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Oxidation of Benzyl Alcohol with Cu^{II} and Zn^{II} Complexes of the Phenoxyl Radical as a Model of the Reaction of Galactose Oxidase[**]

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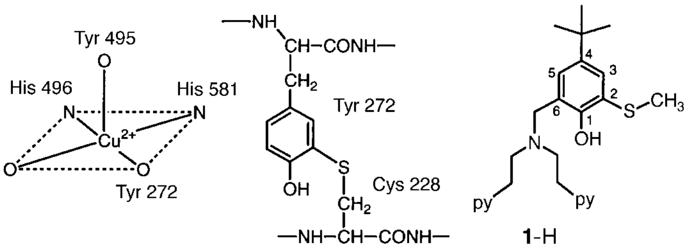
Redox interactions between a transition metal ion and a redox-active amino acid side chain such as the phenol group of tyrosine have been recognized to play a crucial role in several biologically important processes.[1] Recently, a tyrosyl radical directly coordinated to a Cu^{II} center has been discovered in the active site of galactose oxidase (GO, EC 1.1.3.9), which catalyzes the oxidation of D-galactose and primary alcohols to the corresponding aldehydes, coupled to the reduction of O_2 to H_2O_2 [Eq. (1)].[1, 2] The crystal structure of GO at 1.7-Å resolution clearly shows that the tyrosine residue (Tyr272), which is directly coordinated to a Cu^{2+} ion at the equatorial position, is covalently bound to the sulfur atom of the adjacent Cys228, constituting a new organic cofactor (Scheme 1).[2] The active form of GO has recently been suggested to be the Cu^{II}–phenoxyl radical of this organic cofactor, which is converted into the two-electron reduced species [Cu^{I}(phenol)] in the oxidation of alcohols to aldehydes.[3–6]

$$RCH_2OH + O_2 \longrightarrow RCHO + H_2O_2 \qquad (1)$$



Scheme 1. Representation of the coordination at the Cu^{II} center of galactose oxidase (left; the unlabeled O atom is from an acetate ion or water), the organic cofactor (center), and the protonated form of the ligand used in the model complexes **1** (right; py = 2-pyridyl).

Extensive efforts have been made recently to develop synthetic model complexes that can mimic the catalytic function and the spectroscopic characteristics of the Cu^{II}–phenoxyl radical species of the native enzymes.[7–10] Wieghardt et al. have succeeded in developing a very unique functional model of GO using 2,2′-thiobis(4,6-di-*tert*-butylphenol) (LH_2) as the ligand; this complex can oxidize alcohols under aerobic conditions.[7b] In this system the active species has been shown to be the dimeric phenoxyl radical complex $[Cu^{II}_2(L^{\bullet})_2]^{2+}$, in which only the radicals act as oxidation sites and no redox reaction occurs at the Cu^{II} site. Now the important question remains as to the essential role of the Cu^{II} site.

We report herein two synthetic model complexes whose structures and spectroscopic features are similar to those of the active form of GO. One is a Cu^{II}–phenoxyl radical complex that can accept two electrons, and the other is a Zn^{II}–phenoxyl radical complex that can accept only one electron. To our surprise, both model complexes can oxidize benzyl alcohol to yield the same product, benzaldehyde. The important difference between the two model complexes is found to lie in the different kinetic formulation for the oxidation of the alcohol. These features of our model complexes provide valuable insights into the catalytic mechanism of GO.

Addition of $(NH_4)_2[Ce^{IV}(NO_3)_6]$ (ceric ammonium nitrate, CAN) to a solution of the dimeric copper(II)–phenolate complex $[Cu^{II}_2(\mathbf{1})_2](PF_6)_2$ (2.5×10^{-4} M in CH_3CN)[11] caused an immediate decrease in the absorption band at 522 nm due to the dimeric copper(II)–phenolate complex (ligand-to-metal charge-transfer (LMCT) transition from the phenolate to the Cu^{2+} ion), accompanied by the concomitant increase in the new absorption bands at 415 nm ($\varepsilon = 1790\,\text{M}^{-1}\text{cm}^{-1}$) and 867 nm ($\varepsilon = 550\,\text{M}^{-1}\text{cm}^{-1}$) at 25 °C (Figure 1). A similar spectrum was obtained in the oxidation of $[Zn^{II}(\mathbf{1})(CH_3CN)]PF_6$[11] by CAN under the same experimental conditions (418 nm ($\varepsilon = 1250\,\text{M}^{-1}\text{cm}^{-1}$) and 887 nm ($\varepsilon = 510\,\text{M}^{-1}\text{cm}^{-1}$) in CH_3CN at 25 °C). The absorption bands in

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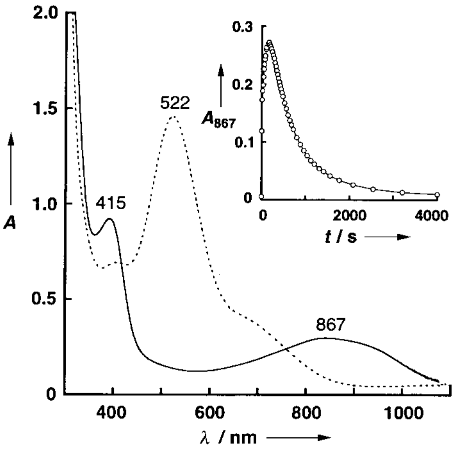


Figure 1. UV/Vis spectra of $[Cu^{II}_2(\mathbf{1})_2](PF_6)_2$ (2.5×10^{-4} M in CH_3CN, dotted line) and $[Cu^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ (solid line) generated from the former by adding CAN (5.0×10^{-4} M) at 25 °C. Inset: Time course of the absorption change at 867 nm due to the formation and decomposition of $[Cu^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$.

the resulting spectra are very similar to those of the phenoxyl radical species of 2-methylthio-*p*-cresol ($\lambda_{max} = 400$ and 830 nm)[12, 13] and of the active form of GO (445 and ca. 800 nm).[3] This indicates that the characteristic absorption bands obtained by the CAN oxidation are due to the phenoxyl radical species ($\mathbf{1}^{\bullet}$) of the ligand. Such an absorption band in the long-wavelength region (above 800 nm), a characteristic of the active form of GO, has never been observed for other synthetic Cu^{II}– and Zn^{II}–phenoxyl radical complexes reported so far except one recent example described by Halcrow et al.[10]

Further evidence in support of the formation of $[M^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ (M = Cu or Zn) was obtained from the resonance Raman spectra ($\tilde{\nu} = 1512$, 1589 cm^{-1} for M = Cu; $\tilde{\nu} = 1517$, 1595 cm^{-1} for M = Zn), the electrospray ionization mass spectra (ESI-MS),[14] and the ESR spectra of the solutions resulting from the CAN oxidation of the corresponding phenolate complexes (ESR-silent at −196 °C in CH_3CN for M = Cu; phenoxyl radical at $g = 2.0052$ at −80 °C in CH_3CH_2CN for M = Zn). The isotropic hyperfine coupling constants ($a_{2\text{-SMe}} = 3.72$ G, $a_{3\text{-H}} = 1.15$ G, $a_{5\text{-H}} = 0.87$ G, $a_{CH(benzyl)} = 2.43$ G) of $[Zn^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ determined by computer simulation indicate that a significant amount of the spin density is delocalized into the methylthio group, as in the case of metal-free phenoxyl radicals.[12] As a result, the structures and spectroscopic characteristics have been found to be almost the same for the Cu^{II}– and the Zn^{II}–phenoxyl radical complexes.

The Cu^{II}–phenoxyl radical complex is relatively stable at ambient temperature, but gradually decomposes to obey first-order kinetics ($k_{dec(Cu)} = 1.62 \times 10^{-3}$ s^{-1}, see inset in Figure 1).[15] Addition of a large excess of benzyl alcohol to a solution of $[Cu^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ in CH_3CN accelerates the decay rate of the phenoxyl radical complex. Formation of $[Cu^{I}(\mathbf{1}\text{-H})]^+$ as a final product was evident, since a) there were no characteristic absorption bands due to the LMCT and ligand field excitation (d–d) of the Cu^{II} complex in the final spectrum of the reaction, and b) the final product was ESR-silent. Furthermore, ligand **1**-H was recovered almost quantitatively after workup with aqueous NH_3, in contrast to the case of self-decomposition.[15] Quantitative formation of benzaldehyde was confirmed by GC-MS.[16] The rate of the oxidation of benzyl alcohol to benzaldehyde obeys pseudo-first-order kinetics, and the plot of the pseudo-first-order rate constant $k_{obs(Cu)}$ versus the concentration of benzyl alcohol gives a straight line (Figure 2a). The second-order rate constant $k_{(Cu)}$ was obtained from the slope as 4.79×10^{-3} $M^{-1}s^{-1}$. A large kinetic deuterium isotope effect ($k^{H}_{(Cu)}/k^{D}_{(Cu)} = 6.8$) was obtained when $PhCH_2OH$ was replaced by $PhCD_2OH$, indicating that the abstraction of the benzylic hydrogen atom is involved in the rate-determining step.

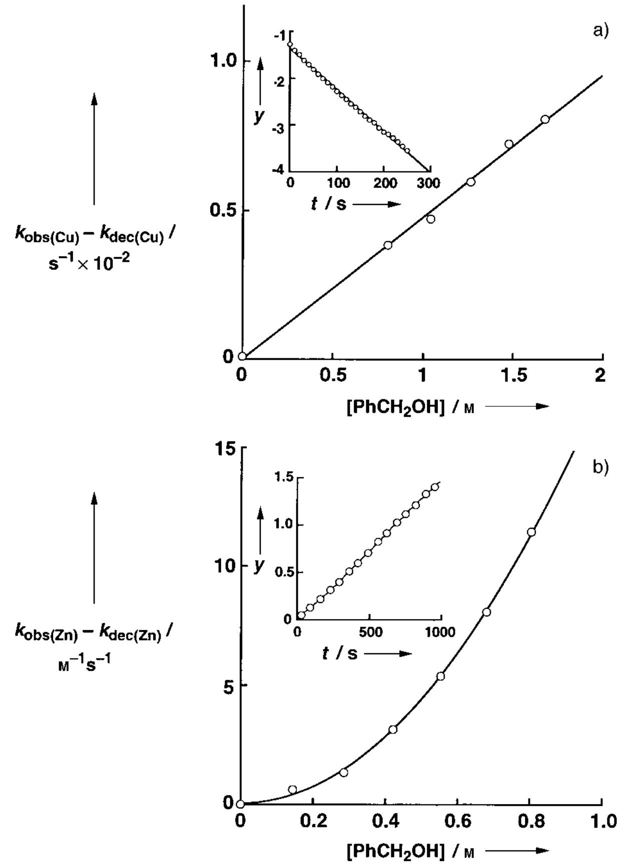
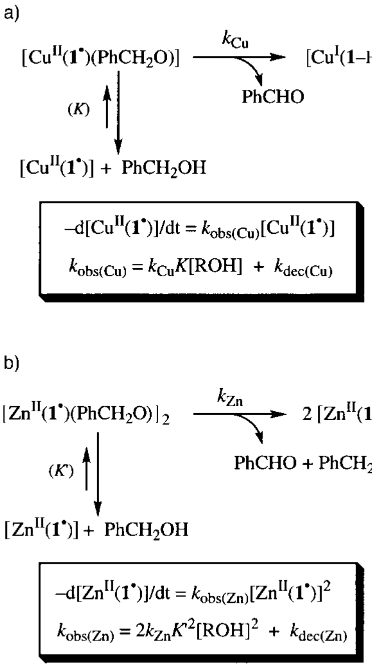


Figure 2. a) Plot of ($k_{obs(Cu)} - k_{dec(Cu)}$) versus the concentration of benzyl alcohol for the oxidation of benzyl alcohol by $[Cu^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ in CH_3CN at 25 °C. Inset: Pseudo-first-order plot for the oxidation; $y = \ln(A - A_{\infty})$. b) Plot of ($k_{obs(Zn)} - k_{dec(Zn)}$) versus the concentration of benzyl alcohol for the oxidation of benzyl alcohol by $[Zn^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ in CH_3CN at 25 °C. Inset: Second-order plot for the oxidation; $y = (A_0 - A)/[Zn(\mathbf{1}^{\bullet})]_0(A - A_{\infty}) \times 10^{-4}$.

The same product, benzaldehyde, was also obtained in the reaction of benzyl alcohol and $[Zn^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$.[16] In contrast to the case of $[Cu^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$, however, the reaction of the zinc–phenoxyl radical complex with benzyl alcohol obeys second-order kinetics with respect to the decay of the absorption at 887 nm, and the second-order rate constant $k_{obs(Zn)}$ shows the second-order dependence on the alcohol concentration (Figure 2b).[17] Furthermore, the yield of benzaldehyde was nearly half that of the copper case.

These results clearly show that the oxidation of benzyl alcohol by $[Cu^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ proceeds by means of coordination of benzyl alcohol in the monomeric form by formally a $2e^-/2H^+$ mechanism to give benzaldehyde and $[Cu^{I}(\mathbf{1}\text{-H})]$ quantitatively (Scheme 2a (type I)). On the other hand, no redox reaction is expected at the Zn^{II} site. In such a case, two electrons can only be accepted by phenoxyl radical sites in a dimeric form (Scheme 2b (type II)). Thus, the reaction obeys second-order kinetics in the case of $[Zn^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$. The latter mechanism of the zinc complex is very close to that of the system of Wieghardt et al. mentioned previously.[7b] The enzymatic reaction has so far been considered to proceed by the type I mechanism. Thus, the different kinetic formulations of $[Cu^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ and $[Zn^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ disclosed in this study clearly demonstrate the importance of the redox cycle between Cu^{I} and Cu^{II} as well as the interconversion between the phenol and phenoxyl radical states for the efficient two-electron oxidation of alcohols at the mononuclear copper site of GO, which is fixed in the protein matrix.



Scheme 2. Type I (a) and type II mechanisms (b) for the oxidation of benzyl alcohol by the copper and zinc model complexes, respectively. (The complexes are given without their charges.)

Experimental Section

Kinetic studies and product analysis. The phenoxyl radical species of the Cu^{II} and Zn^{II} complexes were generated in situ by adding an equimolar amount of $(NH_4)_2[Ce^{IV}(NO_3)_6]$ (CAN, 5.0×10^{-4} M) to a deaerated solution of $[M_2^{II}(\mathbf{1})_2](PF_6)_2$ (M = Cu or Zn,[11] 2.5×10^{-4} M in CH_3CN) (M = Cu or Zn)[11] in a UV cell (1-cm path length, sealed tightly with a silicon cap) at 25 °C. The rate constants for the self-decomposition reaction (k_{dec}) of the phenoxyl radical complexes were determined by following the decrease in the absorption band due to the phenoxyl radical.

The oxidation of benzyl alcohol by the phenoxyl radical species was initiated by adding an excess amount of benzyl alcohol to the CH_3CN solution, after the absorption of the phenoxyl radical species had reached a maximum (a few minutes after the addition of CAN to the phenolate complex solution) at 25 °C. The rate constants (k_{obs}) for the redox reaction were also determined by following the decrease in the absorption due to the phenoxyl radical. The yield of benzaldehyde was determined by GC-MS with mesitylene as an internal standard.

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[14] Coordination of NO_3^- which comes from CAN was confirmed by ESI-MS.

[15] Self-decomposition of the phenoxyl radical species gave a complicated mixture of products. An ESI mass spectrum of the resulting mixture indicated the formation of some S-oxygenated and N-dealkylated products of the ligand.

[16] Benzaldehyde was the only product detected by GC-MS, and no C–C coupling products such as hydrobenzoin [PhCH(OH)CH(OH)Ph] were observed.

[17] Self-decomposition of $[Zn^{II}(\mathbf{1}^{\bullet})(NO_3)]^+$ also obeys second-order kinetics.