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J. Am. Chem. Soc., **2003**, 125 (29), 8716-8717• DOI: 10.1021/ja035837j • Publication Date (Web): 25 June 2003

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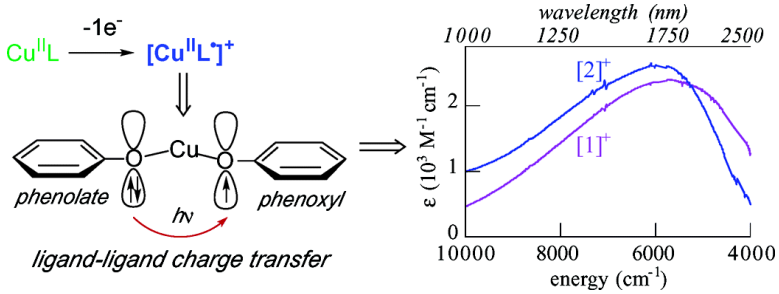

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Published on Web 06/25/2003

Intramolecular Charge Transfer and Biomimetic Reaction Kinetics in Galactose Oxidase Model Complexes

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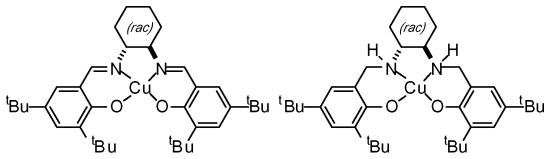
The fungal enzyme galactose oxidase (GOase)[1−3] belongs to the expanding class of known enzymes that use both inorganic and organic cofactors to catalyze transformations.[4] Specifically, a single copper and a modified tyrosine residue couple the two-electron, two-proton oxidation of primary alcohols to the corresponding aldehydes and the two-electron, two-proton reduction of dioxygen to hydrogen peroxide (Figure 1). The apparent simplicity of the active site[5,6] and the attraction of a green, selective method for alcohol oxidation have inspired many efforts to duplicate the properties of GOase in model systems.[7−10] We have reported Cu−Schiff base systems capable of catalytic aerobic oxidation of activated alcohols under basic conditions, but more detailed studies have been thwarted by the complexity of the reaction conditions and a lack of convenient observable "handles".[11,12] Herein we describe two spectroscopically interesting successor compounds, $[\mathbf{1}]^+$ and $[\mathbf{2}]^+$, whose reactions with benzyl alcohol provide insight relevant to the oxidizing half-reaction of GOase.



Adaptations of literature procedures readily provided the neutral precursors **1** and **2**.[13−17] Cyclic voltammetry shows two reversible oxidations for each compound (**1:** +450, +650 mV vs Fc^+/Fc; **2:** +80, +210 mV) ascribed to sequential oxidation of the phenolic moieties; no reduction processes are observed at potentials above −1500 mV. Treatment of **1** with 1 equiv of $AgSbF_6$ in CH_2Cl_2 ($E° = +650$ mV)[16] at room temperature generates an indefinitely stable purple solution of $[\mathbf{1}]SbF_6$.[18] Oxidation of **2** by 1 equiv of $AgSbF_6$ or acetylferricenium hexafluoroantimonate[19,20] ($E° = +270$ mV) immediately produces a deep blue solution of $[\mathbf{2}]SbF_6$ that decays slowly at room temperature (0.1 mM: $t_{1/2} \approx 3$ h).[18] Oxidation of **1** or **2** leads to attenuation of their EPR signals to <15% of their original intensity (77 K), consistent with the formation of antiferromagnetically coupled Cu^{II}−phenoxyl complexes as found in the oxidized form of GOase.

Additional features are found in the near-infrared (NIR) spectra of both compounds (CH_2Cl_2; $[\mathbf{1}]^+$: 1750 nm; $[\mathbf{2}]^+$: 1600 nm).[18] Given the similar low energies and high intensities of these absorptions, the best assignments of these features are to phenolate−phenoxyl charge transfers. This is the first report of spectral features in model complexes resembling the tyrosinate−tyrosyl intervalence transition elucidated for the oxidized form of GOase.[21,22] Furthermore, if **2** is doubly oxidized to form green $[\mathbf{2}]^{2+}$, the intensity of the phenoxyl $\pi-\pi^*$ absorption near 400 nm doubles, but the NIR

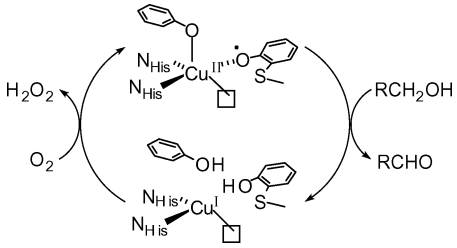


Figure 1. Simplified catalytic mechanism of GOase showing exchange between the oxidized Cu^{II}−tyrosyl (above) and reduced Cu^{I}−tyrosine (below) forms of the enzyme. The coordination site for labile exogenous ligands (square) may be occupied by solvent, alcohol, or oxygen species at different points in the catalytic cycle.

absorption is lost, consistent with formation of a double phenoxyl complex ($[Cu^{II}L^{\bullet\bullet}]^{2+}$) that is incapable of ligand−ligand charge transfer.[18,23]

The stability of $[\mathbf{1}]^+$ or $[\mathbf{2}]^+$ is strongly dependent on solvent and counterion: addition of organic solvents other than CH_2Cl_2 induces slow decay of otherwise stable $[\mathbf{1}]^+$, and the UV−vis spectra of $[\mathbf{1}]^+$ and $[\mathbf{2}]^+$ revert to those of their neutral precursors upon addition of 1 equiv of NO_3^- or Cl^-. The mechanism by which the coordinating anions react with $[\mathbf{1}]^+$ or $[\mathbf{2}]^+$ has not been determined. In reviewing our past results, the lack of reactivity with benzyl alcohol under basic conditions observed for square-planar $[\mathbf{1}]^+$-like complexes could be attributed to their sensitivity to coordinating anions (i.e., benzyloxide).[11,12] We therefore began reactivity studies under neutral conditions to avoid this decomposition pathway.

Addition of the model substrate benzyl alcohol to solutions of $[\mathbf{1}]^+$ or $[\mathbf{2}]^+$ in CH_2Cl_2 induces first-order decay as monitored by UV−vis spectroscopy that correlates directly to the appearance of benzaldehyde, thereby mimicking the oxidizing half-reaction of GOase. UV−vis spectra taken at the completion of the reactions[18] match those of $[\mathbf{1}H]^+$ and $[\mathbf{2}H]^+$, and product yields are consistent with $[\mathbf{1}]^+$ and $[\mathbf{2}]^+$ reacting as one-electron oxidants, giving the following reaction stoichiometry:

$$2[Cu^{II}L^{\bullet}]^+ + PhCH_2OH \rightarrow 2[Cu^{II}LH]^+ + PhCHO$$

Likewise, the absence of an observable reduction in electrochemical studies of **1** and **2** would argue against biomimetic two-electron reduction to a Cu^{I} state. However, the first-order kinetics match results reported for Cu^{II}−phenoxyl complexes that are reduced to Cu^{I} by reaction with benzyl alcohol, not with the second-order dependence of a Zn^{II}−phenoxyl analogue, necessarily a one-electron oxidant.[8,10] Cu^{I} complexes in the present cases may be transiently stabilized by the abstracted H atom(s) as observed in other inorganic systems.[24,25] The final Cu^{II}-containing products could then result from nonrate-limiting comproportionation of Cu^{II}−phenoxyl and Cu^{I}−phenol(ate) species.

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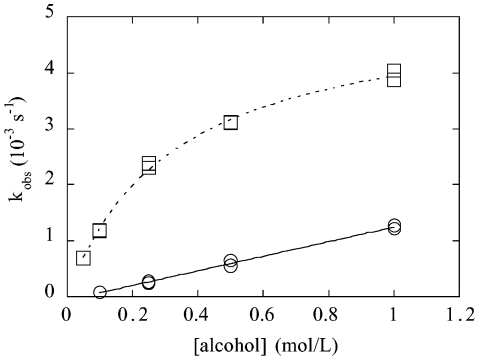

Figure 2. Dependence of the pseudo-first-order rate constant k_{obs} on benzyl alcohol concentration for $[\mathbf{1}]^+$ (circles, shown with linear fit) and $[\mathbf{2}]^+$ (squares, shown with saturation fit) at 25 °C.

The first-order rate constant for $[\mathbf{1}]^+$ is invariant with the initial complex concentration and varies linearly over the range [alcohol] = 0.050−1.0 M (Figure 2), providing a second-order rate constant of $k_2 = 1.3 \pm 0.1 \times 10^{-3}$ M^{-1} s^{-1} at 25 °C. However, the reaction rate of $[\mathbf{2}]^+$ saturates at high substrate concentration (Figure 2), and the data is well fit by a k_{obs} expression including a substrate-binding preequilibrium:

$$[\mathbf{2}]^+ + \text{alcohol} \overset{K}{\rightleftharpoons} [\mathbf{2}(\text{alcohol})]^+ \xrightarrow{k_1} \text{products}$$

$$k_{obs} = \frac{k_1 K[\text{alcohol}]}{1 + K[\text{alcohol}]}$$

The parameters thus obtained for $[\mathbf{2}]^+$ are $K = 3.0 \pm 0.2$ M^{-1} and $k_1 = 5.2 \pm 0.1 \times 10^{-3}$ s^{-1} at 25 °C. We find it remarkable that $[\mathbf{2}]^+$ reacts faster than $[\mathbf{1}]^+$ while noting that $[\mathbf{1}]^+$ is a significantly stronger oxidant ($\Delta E° = +370$ mV). The acceleration is attributable to the mechanistic difference between their reactions: binding should preorganize the substrate and enhance the reactivity of $[\mathbf{2}]^+$, the same means by which enzymes are capable of performing highly selective reactions under mild conditions. To extend the comparison, the oxidation potential for GOase (+410 mV vs NHE) is significantly lower again compared to that for **2**, yet GOase reacts orders of magnitude faster.

Intermolecular and intramolecular rate measurements at 25 °C with α-d_2- and α-d_1-benzyl alcohols give kinetic isotope effect (KIE) values for $[\mathbf{1}]^+$ and $[\mathbf{2}]^+$ of $k_H/k_D = 15$ and 10, respectively. While helpful for confirming that C−H bond cleavage is the rate-determining step in both cases, the large, nonclassical values have only a single precedent in GOase model chemistry.[7] The enzyme itself shows a nonclassical KIE of 22 at 4 °C for the oxidative half-reaction with galactose derivatives,[26] though the KIE is attenuated for reactions with benzyl alcohols.[27] We are currently trying to determine whether the large KIEs in our model systems can be attributed to quantum tunneling or sequential isotope-dependent steps.

In summary, GOase model complexes $[\mathbf{1}]^+$ and $[\mathbf{2}]^+$ have been formed under conditions that maximize their stability and highlight the sensitivity of metalloradical complexes to inappropriate solvents and anions. Both compounds have NIR absorptions assignable to ligand-ligand CT in analogy to GOase; similar features may have been overlooked in other Cu^{II}−phenoxyl−phenolate systems. Comparison of their reactions with benzyl alcohol demonstrates the impact of mechanistic differences on reaction rates, as substrate binding allows the thermodynamically weaker oxidant to react more rapidly. However, complete mechanistic fidelity including catalytic turnover may require a means of site-isolating active species to prevent reaction-terminating comproportionation.

Acknowledgment. We thank J. P. Collman and E. I. Solomon for the use of instruments. R.C.P. is the grateful recipient of an Urbanek Family Stanford Graduate Fellowship. This work was supported by NIH GM50730 (T.D.P.S.).

Supporting Information Available: Experimental details including instrumental methods, synthetic procedures, kinetic measurements, and product analysis; cyclic voltammograms of **1** and **2**; UV−vis−NIR and X-band EPR spectra of **1**, **2**, $[\mathbf{1}]^+$, $[\mathbf{2}]^+$, $[\mathbf{2}]^{2+}$, $[\mathbf{1}H]^+$, and $[\mathbf{2}H]^+$; correlations of UV−vis decay and GC analysis for benzaldehyde (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA035837J