What Can We Learn from Nature about the Reactivity of Coordinated Phenoxyl Radicals?—A Bioinorganic Success Story

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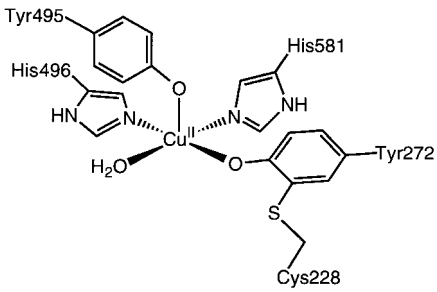
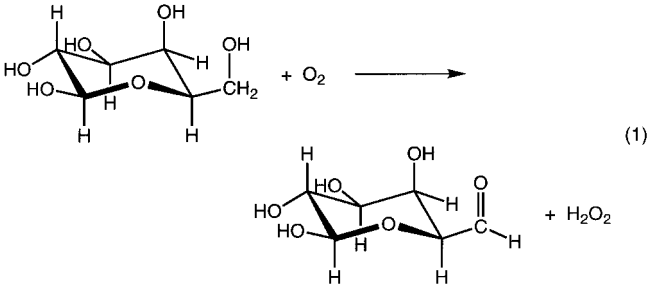
How does nature work on the molecular level? With respect to the function of metal ions in biology, the answer to this important question is intensively pursued by those researchers engaged in the highly interdisciplinary field of bioinorganic chemistry. Inorganic chemists can contribute considerably to the understanding of the structural, electronic, and mechanistic aspects of metal sites in metalloproteins by producing small coordination compounds which mimic the specific properties of those metal sites. Most model complexes concentrate on reproducing specific structural or electronic features; only a few models succeed in displaying stoichiometric or even catalytic reactivity similar to that of the enzymes. But very rarely are both structural *and* functional modeling characters found in a single complex. The latest success story in the development of biomimetic model complexes that mimic structural as well as catalytic aspects of the active sites in metalloproteins was written by Stack et al. with a model for the copper site in the enzyme galactose oxidase.[1] Independently, a different catalytically functioning system was devised by the group of Wieghardt and Chaudhuri, based on the mechanistic principles of the same enzymatic reaction.[2]

Galactose oxidase (GO)[3] 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 [Eq. (1)]. The active site consists of a mononuclear copper ion in a square-pyramidal coordination geometry (Scheme 1).[4] At a pH of 7.0, the copper ion is coordinated to two histidine residues (His 496, His 581), a tyrosinate residue (Tyr 272), and a water molecule in the equatorial plane and to a further tyrosinate residue (Tyr 495) in the apical position. 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 sulfur atom of a nearby cysteine residue.



Scheme 1. Schematic view of the active site of galactose oxidase in the inactive state at pH 7.0.[4]

The mononuclear copper site can be present in three redox forms (Scheme 2); among those, only the fully oxidized and fully reduced redox states, GO_{ox} and GO_{red}, were identified as



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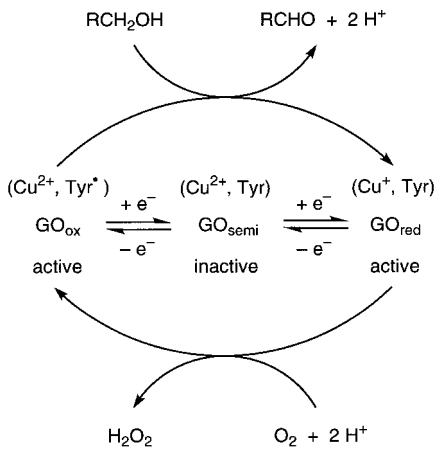

Scheme 2. Redox states of galactose oxidase.

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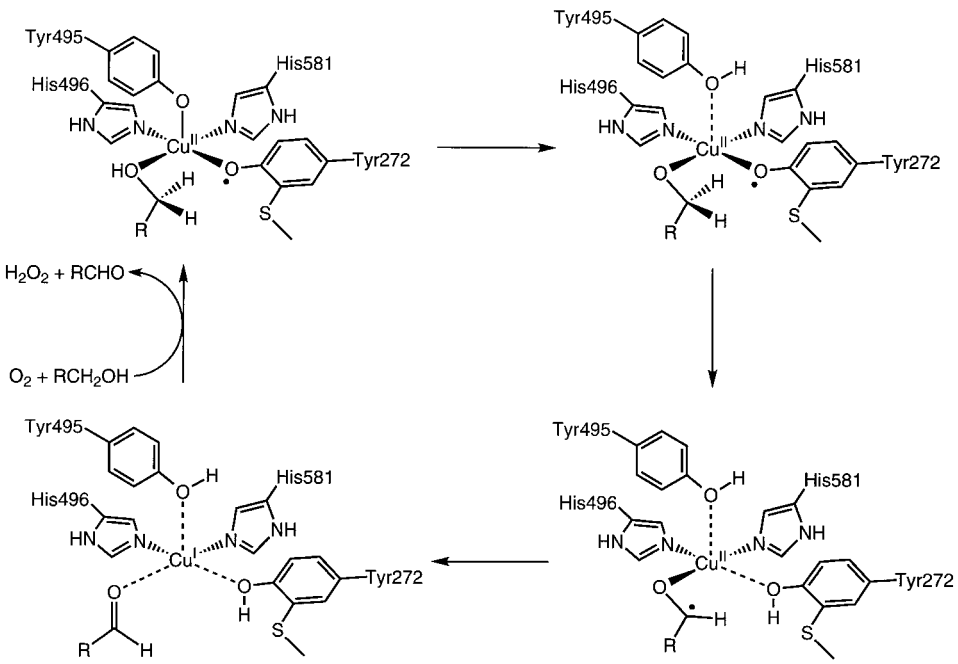
the catalytically active ones. For many years, these redox states were attributed to Cu^{I}, Cu^{II}, and Cu^{III} species, respectively. However, extensive spectroscopic studies of the diamagnetic, fully oxidized enzyme[5] and the oxidation product of the metal-free apo-enzyme unambiguously established[6] that the fully oxidized active site consists of a copper(II) ion antiferromagnetically coupled to a tyrosyl radical instead of the originally proposed copper(III) ion. The tyrosyl radical was determined to be derived from the equatorially bound, covalently modified Tyr272 residue.[7] The covalent modification of the tyrosine residue is thought to ease the oxidation of the coordinated tyrosinate ligand. Also taking the large kinetic isotope effect k_H/k_D[8] and the results from inhibitor studies into account,[9] a radical-based mechanism was proposed for the oxidation of the primary alcohols.[3a] Thus, the catalytic cycle (Scheme 3) starts with the binding of the galactose molecule to an equatorial coordination site of the copper(II) tyrosyl radical species and subsequent deprotonation of the alcohol, whereby the axial Tyr495 residue acts as the base.[10] Then, in a rate-determining step, a hydrogen atom is abstracted from the C-6 methylene carbon atom of the galactose substrate by the tyrosyl radical. The resulting ketyl radical is oxidized to the aldehyde by an intramolecular electron transfer to the copper(II) ion. The original copper(II) tyrosyl radical species is restored by the oxidation of the copper(I) ion and the tyrosine residue with dioxygen, whereby hydrogen peroxide is formed. Interestingly, the reactivity of galactose oxidase serves as an excellent example of the involvement of protein radicals in enzyme catalysis—an emerging theme in the research of enzyme mechanisms which has gained considerable importance in recent years.[3d]

At the time the coordination of a phenoxyl radical to a copper(II) ion was discovered in galactose oxidase, O-bound phenoxyl radical complexes were virtually unknown and represented quite a synthetic challenge to inorganic chemists. Through the study of the redox chemistry of metal phenolate complexes,[11, 12] criteria for the ligand were discerned that facilitate the preparation of such phenoxyl radical complexes. Thus, the phenoxyl radical moiety should be part of a multidentate ligand and should exhibit suitable bulky and oxidation-resistent substituents (e.g. *tert*-butyl or methoxy groups) in *ortho* and *para* position to the phenoxyl oxygen atom. Since the O-bound phenoxyl radical complexes are generally generated from the respective metal phenolate complexes by electrochemical or chemical oxidation, diagnostic tools for identifying the oxidation product as a coordinated phenoxyl radical complex needed to be developed. In addition to UV/Vis and ESR spectroscopy,[11, 12] Resonance Raman[13] and XANES spectroscopy[1] have been proven to be very useful in this process. The study of the phenoxyl radical complexes provides new insights into the structural, electronic, and spectroscopic aspects of the coordination chemistry of phenoxyl radicals. For example, with the help of such structural model complexes for the active site in galactose oxidase, Wieghardt et al. were recently able to provide a plausible explanation for the occurrence of a strong intramolecular antiferromagnetic coupling between electron spins of the coordinated tyrosyl radical and the copper(II) ion in the enzyme.[14] Despite the considerable progress made in synthesizing and characterizing structural model complexes for galactose oxidase, reactivity studies of phenoxyl radicals coordinated to copper(II) ions were scarcely reported presumably due to the instability of the copper(II) phenoxyl radical complexes at room temperature. This lack was recently alleviated by two remarkable studies which established high catalytic activities for the coordinated phenoxyl radicals of mono- and dinuclear copper(II) complexes.

The strategy followed in the first study[1, 15] to achieve an enzyme-like reactivity with small synthetic analogue-complexes consists in reproducing the structural and electronic features of the active site of the enzyme as closely as possible; by this means, catalytic reactivity similar to that of the enzyme should be feasible if the protein matrix itself is not involved in crucial reaction steps of the enzymatic catalysis and/or if the ligand employed in the model study can imitate certain electronic or structural effects of the protein matrix onto the metal site pertinent to the reactivity. Using this approach Stack et al. prepared copper complexes with various diimine-diphenolate ligands. With respect to the type and the electronic properties of the ligand donor atom set, the two imine and the two phenolate donor groups resemble the first coordination shell of the copper site in the enzyme. Based on an



Scheme 3. Postulated reaction mechanism for galactose oxidase (adapted from Whittaker).[3a]

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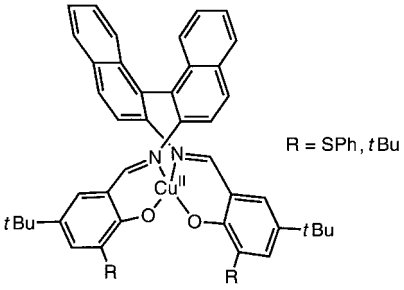
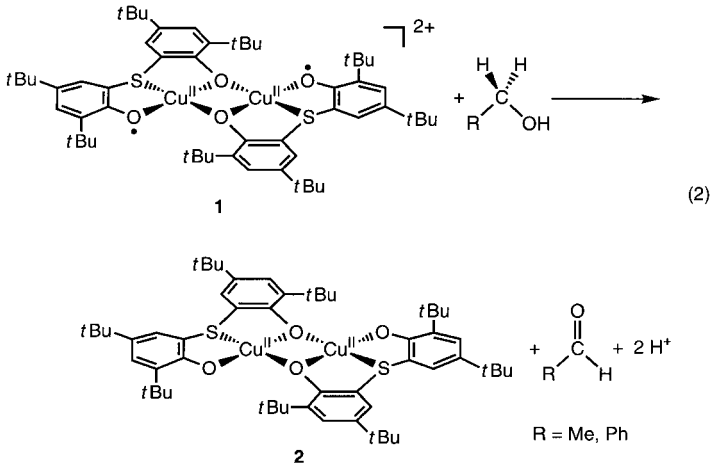
initial study by Kitajima et al.,[16] Stack et al. realized that, for the catalytic reactivity to occur, a nonplanar coordination environment is needed at the copper ion. Thus, they chose to incorporate a binaphthyl unit in the backbone of the ligand to enforce distortion of the square-planar coordination geometry (which is preferred by copper(II) ions) towards a tetrahedral geometry. This distortion is regarded as a way to increase the stability of the corresponding copper(I) species and to facilitate the coordination of a fifth ligand (i.e. the alcoholate substrate) to the copper(II) ion; both effects are considered to be critical in a catalytic mechanism. Further, in accordance with the results previously obtained from the investigation of structural model complexes, appropriate *ortho* and *para* substituents at the phenolate rings are implanted in order to stabilize the copper(II)-phenoxyl radical species. And indeed, with this type of ligands, Stack et al. were able to synthesize nonplanar copper(II) complexes [Cu(L)] from which they could obtain the corresponding copper(I) complexes $[Cu(L)]^-$ and relatively stable phenoxyl-radical copper(II) complexes $[Cu(L^{\bullet})]^+$ by reduction and oxidation, respectively. The three



complexes 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. For example, as in the enzyme, the copper(II) phenolate complex [Cu(L)] is not judged to be involved in the catalytic mechanism because an induction period is observed in which this precursor complex is converted to the catalytically active copper(II) phenoxyl-radical species, while no induction periods occur in the catalytic reactions with the other two copper complexes. A comparison of the catalytic performance of the copper complexes with the various ligands demonstrates that the introduction of a thioether function in *ortho* position to the hydroxy function on the phenol units of the ligand L is not essential for the catalytic reactivity, but does improve the number of achieved catalytic turnovers. In contrast to the enzymatic reaction, simple aliphatic primary alcohols are found to be unreactive under these conditions. This lack of reactivity, however, makes it possible to generate a five-coordinate methoxide adduct from the copper(II) phenoxyl radical complex, thereby providing evidence for the participation of an analogous five-coordinate copper benzylalcoholate species as an intermediate in the catalytic cycle. In summary, the work by Stack et al. has demonstrated in a very impressive and elegant way how the reactivity of an active site in an enzyme can be mimicked by small synthetic inorganic complexes (albeit with slower rates and with a different selectivity) by modeling the structural features of this active site as closely as possible. Essentially they succeeded in making a low-molecular-weight copy of the active site of an enzyme.

More recently, the group of Wieghardt and Chaudhuri produced a second catalytic system employing the ligand 2,2′-thiobis(2,4-di-*tert*-butylphenol).[2] Rather than attempting to reproduce all structural features of the active site of galactose oxidase, they sought with their model to incorporate only those features that are essential to the reactivity in the enzyme. The catalytically active species was recognized as the bis(phenolato)-bridged dicopper(II) complex **1**, in which each copper ion is further coordinated to a phenoxyl radical. In the stoichiometric reaction of **1** with ethanol under anaerobic conditions, a dicopper(II) complex **2** and acetaldehyde is formed [Eq. (2)]. The original complex **1** can be regenerated



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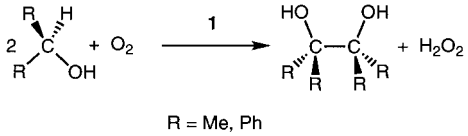
by reaction with dioxygen, which is concomitantly converted to hydrogen peroxide. Using catalytic amounts of **1** in tetrahydrofuran under an atmosphere of air at 20 °C, ethanol is converted to acetaldehyde in a 63% yield after 12 h of reaction time resulting in 630 turnovers of the catalytic cycle. No further oxidation of the acetaldehyde to acetic acid nor any disproportionation of the formed hydrogen peroxide is detected. But in addition to the acetaldehyde, minor amounts (< 5% each) of 2,3-dihydroxybutane, 3-hydroxy-2-butanone, and 2,3-butanedione are formed. Similar results were observed for the corresponding reaction with benzylalcohol. Secondary alcohols like isopropyl alcohol and diphenylcarbinol are catalytically converted with **1** to the corresponding glycol derivatives in up to 68% yields [Eq. (3)]; formation of the corresponding ketones was not observed. Based on kinetic studies of the reactions, the authors postulated a catalytic reaction cycle for the oxidation of primary alcohols



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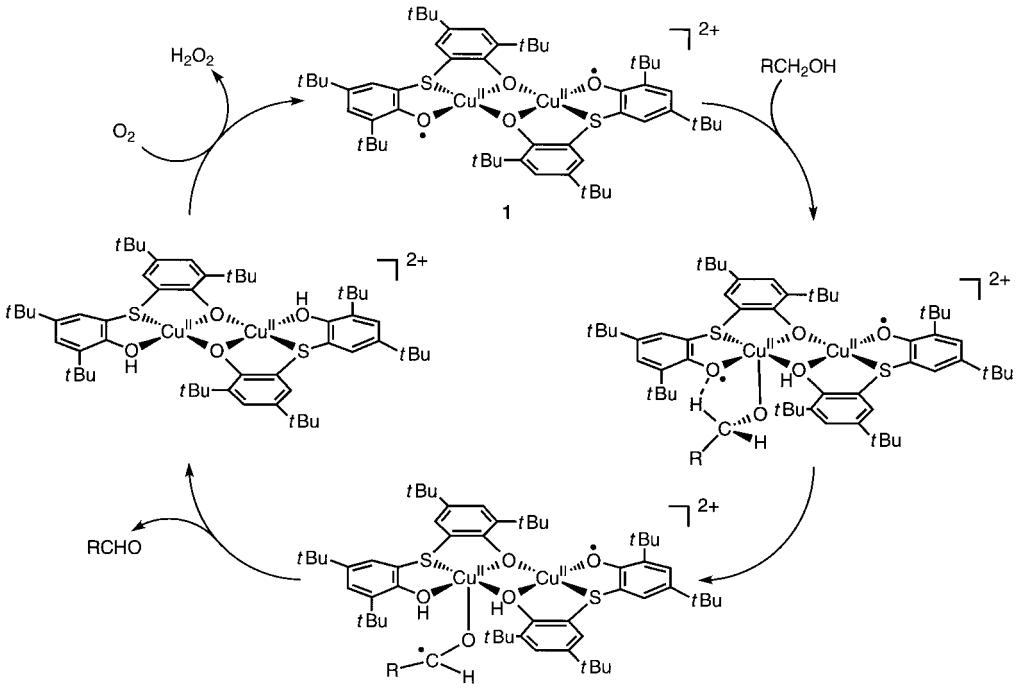

Scheme 4. Postulated mechanism for the catalytic oxidation of primary alcohols by the dinuclear complex **1**.[2]

(Scheme 4) in which an alcoholate ion first binds to one of the copper(II) ions at the axial coordination site. After the rate-limiting hydrogen atom-abstraction reaction has taken place, the resulting ketyl radical is converted to the corresponding aldehyde in an intramolecular electron transfer step. Subsequent oxidation of the coordinated phenol ligands by dioxygen to the phenoxyl radicals regenerates the original catalyst. The difference in products resulting from the oxidation of secondary alcohols is explained by an amendment to the postulated reaction mechanism which states that two alcoholate ions instead of one bind to the two copper ions in a *syn*-facial way and that C–C bond formation takes place between the two resulting coordinated ketyl radicals.

The catalytically active species reported by Wieghardt and Chaudhuri distinguishes itself from the active site in galactose oxidase and from the model complexes of Stack et al. in that it is a dinuclear copper(II) complex with two coordinated phenoxyl radicals, that the coordination geometry at each copper(II) ion is presumably square planar, and that no copper(I) intermediate is involved in the catalytic mechanism. In contrast to the mononuclear model complexes, the dicopper compound can catalyze the oxidation of simple aliphatic primary and secondary alcohols to aldehyde and glycol derivatives, respectively. Although the dinuclear catalyst does not bear any close structural resemblance to the active site of galactose oxidase, it displays a reactivity similar to that of the enzyme because the two fundamental attributes of the enzyme for achieving reactivity have been realized in this synthetic copper complex, namely the presence of a stable phenoxyl radical coordinated to a copper(II) ion and the possibility that the complex can carry out two-electron redox chemistry.

The main objective of bioinorganic research is to learn from nature about the basic principles governing reactivity in a biological system. Chemistry can benefit considerably from this study, since, by applying these principles to small synthetic molecules, novel synthetic methods will be at the disposal of chemists, and new catalysts, which may no longer bear any close structural resemblance to the active site in the enzyme but use the same rationale for the reactivity as the enzyme, can be developed with properties more suitable for practical purposes (e.g. better long-term stability, wider range of application) than the metalloprotein itself. In this context, the study of galactose oxidase will, in my opinion, become a textbook example for how bioinorganic research, working on a highly interdisciplinary level, can give answers to the question of how nature works on the molecular level, thereby breaking new ground in chemistry. In addition to establishing the novel coordination chemistry of phenoxyl radical, two distinct inorganic complexes which very efficiently catalyze the oxidation of alcohols to aldehyde by dioxygen under mild and environmentally friendly conditions have resulted from this research. This feat provides confidence that similar achievements in the biomimetic studies of the active sites of other metalloproteins will be forthcoming.

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Nonsteroidal Antiinflammatory Drugs: A New Generation of Cyclooxygenase Inhibitors**

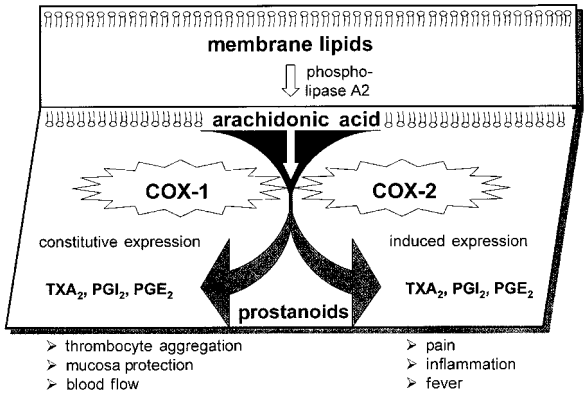
Martin Beuck*

Aspirin®—or acetylsalicylic acid—is synonymous for first aid relief of pain, fever, and inflammation. The 100-year-old and most popular drug is facing new competition. Not simply another compound, but a whole new class with a different spectrum of activities give potential access to indications outside of pain and inflammation.

Therapeutic Basis

The therapeutic effect of acetylsalicylic acid is based on a covalent modification of cyclooxygenase and the inhibition of the first step of prostaglandin synthesis, shown first by Sir John Robert Vane.[1] Cyclooxygenase (COX) exists in two isoforms, COX-1 and COX-2. Serine residue 530 of COX-1 and serine residue 516 of COX-2 are modified by acetylation. Inhibition of COX-1 or COX-2 leads to very different pharmacological effects. The COX-1 inhibition is predominantly responsible for anti-thrombotic effects, while anti-inflammatory effects are mediated mainly through COX-2.

The cyclooxygenase COX-1 is expressed constitutively in all tissues, and is thus always present and active. As far as it is known, this is the case for COX-2 only in kidney, brain, and ovaries. During inflammatory processes COX-2 is increasingly expressed in affected tissues, and consequently production of the pain-mediating prostaglandins is also increased (Scheme 1).



Scheme 1. Role of COX-1 and COX-2 in arachidonic acid metabolism. COX-1/2: cyclooxygenase-1/2; TXA_2: thromboxan A_2; PGI_2: prostacyclin; PGE_2: prostaglandin E_2.

In contrast to self medication of mild headaches and malaise, therapy of pain, for example that caused by rheumatoid arthritis, was treated with high doses of acetylsalicylic acid in the past. This led to undesirable gastrointestinal side effects. These effects in the gastrointestinal tract were mainly mediated by COX-1. Inhibition of COX-1 impairs the synthesis of prostanoids, which have a protective effect on gastric mucosa.

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