EPR-silent copper(II) complex of a thioether-substituted phenoxyl radical $[Cu(L)(Tp^{Ph})]^+$ exhibits a UV–VIS–NIR spectrum very similar to that of the active GOase [LH_2 = 2-hydroxy-3-(methylsulfanyl)-5-methylbenzaldehyde; Tp^{Ph} = tris(3-phenylpyrazolyl)borate]. A comparable model has also been described with LH_2 = 2-(methylsulfanyl)-phenol.[27] In these models, the bidentate ligand L may be considered as an exogenous ligand.

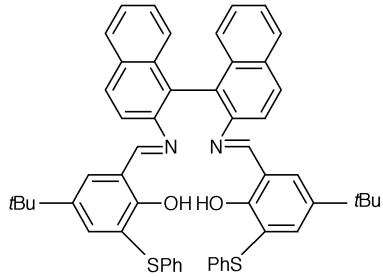
3.4 Model for the reduced form of GOase

Tolman has also structurally mimicked the reduced form of GOase with a three-coordinate Cu(I) complex involving one

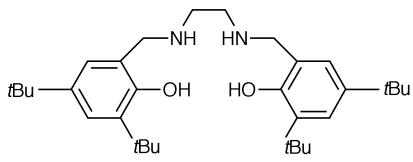
phenolic and two nitrogen donor ligands.[28] This complex is highly reactive toward dioxygen, but the formation of hydrogen peroxide has not been evidenced.

3.5 Catalytic models for GOase chemistry

The first copper(II) complex which may be regarded as a functional galactose oxidase model competent to catalyze the oxidation of primary alcohols to the corresponding aldehydes under oxygen pressure (30 psi) is a copper(I)–bipyridyl complex;[29] 38 turnovers have been obtained in 20 min with ethanol as a substrate and hydrogen peroxide was detected as a product. Another approach was the salen–Cu(II) complex from Kitajima *et al.*:[30] *N*,*N*′-(2-hydroxypropane-1,3-diyl)bis(salicylaldimino)copper(II) was found to be an effective catalyst for the oxidation of ethanol (0.35 mmol of acetaldehyde were formed in 10 h, using 0.06 mmol of the catalyst), propanol or hydroxyacetone in the presence of KOH under O_2. However, no evidence for GOase chemistry (phenoxyl radical occurrence, hydrogen peroxide production) has been shown. Ten years later, in 1996, functional models based on copper complexes of salen type ligands were described: the copper(II) complex with ligand L^1 (Scheme 9) catalyzed the oxidation of benzylic alcohol



L^1

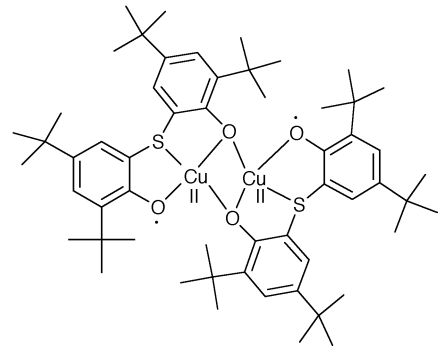


L^2

Scheme 9 Ligands for functional models of GOase.

(which serves as solvent) into benzaldehyde in the presence of 20 equivalents of the oxidant tris(4-bromophenyl)aminium hexachloroantimonate and 20 equivalents of *n*-BuLi (under N_2, at −15 °C) with 9.2 turnovers.[31] The one-electron oxidized copper(II) complex is EPR silent. Further studies with L^1Cu(II) evidenced the ability of this catalyst to oxidize, in the presence of a catalytic amount of KOH, benzyl alcohol, 1-phenylethanol or cinnamyl alcohol to aldehydes with O_2 with high turnovers (1300 in the best case).[32] Unfortunately, unactivated alcohols such as ethanol or methanol are not oxidized. We have shown that the electrochemically one-electron oxidized copper(II) complex of the salen ligand L^2 (Scheme 9) leads, in the presence of KOH, to the catalytic oxidation of methanol, ethanol, propan-1-ol and heptan-1-ol into the corresponding aldehydes with more than 30 turnovers at room temperature in acetonitrile[33] and, moreover, secondary alcohols are not oxidized.

Very recently, a clap of thunder was heard in the field of the research of functional models of GOase: a paper from Wieghardt *et al.*[34] described a binuclear copper(II)–bis(phenoxyl radical) complex (Scheme 10), efficient for the catalytic

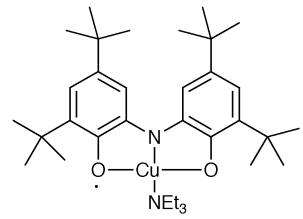


Scheme 10 A functional model for GOase (from ref. 34).

aerobic oxidation of primary non-activated alcohols such as ethanol (but not methanol) into aldehydes and secondary alcohols into 1,2-glycols, with the concomitant production of hydrogen peroxide. More than 600 turnovers of the catalytic cycle were observed in 12 h.

It has to be emphasized that the catalyst uses only the oxidation equivalents stored in the two phenoxyl radical ligands; the Cu(I) state is not involved. *Two non-metallic redox centers have been involved for a two-electron oxidation.* The role of the Cu center is to coordinate the alcoholate anion and to render the coordinated phenolate form reactive towards O_2. The phenoxyl radicals are used for the rate-determining C–H abstraction step, from the coordinated alcoholate anion.

Nine months later, Wieghardt *et al.*[35] described the oxidation of primary alcohols only (*e.g.* ethanol but not methanol) with O_2 to aldehydes and H_2O_2 by a new mononuclear Cu(II)-iminosemiquinone catalyst (Scheme 11). The turnover fre-



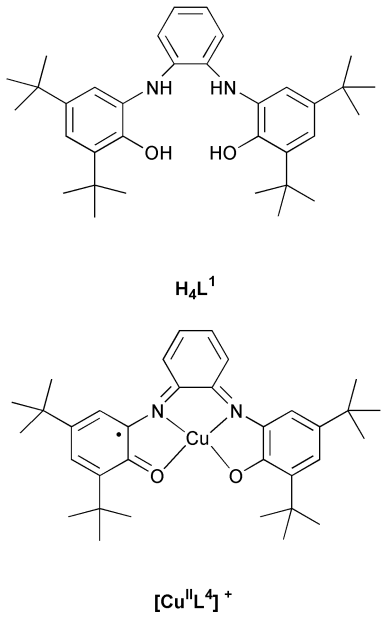
(neutral complex)

Scheme 11 A functional model for GOase and its chemistry (from ref. 35).

quency is 0.5 s^{-1}. The proposed mechanism, in accordance with all the data, is similar to the mechanism proposed for GOase itself: at the beginning, the catalyst binds the alcohol; in the rate-determining step, hydrogen atom transfer from the α-C atom of the alcoholate ligand to the radical-ligand occurs; the resulting coordinated ketyl radical anion transfers very fast an electron to the Cu(II) ion, during which the aldehyde is formed and dissociates; the Cu(I) form can now react with O_2 to form a superoxide complex, which then provides non-coordinated H_2O_2 and regenerates the active catalyst.

Lastly, Wieghardt reported two new catalysts which effectively oxidize (at ambient temperature) primary alcohols, including methanol, with dioxygen to aldehyde and H_2O_2 but not secondary alcohols.[36] These catalysts are the complexes $[Cu(L^4)]PF_6$ and $[Zn(L^4)]PF_6$ obtained from the ligand H_4L^1 (Scheme 12). Up to 5×10^3 turnovers in 50 h have been achieved with the copper catalyst and 170 turnovers in 24 h have been achieved with the zinc catalyst. These catalysts are the most effective and stable ones reported to date, but overall the process is slow.

The dianion $(L^3)^{2-}$ represents the two-electron oxidized form of $(L^1)^{4-}$ (diiminoquinone) and the $[Cu^{II}(L^3)]\cdot CH_3CN$ and the $[Zn^{II}(L^3)]\cdot CH_3CN$ square planar complexes have been characterized by X-ray crystallography. The ligand $(L^4)^-$ is a



Scheme 12 Ligand and functional model of GOase (from ref. 36).

paramagnetic organic radical ($S = \frac{1}{2}$); its cupric complex is diamagnetic ($S = 0$) and its zinc complex is paramagnetic ($S = \frac{1}{2}$). These complexes are the one-electron oxidized forms of $[M(L^3)]$ (M = Cu, Zn) respectively. *The redox chemistry during the catalytic process is ligand-based*: the two-electron reduced form of the catalysts is $[M^{II}(L^2)]^-$, in which $[L^2]^{3-}$ is the trianionic form of the diiminosemiquinone. $[M^{II}(L^2)]^-$, electrochemically generated, reacts with O_2, yielding quantitatively H_2O_2 and $[M(L^4)]^+$ in the presence of protons. $[Cu(L^4)]PF_6$ and $[Zn(L^4)]PF_6$ are not only highly efficient functional models for GOase, but also underline the interest in non-metallic redox centers as a source of oxidizing equivalents and the possible competence of their reduced state to activate dioxygen.

Of course, the Wieghardt models are to date the best models for the chemistry of GOase. Other approaches based on non-metallic redox centers effective for the oxidation of alcohols may nevertheless be envisaged. In particular, attention has to be drawn to the functional competence of the adduct of copper(II) salts with TEMPO to oxidize alcohols.[37] We have observed the catalytic oxidation of benzyl alcohol into benzaldehyde (no overoxidation into benzoic acid has been detected) by the system *N*-hydroxyphthalimide–CuCl–air.[38]

Some of the biomimetic functional models of GOase described in this review can effectively be used in organic synthesis, for chemoselective oxidation of primary alcohols. They can also be used as *a factory of hydrogen peroxide*. The biomimetic approach has led to efficient catalysts; however, some features of the enzyme have not yet been investigated, such as the stereoselectivity between *pro R* and *pro S* hydrogen atoms of the substrate, during their abstraction step by the radical species; this selectivity would involve the use of chiral models.[39] On the other hand, models without any metal center may also be envisaged. So, we think that the story of GOase biomimetic models is not at an end.

4 Why tyrosyl radicals in metalloenzymes?

Hydrogen atom abstraction is a key step in numerous oxidation processes and is implicated in the catalytic cycles of a variety of metalloenzymes. The abstracting agent is often a metal–oxo complex. A necessary requirement is radical character at the oxygen that accepts the H and the analogy of these metal–oxo intermediates with organic radicals has been underlined.[40] Iron–oxo species were probably the first metal–oxo species used by living systems and remain the most frequently used. There is now ample evidence that organic radicals, and especially tyrosyl radicals, may serve important functions in oxidation catalysis.[41] It is thus tempting to suggest that tyrosyl radicals appeared in redox active metal-containing proteins as a consequence of the increased oxidizing atmosphere, once molecular oxygen appeared on the surface of the earth. The different radicals possess various stabilities and reactivities. In most cases, the resulting radical proteins were useless and were destroyed. Nevertheless, a few tyrosyl radicals served new functions. What could be the advantages of maintaining a tyrosyl radical within an enzyme? A key property of a tyrosyl radical is its high and easy-to-modulate redox potential. As a consequence, it is an elegant way to preserve oxidizing equivalents, provided by metal centers. H abstraction by radicals such as alkoxyl, phenoxyl or hydroxyl radicals is energetically favored over a hydrogen atom transfer to an inorganic metal-based oxidant. The thermodynamic affinity of a tyrosyl radical for a hydrogen atom is large. Another advantage of a tyrosyl radical resides in the possibility it offers to the metalloenzyme to delocalize, through well-defined long-range electron transfer pathways, oxidizing equivalents and storing them at variable distances from the primary metal site. The polypeptide chain thus provides a mechanism for regulating the oxidizing reactivity of the system.

5 Conclusion and outlook

Oxidases or oxygenases use Cu or Fe, and, to a lesser extent, other metals (Mn, V, Ni, …). The use of non-metallic redox centers is an alternative route used by nature and open to the imagination of chemists. Cu(II)-tyrosyl radical species are a fascinating class of metal–radical arrays in proteins that delocalize multiple oxidizing equivalents. By incorporating in their models only those features that are essential to the reactivity in the enzyme rather than attempting to reproduce the structural features of the active site of GOase, Wieghardt *et al.* obtained undoubtedly the best results. Fascinating perspectives are opened up by the design of molecular species containing oxidizing equivalents (two and even more) in a unique 'molecular box' involving metallic and non-metallic, or even only non-metallic redox centers, held together in an adequate manner allowing synergism towards the two required basic chemical acts: C–H abstraction and electron transfer. This bioinspired approach is, of course, not limited to copper and phenoxyl, nor to the oxidation of alcohols.

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7 References

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