Galactose Oxidase Model Complexes: Catalytic Reactivities

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Protein-based radicals in enzymatic reactions are no longer considered suspect, since numerous enzymes are now known to function through organic side chain radicals.[1,2] Such is the case of galactose oxidase (GOase),[3] a mononuclear copper enzyme which uses a modified tyrosyl radical to facilitate the two-electron oxidation of primary alcohols to aldehydes with subsequent reduction of dioxygen to peroxide.[1] This modified tyrosine residue contains a covalent cross-link between an aromatic carbon atom and a cysteine thiolate (Figure 1).[4] The hydroxyl group of the tyrosyl radical is ligated directly to the copper center as revealed by spectroscopic[5,6] and crystallographic studies.[4] In its fully oxidized form, the copper center is EPR-silent, consistent with strong antiferromagnetic coupling between a ligand-based radical and a d^9 Cu(II) center. Mechanistic proposals[6,7] suggest that the square pyramidal coordination of the copper in GOase serves to position the methylene hydrogen of the substrate alcohol in close proximity to the oxygen atom of the tyrosyl radical (Figure 1).

Our aim is to elucidate the structural features essential for GOase reactivity through functional model chemistry. Many structural and spectroscopic model complexes of GOase have recently been reported;[8,9] however, reactivity studies are precluded in many cases because the complexes exist as unreactive phenolate-bridged dimers.[9] One study has reported a nonsquare planar copper complex that exhibits catalytic oxidase reactivity under extremely basic conditions.[10] Presented here are GOase model complexes that reproduce many of the unique properties of GOase, including stabilization of EPR-silent species obtained by one-electron oxidation of Cu(II) complexes, and catalytic conversion of an alcohol to an aldehyde in the presence of a suitable oxidant. A correlation is found between Cu(II) complexes that exhibit a moderately stable EPR-silent state and those that exhibit oxidase reactivity.

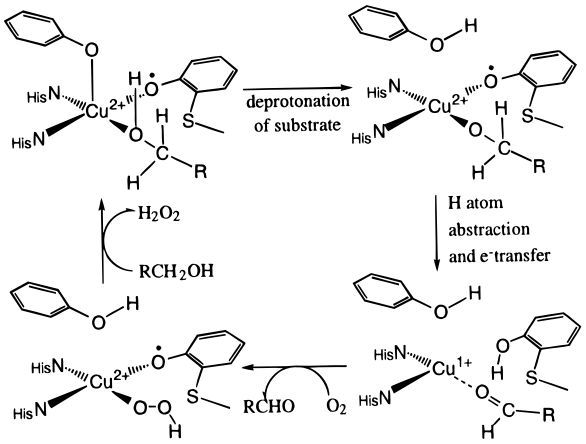

Figure 1. Proposed catalytic cycle of galactose oxidase.[6]

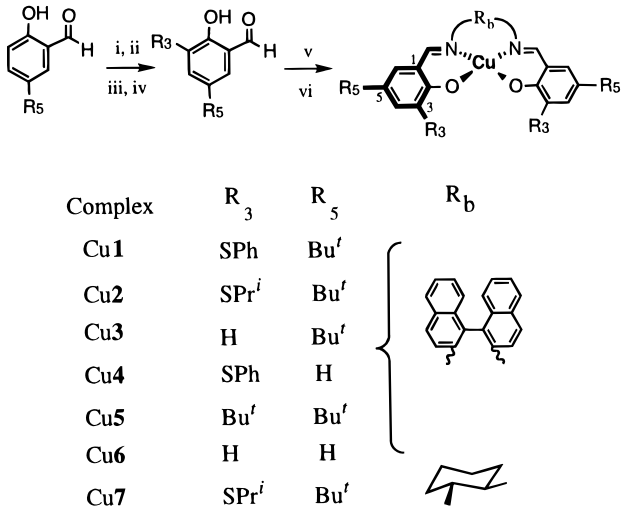

Figure 2.[15] Ligand synthesis. The key step is the *ortho*-lithiation of a 1,3-dimethylimidazolidine-protected salicylaldehyde[14] followed by electrophilic attack of a suitable disulfide. (Ligands H_2**3**, H_2**5**, and H_2**6** do not require steps i−iv.) Condensation of the aldehyde with diamine followed by metal incorporation gives the complexes. Reagents: i. dimethylethylenediamine, EtOH, 25 °C; ii. 2 equiv of *n*-BuLi, TMEDA, Et_2O, 25 °C; iii. R_3-R_3; iv. HCl (2 M), H_2O; v. $H_2N-R_b-NH_2$, EtOH, reflux; vi. $Cu(OAc)_2$, MeOH, reflux.

The ligands in Figure 2 provide a single metal ion with an N_2O_2 coordination environment with various degrees of distortion from a square planar geometry depending on the backbone diamine. Two-carbon-bridged diimines generate nearly planar Cu(II) complexes,[11] while three-[10] and four-carbon-bridged diimines[12] induce significant distortions toward a tetrahedral coordination. Such tetrahedral distortions should enhance not only the affinity of the metal center for a fifth ligand (potential substrate molecule)[13] but also the stability of the Cu(I) form. A succinct, yet versatile synthesis of the appropriately substituted salicylaldehydes is outlined in Figure 2. The modular nature of this synthesis allows a systematic variation of the ligands to

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(13) Ligand exchange in four-coordinate complexes generally proceeds through an associative mechanism. The idealized geometry of a five-coordinate intermediate is trigonal bipyramidal. A 45° distortion from a square planar array is necessary to achieve this geometry, a distortion similar to that observed in the crystal structures reported.
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Table 1. EPR Data and Reactivity Study Results

complex	oxidative peak potentials[a]	EPR signal quenched	turnovers[22]
Cu^{II}**1**	1.00	yes	9.2 ± 0.8, 1.0 ± 0.2[b]
Cu^{II}**2**	0.92	yes	1
Cu^{II}**3**	1.08	no	0
Cu^{II}**4**	1.00	no	0
Cu^{II}**5**	0.81,[c] 0.98[d]	yes	0, 1.2 ± 0.4[b]
Cu^{II}**7**	1.00[e]	no	0

[a] Potentials (vs SCE) were measured at −40 °C in MeCN with 0.1 M TBAP. [b] Stoichiometric reaction: 1 equiv each of copper complex, (TPA^+), and base. [c] $E_{1/2}$, ΔE_p = 80 mV. [d] $E_{1/2}$, ΔE_p = 67 mV. [e] Estimated value.[15]

identify the features which are necessary for oxidase reactivity.

The crystal structure of Cu^{II}**2**[15] confirms its existence as a monomeric, four-coordinate, distorted square planar Cu(II) complex.[16] The tetrahedral distortion results from the binaphthyl backbone rather than the steric interaction between the SPr^i groups, as a similar distortion is observed in the unsubstituted derivative Cu^{II}**6**.[17] The EPR spectra of the Cu(II) complexes are similar to that of GOase,[5e] whereas the electronic spectra and the electrochemical properties significantly differ. These Cu(II) complexes all display oxidative responses in the 0.80−1.10 V range (Table 1),[18] much higher than that of GOase ($E_{1/2}$, 0.23 V vs SCE).[19] Addition of 1.1 equiv of the oxidant tris(4-bromophenyl)aminium hexachloroantimonate, (TPA^+)-[$SbCl_6^-$],[20] to these complexes at −40 °C yields varied results as assessed by EPR (Table 1).[15] Only the EPR signals of the complexes with 3,5-disubstituted salicylates and a binaphthyl backbone are quenched (e.g., Figure 3, inset), while the signals of the other complexes are significantly altered.[15] The three EPR-silent species are moderately stable at room temperature.[21] Associated with oxidation of these complexes is a change in the absorption spectra (Figure 3). The spectra are further altered by the addition of 1 equiv of lithium benzenemethoxide, which suggests the binding of substrate to the oxidatively activated species.

The ability to oxidatively quench the Cu(II) EPR signal directly coincides with the oxidase activity of these complexes with benzyl alcohol (Table 1).[22] The oxidized forms of both Cu^{II}**1** and Cu^{II}**5** are capable of a stoichiometric conversion under anaerobic conditions.[23] When 20 equiv of oxidant and base

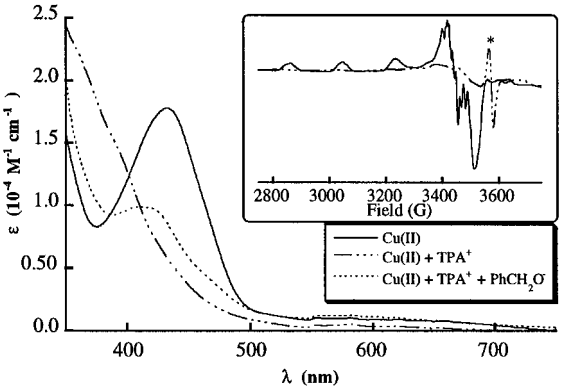
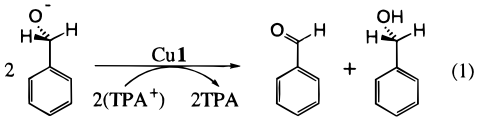


Figure 3. Electronic spectra for Cu^{II}**1**, its oxidized product (1 equiv of TPA^+), and the lithium benzenemethoxide adduct in toluene at −80 °C. Inset: EPR spectra for 1.1 mM Cu^{II}**1** and its oxidized product in toluene/acetonitrile solvents. Instrumental parameters: microwave power, 13 dB; microwave frequency, 9.505 GHz; modulation amplitude, 10 G; temperature, 77 K. The asterisk (∗) denotes excess (TPA^+).

are used, only Cu^{II}**1** displays catalytic behavior [9.2(± 0.8) turnovers], consistent with the consumption of all oxidizing equivalents (eq 1).[24]



(1)

Systematic variation of structural features of these model complexes reveals that specific ligand attributes are necessary for oxidase reactivity, namely, a nonsquare planar coordination geometry and 3,5-disubstitution of the salicylate ring. The latter likely precludes ligand radical coupling in the oxidized form, while the former promotes substrate binding. These structural attributes enable the generation of a moderately stable EPR-silent species by one-electron oxidation of the Cu(II) complexes. The stoichiometric turnover experiments indicate that the oxidized forms must accept two electrons to accomplish the oxidation of an alcohol to an aldehyde. This reactivity is reminiscent of GOase. Although the complex that exhibits catalytic behavior has similar coordination and structural properties to GOase, the thioether linkage on the phenolate group is not necessary for oxidase reactivity, as indicated by the reactivity of Cu^{II}**5** (Table 1).

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Supporting Information Available: Crystallographic data for Cu^{II}**2** and Cu^{II}**6** and spectral data and synthetic details (19 pages). See any current masthead page for ordering and Internet access instructions.

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(15) See Supporting Information.

(16) Crystal data for Cu^{II}**2**: a small brownish green crystal from THF/pentane; triclinic *P*1 (No. 2), a = 10.4865(9) Å, b = 15.996(1) Å, c = 17.986(2) Å, α = 69.347(1)°, β = 88.254(1)°, γ = 81.377(1)°, V = 2790.2(4) Å^3, Z = 2.0; 3579 reflections ($I > 4\sigma(I)$, 4.5° < 2θ < 45.5°), $R(R_w)$ = 0.087(0.097). The dihedral angle between the two O−Cu−N planes is 30°.

(17) Crystal data for Cu^{II}**6**: brownish green rhombic blocks from CH_2Cl_2/ether; monoclinic *C*2/*c* (No. 15), a = 17.354(8) Å, b = 17.479(6) Å, c = 16.664(6) Å, β = 100.66(4)°, V = 4967(3) Å^3, Z = 8.0; 1974 reflections ($I > 3\sigma(I)$, 17° < 2θ < 36°), $R(R_w)$ = 0.049(0.043). The dihedral angle between the two O−Cu−N planes is 35°.

(18) These Cu(II) complexes all have similar spectroscopic properties (UV-vis, EPR).

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(20) The potential of tris(4-bromophenyl)aminium hexachloroantimonate, (TPA^+)[$SbCl_6^-$], is 1.15 V versus SCE in acetonitrile with 0.1 M tetrabutylammonium perchlorate.

(21) Room temperature stable phenolic radicals ligated to metal centers have been recently identified. See: Hockertz, J.; Steenken, S.; Wieghardt, K.; Hildebrandt, P. *J. Am. Chem. Soc.* **1993**, *115*, 11222−11230.

(22) Reaction conditions: under N_2, 1 equiv of the copper complex is dissolved in benzyl alcohol, cooled to −15 °C and 20 equiv of (TPA^+)[$SbCl_6^-$] is added followed by 20 equiv of *n*-BuLi. The solution is stirred at 35 °C for 5 h, passed through a short silica column, and analyzed by gas chromatography.[15]

(23) In the absence of copper complexes under otherwise identical conditions, the oxidation of benzyl alcohol is negligible.

(24) Due to the limited solubility of the reactants, no more than 20 equiv of oxidant and base were used.