Copper Monooxygenase Models

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Benzylic Ligand Hydroxylation Starting from a Dicopper μ-η²:η² Peroxo Intermediate: Dramatic Acceleration of the Reaction by Hydrogen-Atom Donors**

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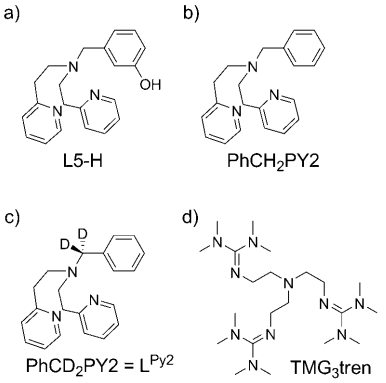
The ubiquitous copper enzyme tyrosinase catalyzes the *o*-hydroxylation of monophenols to catechols along with subsequent two-electron oxidation to *o*-quinones.[1–3] Its physiological function is to initiate melanin synthesis by converting tyrosine to dopaquinone, which spontaneously polymerizes in a non-enzymatic reaction cascade.[4,5] Various recent crystal structures of different tyrosinases revealed a binuclear copper type 3 active site, with each copper ion coordinated by three histidine residues.[6–9] Two related metalloproteins belonging to this class are hemocyanin (Hc), which functions as oxygen-carrying protein in mollusks and arthropods, and catechol oxidase (CO), which mediates the oxidation of catechols to *o*-quinones.[5] In keeping with their very similar active sites, all of these copper proteins bind dioxygen as peroxide in a distinctive side-on bridging (μ-η²:η²) geometry, whereby both Cu^{I} ions are oxidized to Cu^{II}.[1,10] Starting from the oxy state of tyrosinase, monophenolic substrates are converted to catechols within the monophenolase cycle, which is commonly interpreted as the result of an electrophilic substitution.[1,7] The substrate is then released as *o*-quinone, thus restoring the deoxy state. The diphenolase reaction represents the second type of reactivity of oxy tyrosinase, oxidizing external catechols to *o*-quinones. In contrast to tyrosinase, the catalytic activity of CO is restricted to the latter reaction.[11]

The biological function of tyrosinase has successfully been reproduced with low-molecular-weight copper complexes that hydroxylate monophenolic substrates or the ligand framework.[1,2,12–15] The latter type of reaction was discovered by Karlin and co-workers upon investigation of the Cu_2**XYL** system.[16] Starting from a dicopper μ-η²:η²-peroxo intermediate, the *m*-xylene spacer of the ligand was hydroxylated in the 2-position, presumably in the course of an electrophilic attack of the side-on peroxo dicopper unit on the arene.[17] Tolman and co-workers later established the bis(μ-oxo) dicopper(III) intermediate as an alternative mechanistic scenario for the aromatic hydroxylation.[18] Recent studies of the ligand hydroxylation in a dicopper bisimine system have shown that the peroxide σ* orbital has to overlap with the π orbitals of the substrate, determining the orientation of the aromatic substrate relative to the dicopper peroxo or bis(μ-oxo) unit in the corresponding transition states.[1,19]

Despite the considerable number of model systems revealing the hydroxylation of an arene unit within the ligand framework,[15] no evidence for aromatic ligand hydroxylation of an appended phenol has been found to date. To induce this reaction, which would represent the true counterpart of the chemical reactivity of tyrosinase, we synthesized the new ligand [*N*-(3-hydroxyphenyl)methyl]bis[(2-pyrid-2-yl)ethyl]amine (**L5-H**, Scheme 1 a). The copper(I) complex of



Scheme 1. Ligands for copper monooxygenase model complexes: a) **L5-H** of this study, b) **$PhCH_2PY2$**,[20] c) **$PhCD_2PY2$** (also named **L^{Py2}**),[20–22] and d) **TMG_3tren**.[23,24]

L5-H (**1**) was converted to a highly reactive dicopper μ-η²:η² peroxo intermediate by low-temperature oxygenation in acetone. In contrast to the expected tyrosinase-like hydroxylation of the attached phenol, we found the formation of *m*-hydroxy benzaldehyde as the product of a benzylic ligand hydroxylation with subsequent N-dealkylation (Scheme 2). This reactivity had already been described in a slower variant by Karlin and co-workers after oxygenation of the related complex [Cu(**$PhCH_2PY2$**)]$^+$ in dichloromethane (Scheme 1 b).[20] As shown by Itoh and co-workers, this reaction can be suppressed by benzylic deuteration of the ligand **$PhCH_2PY2$**. The μ-η²:η²-peroxo intermediate of the dideuterated analogue [Cu(**$PhCD_2PY2$**)]$^+$ (also named [Cu^{I}**L^{Py2}**]; Scheme 1 c) in fact turned out to be stable towards benzylic hydroxylation, and the substoichiometric *o*-hydroxylation of

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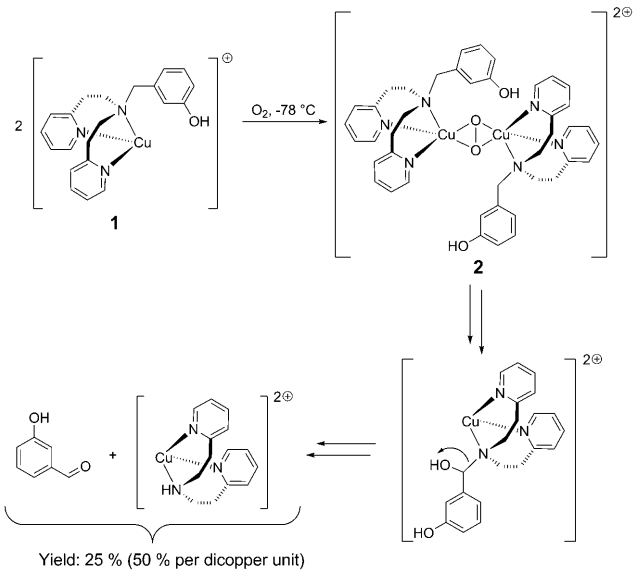
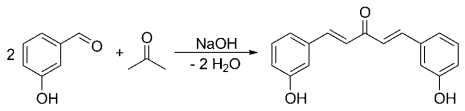


Scheme 2. Reaction sequence leading to the formation of *m*-hydroxybenzaldehyde. Intermediate **2** is generated upon reaction of **1** with dioxygen at low temperature. The benzylic ligand hydroxylation with subsequent N-dealkylation was observed instead of the intended aromatic hydroxylation. Counterions and the equilibrium of the Cu^{II}_2 μ-η^2:η^2-peroxo species with its bis(μ-oxo) dicopper(III) analogue in compound **2** are neglected.

various *para*-substituted external phenolates could be observed.[21,22] This result indicated that the abstraction of H-atoms plays a key role in the benzylic hydroxylation reaction. However, the detailed mechanism of this reaction (which is undesired if the aromatic tyrosinase-like hydroxylation is intended) remained unclear.

First, the reactivity of **1** with O_2 in acetone was investigated in situ by UV/Vis spectroscopy (Figure 1) to gain mechanistic insight into the benzylic hydroxylation of **L5-H**. During the first 8 min of O_2 uptake, four absorption bands at 364 ($\varepsilon \approx 14000$), 430 ($\varepsilon \approx 4000$), 510 ($\varepsilon \approx 1700$), and approximately 650 nm ($\varepsilon \approx 900\,M^{-1}\,cm^{-1}$) evolved, thus indicating the formation of a new intermediate (**2**). Similar spectroscopic features have been described for the copper(I) complexes of related bis[(2-pyrid-2-yl)ethyl]amine ligands after low-temperature oxygenation[25,26] and are characteristic for a Cu^{II}_2 μ-η^2:η^2-peroxo species that is in equilibrium with its bis(μ-oxo) Cu^{III}_2 isomer.[14] In contrast to the relatively stable Cu^{II}_2 μ-η^2:η^2-peroxo intermediate of **PhCH$_2$PY2**,[20] compound **2** starts to decompose after 8 min of O_2 uptake at 195 K, as evident from a decrease in the intensity of the absorption bands at 364, 430, 510, and 650 nm. The decomposition of **2** results in the formation of *m*-hydroxy benzaldehyde after benzylic hydroxylation with subsequent N-dealkylation (Scheme 2). After extraction of the organic phase, only the corresponding secondary amine bis[(2-pyrid-2-yl)ethyl]amine and the intact ligand **L5-H** are detected by NMR spectroscopy as further products of the described reaction.

To allow monitoring of the reaction progress as a function of time, a new protocol for the quantitative analysis of the final product by derivatization of the formed aldehyde to the UV/Vis detectable compound 1,3-bis(*m*-hydroxybenzylidene)acetone was developed (Scheme 3; see the Supporting Information for details). The time course of the benzylic hydroxylation of **L5-H** is depicted in Figure 2. This reaction turned out to be strikingly faster than the corresponding benzylic hydroxylation of **PhCH$_2$PY2**. The Karlin group's system formed benzaldehyde in a yield of 40 % per dicopper



Scheme 3. Derivatization of *m*-hydroxybenzaldehyde with acetone to 1,3-bis(*m*-hydroxybenzylidene)acetone.

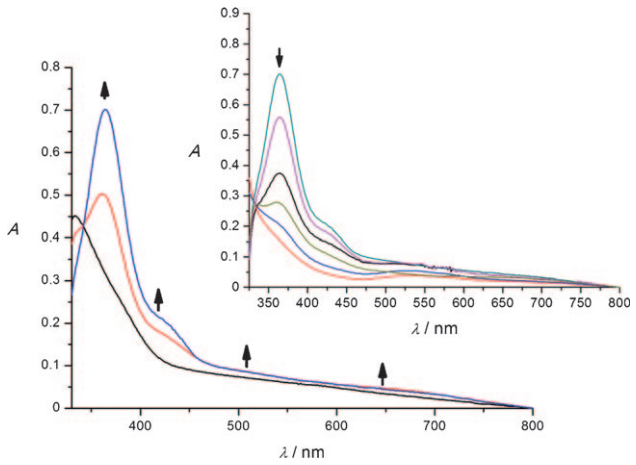


Figure 1. UV/Vis spectra of a 1 mM solution of the precursor complex **1** in acetone under argon atmosphere (black) and its oxygenation product **2** after reaction of **1** with O_2 at −78 °C for 3 min (red) and 5 min (blue); $l = 0.1$ cm. Inset: Decomposition of **2** beginning after 8 min of oxygenation and measured in 30 min intervals.

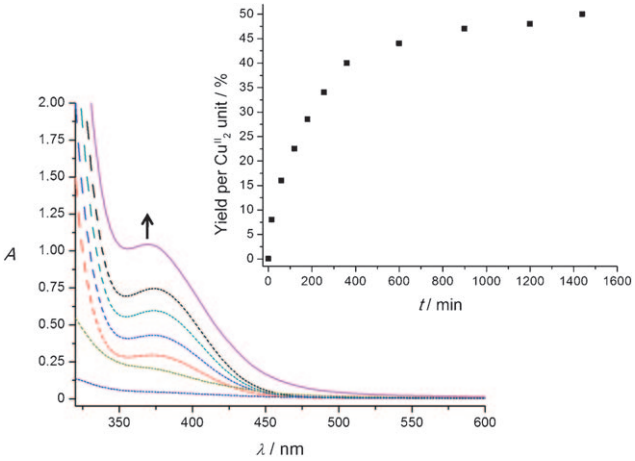


Figure 2. UV/Vis spectroscopic monitoring of the formation of 1,3-bis(*m*-hydroxybenzyliden)acetone after oxygenation and derivatization (see text for additional information) of a 12.5 mM solution of **1** during the first 4 h (dotted lines; navy blue: $t = 0$ min, dark yellow: $t = 30$ min, red: $t = 1$ h, blue: $t = 2$ h, cyan: $t = 3$ h, black: $t = 4$ h) and after one day (solid line; magenta); $l = 1$ cm. Inset: Yield per dicopper unit as a function of time.

unit after oxygenation in dichloromethane for four days,[20] whereas for **L5-H** the same amount of *m*-hydroxybenzaldehyde was already produced after 6 h of oxygenation in acetone, as determined by HPLC analysis and derivatization. Furthermore, **L5-H** revealed a higher overall yield of the reaction (50% per dicopper unit after one day). The ultimate yield of *m*-hydroxybenzaldehyde was further confirmed by workup of the organic phase.

To make sure that the different reaction times are not caused by the use of different solvents, the low-temperature oxygenation of **1** was repeated in dichloromethane. Although no reactive Cu_2–O_2 intermediate was detected for this solvent, the same yield and the same reaction course as evidenced for acetone were observed. These results imply a dramatic acceleration of the hydroxylation reaction caused by the additional phenolic hydroxy group in **L5-H**, which can act either as proton donor or as H-atom donor.

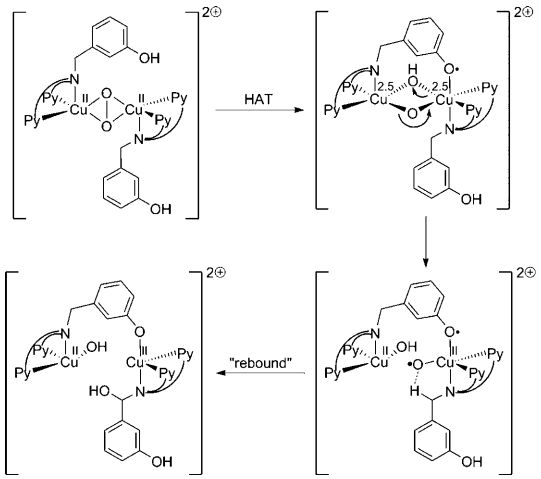
To clarify the exact role of the hydroxy group, the low-temperature oxygenation of **1** was repeated in the presence of the H-atom donor TEMPO-H in acetone. To this end, two aliquots were taken from the low-temperature oxygenation mixture after 15 min. One equivalent of TEMPO-H relative to the amount of the peroxo intermediate **2** was added to one of the solutions before both samples were warmed up and derivatized under identical conditions. Importantly, the presence of the additional H-atom donor lead to a fourfold higher yield of *m*-hydroxybenzaldehyde compared to the reference without TEMPO-H, indicating a further increase of the reaction rate. The ultimate yield of *m*-hydroxybenzaldehyde (50%) was not influenced.[27] This result confirms the pivotal role of H-atom transfer in the benzylic hydroxylation starting from μ-η^2:η^2-peroxo dicopper(II) intermediates. Moreover, it strongly supports the hypothesis that the acceleration of the benzylic hydroxylation of **L5-H** compared to the **PhCH$_2$PY2** system is caused by the additional phenolic residue, which acts as an H-atom donor.

The conversion of phenols to phenoxyl radicals upon reaction with Cu^{II}_2 μ-η^2:η^2-peroxo and Cu^{III}_2 bis(μ-oxo) intermediates is a well-known phenomenon in type 3 copper model chemistry. In fact, this reaction is the root cause of the difficulties in establishing low-molecular-weight model systems of tyrosinase that transform external phenols to *o*-quinones in a biomimetic and catalytic fashion.[1] In the present Cu^I **L5-H** system, H-atom transfer from the phenolic residue in the ligand framework (or alternatively from the added TEMPO-H) to the μ-η^2:η^2-peroxo intermediate apparently triggers a reaction sequence that ultimately leads to the selective benzylic ligand hydroxylation. Important information with respect to this chemistry can be inferred from the mechanism of aliphatic hydroxylation reactions mediated by the binuclear, uncoupled copper monooxygenases PHM and DβM.[28]

Given the considerable number of mechanisms that have been advanced for PHM,[28–32] we limit the discussion to the scenario which was proposed by Amzel and co-workers on the basis of a crystal structure and DFT calculations.[33,34] Starting from a mononuclear end-on-bound η^1-superoxo Cu^{II} species, the authors postulated an initial transfer of a proton and an electron to the superoxide, leading to an η^1-hydroperoxo Cu^{II} intermediate. This species undergoes a heterolytic O–O bond cleavage, thus generating a highly reactive $[LCuO]^{2+}$ intermediate (L = ligand sphere), which is responsible for the incorporation of oxygen into the substrate. More precisely, this reactive Cu–O unit was formulated as $[L^{\bullet+}\,Cu^{III}\text{–}O^{2-}]^{2+}$, corresponding to a Cu^{III} oxo species with a bound ligand radical cation.[29] However, the $[CuO]^+$ unit was defined as a Cu^{II} oxyl structure by Cramer and co-workers on the basis of DFT calculations on simplified model systems.[35,36]

Additional evidence for the mechanism above was provided by the investigation of a low-molecular-weight model system based on the ligand **TMG$_3$tren** (Scheme 1 d).[23,24] Karlin and co-workers demonstrated in 2008 that the addition of phenol or TEMPO-H to the mononuclear η^1-superoxo Cu^{II} complex supported by this ligand leads to the hydroxylation of the aliphatic ligand framework. A high-valent copper oxo species was postulated as the relevant intermediate.[24]

By analogy with these scenarios, we propose a similar mechanism for the benzylic hydroxylation of **L5-H** (Scheme 4): The reaction is initiated by H-atom transfer



Scheme 4. Mechanism proposed for the benzylic hydroxylation of the **L5-H** system.

(HAT) from the phenol to the peroxide with concomitant O–O bond cleavage, whereby a μ-hydroxo-μ-oxo species is formed with both copper ions formally in the oxidation state +2.5; the resulting phenoxyl radical is stabilized by coordination to copper (see below). This intermediate spontaneously rearranges to a highly reactive $[PhO^{\bullet}\,Cu^{II}\text{–}O^{\bullet-}]^+$ species, which inserts oxygen into the benzylic C–H bond of **L5-H** in a rebound-like mechanism. The resulting product finally undergoes N-dealkylation along with generation of *m*-hydroxybenzaldehyde. Detailed spectroscopic and quantum chemical experiments are currently underway to gain further insight into the fundamental steps of the described reaction mechanism.

In summary, the benzylic ligand hydroxylation mediated by the **L5-H** system is dramatically accelerated relative to related model systems without phenol. It was also demonstrated by means of control experiments with the H-atom

donor TEMPO-H that this reaction is triggered by an initial H-atom transfer to the μ-η^2:η^2-peroxo core. Phenoxyl radicals are commonly formed upon reaction of phenols with μ-η^2:η^2-peroxo dicopper complexes and subsequently undergo C–C or C–O coupling reactions.[37,38] In the present case, the corresponding coupling products were not observed, thus indicating that the intermediary generated radical species remain coordinated to copper. Thus, the benzylic hydroxylation of the ligand occurs with unprecedented efficiency. A highly reactive $[CuO]^+$ species is assumed to be a key intermediate in this reaction, by analogy with the binuclear, uncoupled copper monooxygenases.

Of fundamental importance is the result that this reactivity of the μ-η^2:η^2-peroxo unit can be induced specifically. With regard to the biological system, it is remarkable that the unphysiological reaction of a tyrosinase model system is brought about through the reaction of a physiological intermediate (oxy site) with an analogue of the natural substrate tyrosine (phenol). These findings again pose the question as to how the H-atom transfer from phenolic substrates to the μ-η^2:η^2-peroxo unit is avoided in the enzyme tyrosinase.[10] An internal base in close proximity to the active site of the enzyme would on the one hand ensure the deprotonation of the phenol and thus provide for a better coordination of the substrate to the copper center and on the other hand would suppress the formation of phenoxyl radicals. Such an internal base has repeatedly been postulated but never been unequivocally identified.[1] Consequently, this topic will continue to provide interesting results and to raise controversial scientific discussions.

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