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Copper-Based Bioinspired Oxygenation and Glyoxalase-Like Reactivity

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In Nature, enzymes perform specific reactions. Structurally abbreviated enzyme models may extend their biomimetic functionality to bioinspired reactivity.[1] In particular, external substrates oxygenation remains an important objective of biomimetic and bioinspired catalysis.[2] We have reported that [(Cu(I)Tp^{CF_3,CH_3})$_2$], **1**,[3] (Tp^{CF_3,CH_3} = 3-trifluoromethyl-5-methyl-1-pyrazolyl borate) binds O_2 at 25 °C in CH_2Cl_2 to give [(Cu(II)Tp^{CF_3,CH_3})$_2$(O_2)], **2**.[3,4] NMR studies[3] in CH_2Cl_2 show that, in the absence of O_2, **1** is in equilibrium with mononuclear [Cu(I)Tp^{CF_3,CH_3}], **3**, a likely intermediate in the formation of **2**. Acetone addition to **1** or **3** produces [CuTp^{CF_3,CH_3}(acetone)], **4**, which gives **2** at 25 °C if the [acetone]/[Cu] ratio is below ~200, or at higher ratios below −30 °C. Acetone is a poor ligand for Cu(I) and peroxo Cu(II) complexes,[5] the formation and stability of its CuTp complexes depends on the electronic structure of the Tp ligand. Thus, the relatively electron rich Cu(I)Tp^{R_1,R_2} complexes (R_1, R_2 = alkyl) do not coordinate acetone; the electron poor ones, R_1, R_2 = phenyl, bind acetone in solid state but cannot prevent its loss upon dissolution in noncoordinating CH_2Cl_2.[6] Similarly, CuTp^{R_1,R_2} peroxo complexes decompose in acetone upon warming.[6,7] In contrast, **1** at [acetone]/[Cu] > ~300, turns blue in air and deposits crystals[8] of [CuTp^{CF_3,CH_3}-(lactate)], **5**[10] (Figure 1).

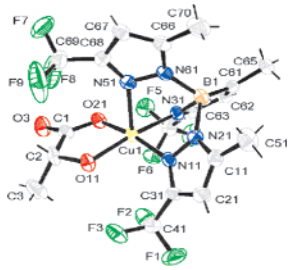
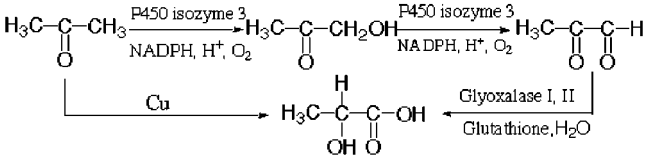


Figure 1. X-ray structure of **5** at 40% probability. Selected bond length (Å) and angles (deg): Cu(1)−O(21) 1.923(4), Cu(1)−O(11) 1.925(4), Cu(1)−N(31) 1.972(5), Cu(1)−N(11) 2.002(5), Cu(1)−N(51) 2.349(5), O(21)−Cu(1)−O(11) 83.9(2), O(11)−Cu(1)−N(31) 170.4(2), O(21)−Cu(1)−N(11) 169.7(2), N(31)−Cu(1)−N(11) 88.0(2), O(21)−Cu(1)−N(51) 100.0(2), O(11)−Cu(1)−N(51) 101.0(2), N(31)−Cu(1)−N(51) 88.6(2), N(11)−Cu(1)−N(51) 90.1(2).

The CuN_3O_2 square pyramidal geometry of **5** resembles that of **2** and [CuTp^{CF_3,CH_3}(acetate)], **6**,[11] but is unique in lactate complexes.[12] The one-pot formation of lactate, CH_3-C(H)(OH)-COO^-, from acetone, CH_3-CO-CH_3, appears unprecedented. Usually, methyl ketones yield upon oxidation α-keto acids or acids with one less carbon.[13] Formally, acetone incorporates two oxygen atoms to yield lactate, but the number of hydrogen atoms is unchanged (considering the lactic acid). Perdeuterated acetone gives perdeuterated lactate, indicating that all hydrogen atoms are derived from acetone. One acetone CH_3 group is oxidized to COO^-, while the carbonyl group is reduced to alcohol level. The possibility that a peroxo complex is the oxidizing agent seems unlikely since **2** yields **5** only *after* its reduction in acetone to colorless **4**. A transient Cu(III) species, favored by acetone,[14] and possibly with oxo radical character[15] cannot be excluded a priori. Such species could be stable,[16] or decompose to unknown products.[17] However, both UV−vis and ESR monitoring[18] of the oxidation detects only mononuclear Cu(II) species,[19] consistent with the tendency of fluorinated Tp ligands to stablize lower Cu oxidation states.[4] Thus, it appears that the highest[6] catalytically relevant Cu oxidation state is II.

While chemically uncommon, the conversion of acetone to lactate is part of the gluconeogenic pathway, Scheme 1.

Scheme 1. Synthetic and Gluconeogenic Conversion of Acetone to Lactate



P450 isozyme 3a catalyzes the acetone oxidation to acetol (hydroxy acetone), then to methyl glyoxal (MG),[20] followed by the Ni (*Escherichia coli*) or Zn (human) glyoxalase I (GlxI)-catalyzed isomerization of the latter (as the glutathione thiohemiacetal) to the lactic thioester, which is hydrolyzed by glyoxalase II (GlxII).[21]

Does the Cu pathway to lactate involve the biochemical intermediates? Attempts to oxidize (i) a solution of **1** in acetol and (ii) a mixture of acetone:acetol:**1** in 900:100:1 ratio failed. No Cu(II) was formed, and as expected, no MG or lactate was detected either, suggesting that acetol is *not* a significant reaction intermediate in the Cu pathway.[22−24] Experiment (ii) suggests that acetol actually inhibits the oxidation step.

Is MG an intermediate? In the absence of air **1** does not react with MG, but, aerobically, MG and **1** yield **5**.[25] MG might thus be an intermediate in Cu(II) (not Cu(I)) glyoxalase reactivity.

The *direct* oxidation of acetone to MG (i.e. skipping the acetol intermediate) might proceed via an acetonyl radical, CH_3COCH_2•, formed during the copper-assisted oxidation of acetone. O_2 capture by acetonyl yields the acetonylperoxy radical, CH_3COCH_2OO•. MG forms either by its decay[26] or by dehydration of the hydroperoxide, CH_3COCH_2OOH, produced by H• abstraction from another acetone molecule, or H^+ and electron binding. Insights into the mechanism were obtained by the oxidation of a 1:1 mixture of acetone:d_6-acetone with **1**, which afforded only nondeuterated and perdeuterated lactate.[27a] No isotope scrambling was observed, suggesting that a classical radical chain mechanism is not operational. From the ratio of protio/deuterated **5**, a preliminary estimation of the overall kinetic isotope effect (KIE) gives KIE ≈ 5. This value, lower than the KIE of **7** for tautomerization,[28] but higher than the KIE of 1.15 for acetol oxidation,[22] suggests a rate-determining C−H bond splitting, consistent with a (coordinated?) acetonyl radical intermediate. For the MG isomerization, the above-mentioned lack of

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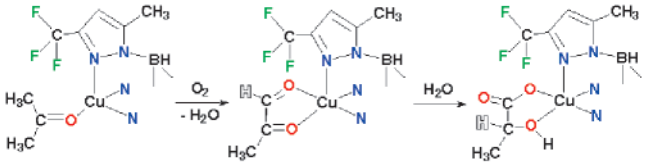
deuterium scrambling in the lactate suggests an *intramolecular* Cannizzaro-like pathway,[29] perhaps via a Lewis-acid catalyzed 1,2 H^-,[30,31] or GlxI-like 1,2 H^+ shift.[32] If a H^+ shift occurs, a [Cu(III)Tp^{CF_3,CH_3}(enediolate)] intermediate, an anionic Cu(II) analogue, or a dinuclear xylose isomerase type[31c,d] complex is required. The UV−vis, ESR, and lack of cations, however, argue against the presence of these species in solution while suggesting that the Cu in $CuTp^{CF_3,CH_3}$ is the Lewis-acid. The overall inner-sphere reaction may thus include a 1,2-hydride transfer at a three-center, Cu(II) transition state, Scheme 2.

Scheme 2. Proposed Overall Reaction and the 1,2-Hydrogen (highlighted) Shift[a]



[a] Only one boron-bonded pyrazole ring is shown in full.

This view is consistent with the quantitative formation of a 1:1 Cu:lactate complex and with the lack of a radical chain mechanism and formation of other acetone-derived products. The H_2O addition step was verifyed by performing the reaction in the presence of $H_2{}^{18}O$. As expected, the labeled lactate results.[27b]

The Cu-catalyzed oxygenation of acetone to lactate does not *formally* "waste" reducing equivalents as water, typical of aerobic P450 monooxygenases (Scheme 1): the two hydrogen atoms, eliminated as H_2O during the formation of MG from acetone, are reincorporated into the lactic acid product.

The Cu-based and genuine GlxI activity may have a common structural basis. Enediolate mimics occupy cis sites of the square-pyramidal Zn of human GlxI,[33] while cis sites of the square-pyramidal copper in **5** are occupied by the lactate. The Ni in *E. coli* GlxI also exhibits accessible cis sites.[21a] Interestingly, the GlxI-like activity we observe is inconsistent with the inactive, Cu-substituted, *human* GlxI.[34] The structural and functional similarities noted above suggest that the Cu-substituted *E. coli* GlxI might be active.

In summary, an external substrate, acetone, is oxygenated aerobically using a robust Cu complex whose metal environment exhibits no C−H bonds. A similar biological process requires oxygenating (Fe) and glyoxalatic (Zn, Ni) enzymes, and extra H^+ and electrons.

Future work will shed light on the active Cu species and mechanistic details, seek the catalytic production of lactic acid (a valuable chemical and precursor to biodegradable polymers[35]), and extend this chemistry to other substrates.

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Supporting Information Available: Preparation, reactivity details, X-ray tables and mass spectra fits for **5** (PDF). This material is available free of charge via the Internet at http://pub.acs.org.

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