Copper Monooxygenases

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How Do Copper Enzymes Hydroxylate Aliphatic Substrates? Recent Insights from the Chemistry of Model Systems**

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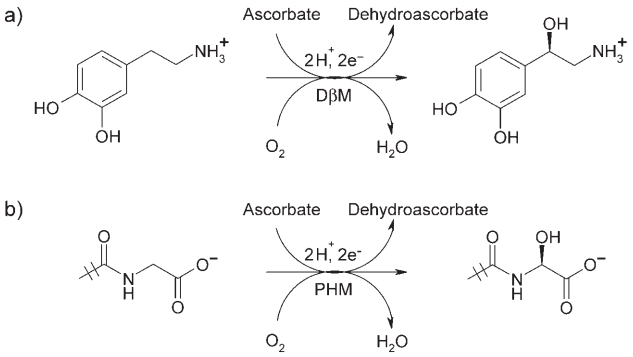
Copper enzymes play an important role in many biological processes. Classically, the involved active sites are divided into type 1, type 2, and type 3 according to the number of metal atoms and the chemical composition.[1] Although this division of copper centers was made originally on the basis of structural and spectroscopic properties, it also reflects a clear-cut differentiation in function: whereas type 3 centers in the enzymes tyrosinase and catechol oxidase mediate the oxygenation (hydroxylation) and/or two-electron oxidation of phenolic substrates, type 1 (blue) copper centers are involved in electron transfer reactions. Type 2 centers can exhibit superoxide dismutase, oxidase/reductase or oxygenase activity. Copper-containing oxidases, such as amine oxidase and galactose oxidase, contain a single type 2 center, and couple the oxidation of a substrate to the two-electron reduction of O_2 with the formation of H_2O_2. Monooxygenase activity, on the other hand, is found in copper enzymes with two type 2 centers. One type 2 center (Cu_A) is involved in electron transfer whereas the other type 2 center (Cu_B) mediates the incorporation of oxygen into the substrate. In contrast to type 3 copper proteins, however, these enzymes do not hydroxylate aromatic, but rather aliphatic substrates; moreover, the two copper centers are not coupled. This situation applies in particular to dopamine β-monooxygenase (DβM) and peptidylglycine-α-hydroxylating monooxygenase (PHM).[2] The former enzyme plays a role in the biosynthesis of adrenaline (conversion of dopamine into noradrenaline), whereas PHM is involved in the post-translational modification of peptides. Both enzymes convert the nonincorporated oxygen into water using two equivalents of ascorbate (Scheme 1).

Although an understanding of the mode of action of DβM and PHM on a molecular level is of significant interest, the exact reaction mechanism of these enzymes has not yet been elucidated. A decisive hint came in 2004 from a publication which presented the X-ray structure of the dioxygen-bound form of PHM along with a substrate analogon (Figure 1).[3] Importantly, O_2 is bound in an end-on (η^1) geometry to Cu_B. The Cu_B–O–O angle is 110° and the O–O distance is 1.23 Å. This geometry is compatible with a dioxygen or a superoxide



Scheme 1. Conversion of substrates catalyzed by a) DβM, and b) PHM.

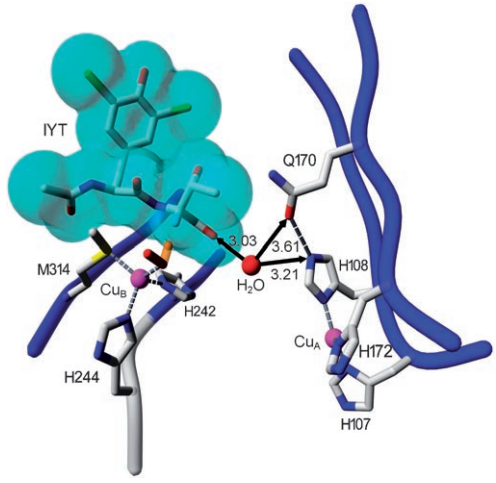


Figure 1. Active site of PHM with the two copper atoms (violet) and bound substrate analogon (IYT, cyan). Dioxygen (red bar) is coordinated in an end-on terminal (η^1) fashion as superoxide and in principle can rotate (orange) towards an H atom of IYT (light blue) to cause an H-atom abstraction (see text). N blue, S yellow, I green.

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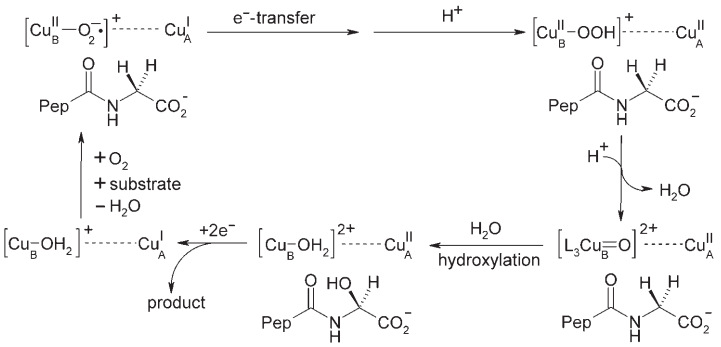
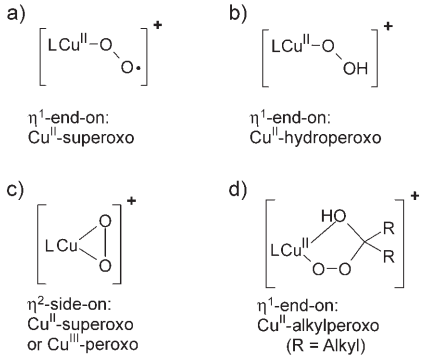
species bound to Cu_B, but not with a copper peroxo (or hydroperoxo) unit (Scheme 2a,b). The authors initially assumed that, as a first step, an electron is transferred from Cu_A to Cu_B, converting the copper(II) into a copper(I) superoxo species. This intermediate would attack the aliphatic substrate under hydrogen-atom abstraction, which could be initiated by rotation of the O_2 ligand in the direction of that hydrogen atom (Figure 1). On the basis of theoretical calculations, however, this reaction proposal was later modified.[4] According to the more recent proposal, one electron and one proton are first transferred to the superoxide intermediate, forming a copper(II) hydroperoxo species. This intermediate spontaneously



Scheme 3. Catalytic cycle proposed for PHM by Amzel et al. (2006). Pep = peptide residue.



Scheme 2. Several mononuclear copper–dioxygen species a)–d); L = ligand.

cleaves the O–O bond under formation of a highly reactive copper oxo moiety and water. This $\{Cu{=}O\}^{2+}$ species, which has been formulated as a $[L_3^{\bullet+}\text{-}Cu^{II}\text{-}O^{\bullet-}]^{2+}$ unit (L = ligands), subsequently hydroxylates the substrate in a fashion that is analogous to the $[(Por^{\bullet-})Fe^{IV}{=}O]$ intermediate ("compound I"; $Por^{\bullet-}$ = porphyrin radical anion) of cytochrome P450. Thereafter the two copper(II) centers have to be reduced again; the catalytic cycle is closed with the liberation of the product. It should be mentioned, however, that other proposals for the mechanism exist which differ from that described above.[5]

The imitation of the enzymatic reaction with simple model systems and the characterization of the involved copper–oxygen intermediates are fascinating and intensively studied areas of current bioinorganic chemistry. An important issue in this respect has been the preparation and characterization of mononuclear copper–dioxygen complexes. This is not trivial, as these intermediates have the tendency to dimerize to binuclear, O_2-bridged complexes. These, however, are model systems for type 3 copper centers; the O_2 reactivity with monophenolic substrates is nowadays interpreted as an electrophilic aromatic substitution.[6] Correspondingly, much effort has been made to protect the mononuclear $Cu\text{–}O_2$ adducts from dimerization with sterically demanding ligands. An early representative of this class of compounds is the side-on superoxo complex (Scheme 2c) of Kitajima et al., which had been obtained by reaction of the copper(I) complex with O_2 in solution at low temperatures (Scheme 4a).[7] The key to the successful synthesis of this complex had been the modification of a ligand known for the formation of $\mu{:}\eta^2{:}\eta^2$ peroxo-dicopper(II) complexes towards stronger sterical hindrance, prohibiting the dimerization of two copper centers. Further examples of η^2-dioxygen-bound, mononuclear copper complexes are the systems prepared and investigated by Tolman et al.[8] These species have been obtained by low-temperature oxygenation of sterically hindered β-diketiminato and anilido-imine copper(I) complexes.[8b–d] In this case too, the steric demand of the ligands had been necessary to prevent formation of the binuclear Cu_2–bis(μ-oxo) complexes which, in particular, are obtained in an excess of the respective copper(I) complexes. Although the η^2 coordination has been confirmed by X-ray structure analysis, the question remains as to whether these systems should be described as copper(II) superoxo or copper(III) peroxo complexes.[8b,c] Recent spectroscopic studies and DFT calculations suggest a $Cu\text{–}O_2$ unit in the continuum between both limiting structures.[8a] Furthermore, Tolman et al. synthesized mononuclear copper complexes with 2-pyridinecarbaldehyde imine ligands containing differently substituted arenes in the 6-position (Scheme 4b). Upon reaction of two of these systems with O_2, a hydroxylation of the arene ring occurred, which presumably is mediated by a high-valent $\{Cu{=}O\}^{+}$ species. The other oxygen atom of dioxygen (which is initially bound as end-on superoxide) is transferred to an α-ketocarboxylate is coordinated as a cofactor after CO_2 has been released.[8e]

In contrast to Tolman et al., S. Itoh and co-workers synthesized mononuclear copper(II) hydroperoxo complexes (Scheme 2b) by reacting copper(II) complexes of sterically demanding, tridentate ligands with an excess of a mixture of triethylamine (NEt_3) and hydrogen peroxide (H_2O_2) at low temperatures.[9] On the basis of kinetic measurements, this research group proposed a deprotonation pre-equilibrium of H_2O_2, with subsequent coordination of the hydroperoxide anion to the copper center.[9b,c] Furthermore, Itoh et al. observed that the solvent has a great influence on the formation of the reactive oxygen species, as conversion of the mononuclear copper complex with NEt_3 and H_2O_2 in different solvents was found to lead to different products. For example, reaction of a mononuclear copper complex with NEt_3 and H_2O_2 in acetone at low temperatures, an alkylperoxo complex (Scheme 2d, Scheme 4c) was formulated as an

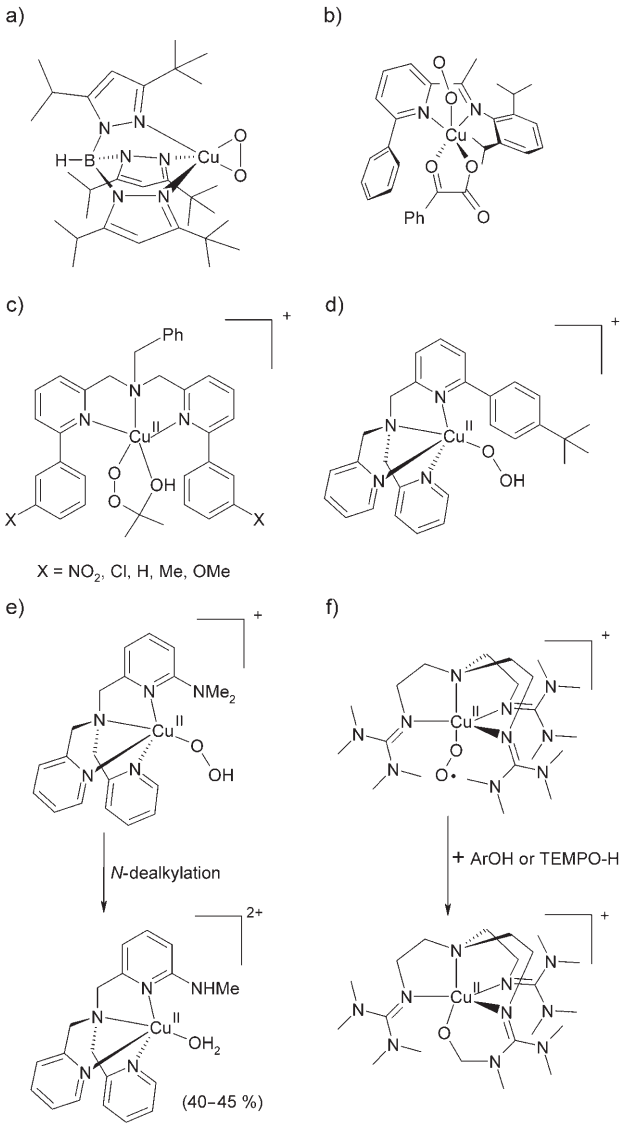
intermediate, which after warming up to room temperature causes an aromatic ligand hydroxylation and formation of the corresponding phenolato complex.[9a] The analogous reaction in propionitrile, in contrast, leads to the hydroperoxo complex for which, after warming to room temperature, no ligand hydroxylation was observed. A mechanism for the hydroxylation mediated by the alkylperoxo complex has not yet been formulated.[9a]

Karlin et al. were also able to detect an aromatic ligand hydroxylation of a mononuclear copper complex with the ligand 6tBP by reaction with H_2O_2 in the presence of NEt_3 in acetone.[10] In contrast to Itoh's group, however, a hydroperoxo complex (Scheme 4d) and not an alkylperoxo complexes was formulated as the intermediate for this reaction in acetone. Another reaction class which has been observed by Karlin et al. for mononuclear copper complexes is the *N*-dealkylation of an NMe_2-TMPA ligand by a hydroperoxo complex (Scheme 4e).[11] The NMe_2-TMPA system resembles the H_2bppa system (H_2bppa = bis[(6-pivalamido-2-pyridyl)-methyl][(2-pyridyl)methyl]amine) of Masuda et al., the difference being that the ligand investigated by Karlin contains a potentially oxidizeable substrate in the form of a dimethylamino group.[12]

In none of these cases, however, could an aliphatic ligand hydroxylation in analogy to DβM and PHM be observed. This gap has now been closed by Karlin et al. who were able to detect the hydroxylation of a methyl group of a ligand coordinated to a mononuclear Cu^{II}–O_2 complex (Scheme 4f).[13] A 1:1 Cu–O_2 complex with the superbasic ligand tris[2-(*N*-tetramethylguanidyl)ethyl]amine (TMG_3tren) was investigated. This adduct had been synthesized in the group of J. Sundermeyer by low-temperature oxygenation of the corresponding copper(I) complex. S. Schindler et al. achieved the X-ray structural characterization of the SbF_6 salt of this complex.[14] In the molecular structure, dioxygen has an end-on coordination, as was detected for the enzyme PHM (Scheme 4f, Figure 1). The O–O bond length and the O–O stretching frequency also indicate an end-on superoxo configuration (Scheme 2a).

As Karlin et al. were able to show recently, this superoxo complex reacts with a number of mono- and diphenolic substrates to the corresponding oxygenated and oxidized products, respectively, including phenoxyl radicals and the C–C coupled products that are typical for copper–dioxygen systems. This chemistry, however, had already been known for mononuclear copper complexes from the NMe_2-TMPA complex (Scheme 4e).[11] More of interest was the observation that, as a side-reaction of all of these transformations, the TMG_3tren ligand was always found to be hydroxylated, corresponding to an aliphatic hydroxylation, in analogy to the enzymatic system (Scheme 1). The authors concluded from their observations that the initial step of all hydroxylation reactions is abstraction of a hydrogen atom from a phenol. To turn the ligand hydroxylation into the main reaction, they accordingly used an hydrogen-atom donor which does not cause secondary reactions, namely *N*-hydroxy-2,2,6,6-tetramethylpiperidine (TEMPO-H). Treatment of the superoxo complex with this reagent first leads to a hydroperoxo complex, as shown by low-temperature (ca. −100 °C) absorption spectroscopy. This intermediate, which also can be generated by reaction of the copper(II) hydroperoxo complex with H_2O_2, further reacts upon warming to the hydroxylated final product (Scheme 4f, bottom).

The decisive question is now whether the hydroperoxo intermediate itself is capable to mediate the hydroxylation, or whether O–O cleavage has to occur first. In the latter case, a $\{Cu{=}O\}^{2+}$ species ($\{Cu^{III}{-}O^{\bullet-}\}$or $\{L^{\bullet+}Cu^{II}{-}O^{\bullet-}\}$) would form which subsequently attacks the substrate. This scenario, which



Scheme 4. Copper–dioxygen model systems for DβM and PHM: a) side-on copper(II) superoxocomplex from Kitajima et al.,[7] b) end-on copper–dioxygen complex with a substituted 2-pyridincarbaldehyde-imine- and an α-ketocarboxylate ligand of Tolman et al.,[8e] c) copper(II) alkylperoxo complex of different substituted *N*,*N*-bis[(6-phenyl-2-pyridyl)methyl]benzyl amine ligands of Itoh et al.,[9a] d) copper(II) hydroperoxo complex of the 6tBP ligand of Karlin et al.,[10] e) copper(II) hydroperoxo complex of the NMe_2-TMPA (TMPA = tris(2-pyrdylmethyl)-amine) ligand and subsequent *N*-dealkylation of Karlin et al.,[11] and f) copper(II) superoxo complex of the TMG_3tren ligand and subsequent aliphatic hydroxylation initiated by a hydrogen atom donor of Karlin et al.[13]

would be entirely analogous to the reaction mechanism of PHM (Scheme 3), has been explored by Karlin et al. by reaction of an oxygen atom donor (PhIO) with the copper(I) complex. Also in this case a ligand hydroxylation was observed.[15] The authors point out, however, that this hydroxylation could also be due to the reactivity of a PhIO molecule coordinated to the copper(I) center.

In summary, by detection of a ligand hydroxylation in Sundermeyer's copper–TMG_3tren complex, Karlin et al. have obtained new insights into the reaction course of aliphatic hydroxylations mediated by copper centers. A simple model system is now available which allows this reaction to be followed in detail, leading to a better understanding of the enzymatic pathway. Nevertheless, the strongly different ligand systems in the enzyme and the model complex should not be overlooked: while the active Cu_B center in the enzyme contains two histidine units and one methionine ligand, the copper center of the TMG_3tren complex is coordinated in a trigonal-bipyramidal geometry by one amine nitrogen atom and three strongly electron-donating imine groups. These different coordination spheres could very well cause different reactivities of the involved copper-oxygen species and, as a consequence, entail different reaction mechanisms for the hydroxylation of aliphatic substrates. A systematic understanding of these structure–function relationships is lacking. This subject will therefore continue to provide opportunities for new, surprising results and interesting scientific controversies.

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[15] If PhIO acts as an oxygen donor, this, however, would formally lead to a $\{Cu{=}O\}^{+}$-unit.

