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Theoretical Exploration of the Oxidative Properties of a $[(tren^{Me1})CuO_2]^+$ Adduct Relevant to Copper Monooxygenase Enzymes: Insights into Competitive Dehydrogenation versus Hydroxylation Reaction Pathways

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Abstract: Singlet and triplet H-transfer reaction paths from C–H and N–H bonds were examined by means of DFT and spin-flip TD-DFT computations on the $[(tren^{Me1})CuO_2]^+$ adduct. The singlet energy surfaces allow its evolution towards H_2O_2 and an imine species. Whereas N–H cleavage appears to be a radical process, C–H rupture results in a carbocation intermediate stabilized by an adjacent N atom and an electrostatic interaction with the $[Cu^IOOH]$ metal core. Upon injection of an additional electron, the latter species straightforwardly forms a hydroxylated product. Based on these computational results, a new mechanistic description of the reactivity of copper monooxygenases is proposed.

Keywords: copper · density functional calculations · dioxygen · enzyme models · hydroxylation · oxidation

Introduction

Dioxygen activation for functionalization of a C–H bond or any other chemical group is of tremendous importance in chemistry[1–3] and covers topics ranging from fuel combustion to enantioselective monooxygenation by enzymes. Oxidation by molecular oxygen is kinetically difficult even though thermodynamically favored. In biological systems, metalloenzymes, iron and copper being the metals of choice, most frequently carry out promoted oxidation by molecular dioxygen.[1–11] Starting from a mono- or polymetallic core $[M_n]^{m+}$ (classically, n ranges from 1 to 4), this activation process proceeds through initial binding of O_2 to yield a $[M_nO_2]^{m+}$ adduct and subsequent formation of strongly oxidative species such as metal hydroperoxo, metal oxyl, or metal oxo entities. As a consequence, a general view of activation of dioxygen by metalloenzymes does not seem possible. Nevertheless, subclasses of enzymes that use the same strategies can be identified. Among them, two monooxygenases,[12] namely, peptidylglycine α-hydroxylating monooxygenase (PHM) and dopamine β-hydroxylase (DβH; Figure 1, middle) appear particularly interesting, as both involve a common strategy relying on two noncoupled mononuclear copper centers separated by a solvent cleft (Figure 1, left). Many theoretical investigations have been devoted to these systems and have addressed the capability of various oxygenated intermediates such as copper hydroperoxo ($[Cu^{II}(^-OOH)]^+$),[13–16] copper oxyl ($[Cu^{II}(O^{\bullet-})]^+$),[15–17] or copper oxo ($[CuO]^{2+}$)[14] moieties to achieve the hydroxylation of aliphatic substrates (Figure 1, right).[18–20] However, more and more biochemical evidence suggests that the oxidation mechanism proceeds by direct attack on the C–H bond by a copper superoxo intermediate $[Cu^{II}(O_2^{\bullet-})]^+$.[12,21–26] This possibility was recently explored by quantum chemistry.[12,13,27]

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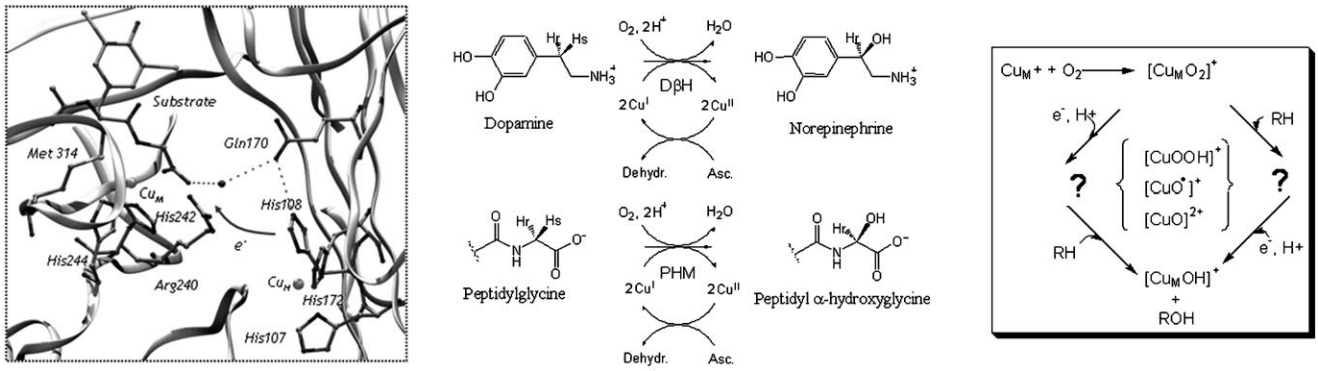


Figure 1. DβH and PHM net reaction (middle; Asc. and Dehydr. stand for ascorbate and dehydroascorbate, respectively); the two uncoupled copper centers of PHM and long-range electron transfer from the Cu_H to the Cu_M site of PHM (left); and the potential reaction paths proposed for hydroxylation mechanisms (right).

Whereas coupled dinuclear copper systems have been the subject of a large number of theoretical and experimental studies,[28–30] investigations of the oxidative processes induced by mononuclear copper–O_2 adducts are less common. The main reason is that model compounds usually undergo fast dimerization or exhibit slow reactivity.[4,31] Mononuclear complexes built on sterically hindered bidentate anionic ligands exhibit a pronounced charge transfer from copper to O_2 and have been successfully accumulated at low temperature, but they appeared to be inert. This suggests that a balance should be found in the charge transfer from copper to dioxygen when designing ideal biomimetic systems: reactive complexes should permit sufficiently strong coordination of dioxygen but should also retain superoxide character to promote C–H abstraction.[31–35] A first illustration of this aspect was recently reported in a work dedicated to the oxidation of an exogenous substrate by a Cu^I mononuclear complex relying on a neutral but strongly donating (N_4) ligand.[36–38] This shows that the catalysis of oxygen incorporation by a copper center might not be as unusual as thought. Indeed, degradation of the copper ligands in the presence of O_2 has been described previously, but most logically was considered to be an undesirable side reaction, not deserving of any further investigations.[31,36] Such reactivity towards O_2 has also been observed in the Cu^I complex of a tren-capped calix[6]-arene ligand (Figure 2, left).[39] In this system, the cavity of calixtren constrains the copper center in a mononuclear environment, as dimerization is prohibited by the calixarene structure. It has been noted, however, that the [(calixtren)Cu^I] complex reacts very rapidly and irreversibly with O_2, even at very low temperature, to yield the corresponding Cu^{II} derivative with partial oxidation of the ligand itself. Notably, very few degradation products have been observed, and their exact chemical identification has not been fully accomplished yet. It has also been noticed that in the presence of KO_2 no oxidation of the calixtren is observed, which suggests that the species obtained by coordination of O_2 to a Cu^I species, further referred to as a $[CuO_2]^+$ adduct, is responsible for degradation of the ligand.[39] Since this oxidative process occurs within a mononuclear species, efforts have been made to understand the origins of this side reaction, and examination of this problem from a theoretical point of view is appealing. Therefore, we performed computations based on copper(I) model complex $[(tren^{Me1})Cu]^+$ (Figure 2, right) which has the same N_4 coordination core around the metal center but contains no calixarene cavity. Investigating the reactivity of the dioxygen adduct $[(tren^{Me1})CuO_2]^+$ (**A**) requires in principle consideration of a number of competitive mechanisms, including associative or dissociative steps.[40] However, the examination of intramolecular oxidative process carried out here avoids these aspects and focuses on the intrinsic oxidative properties of the $[CuO_2]^+$ core.

The first part of the present paper describes the reactive pathways via which starting complex $[(tren^{Me1})CuO_2]^+$ (**A**) undergoes intramolecular redox reactions towards formation of an imino Cu^I complex and H_2O_2. Initial breaking of either a N–H or a C–H bond by $[CuO_2]^+$ is successively considered. In the second part, comparison of the two oxidative pathways is supplemented by a discussion on spin states and energetic aspects. Finally, based on these results, an original enzymatic mechanism for CH hydroxylation by noncoupled copper monooxygenases is pro-

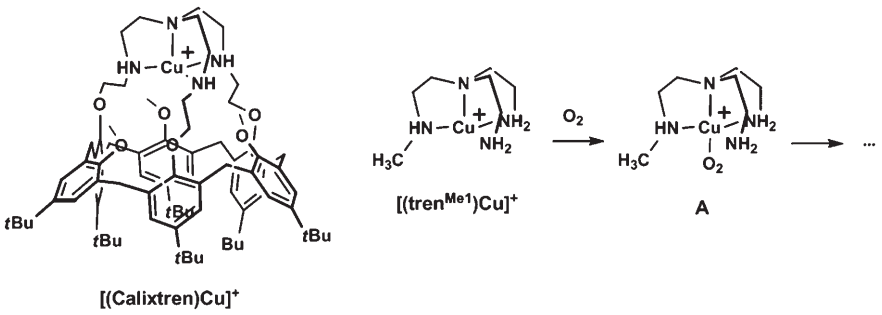


Figure 2. Schematic representation of $[(calixtren)Cu]^+$ (left), and the computed model reactivity of the $[(tren^{Me1})Cu]^+$ complex in the presence of O_2 (right).

posed. It is shown that injection of a supplementary electron into the product resulting from initial C–H bond cleavage may be the key to achieving hydroxylation of an alkyl group.

The computational details of the present study are described in the Supporting Information and in references [41–43].

Results

N–H and C–H cleavage pathways

The entire study relies on the preliminary interaction of the copper(I) complex $[(tren^{Me1})Cu]^+$ with dioxygen to form adducts $[(tren^{Me1})CuO_2]^+$ (**A**). Due to the triplet character of molecular dioxygen, the two possible spin states of **A**, triplet and singlet, are considered (Figures 2 and 3). All corresponding starting $[Cu^IO_2]$ adducts ($\mathbf{A_B}$ and $\mathbf{A_C}$ for the triplet states, $\mathbf{A_D}$ and $\mathbf{A_F}$ for the singlet states, see Figures 4 and 6 below) have pronounced superoxide character, as testified by O–O bond lengths of about 1.29 Å (singlet states, Table 1) and 1.27 Å (triplet states[44]). The triplet adduct was previously shown to be the most stable state for the non-methylated analogue $[(tren)CuO_2]^+$.[43] The same conclusion applies here. This is in agreement with the experimental data reported for the related $[(tren^{TMG})CuO_2]^+$ adduct, which has an O–O distance of 1.280 Å,[45] and was recently shown to have a triplet fundamental state.[12]

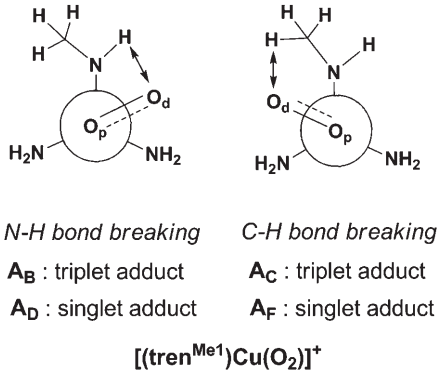


Figure 3. Conformers of the starting $[(tren^{Me1})CuO_2]^+$ adduct leading to initial intramolecular attack on the N–H or C–H bond.

Table 1. Characteristic distances [Å] and NPA charges [e] for the singlet paths.

	N–H cleavage: X = N			C–H cleavage: X = C		
	$\mathbf{A_D}$	$\mathbf{TS(A_D\text{-}D)}$	**D**	$\mathbf{A_F}$	$\mathbf{TS(A_F\text{-}F)}$	**F**
d(O–O)	1.296	1.369	1.417	1.292	1.375	1.503
d(X–H)	1.021	1.349	3.208	1.090	1.495	2.250
d(H–O)	3.001	1.167	0.975	2.569	1.087	0.977
d(Cu–O)	1.892	1.930	1.866	1.903	1.895	1.957
d(Cu···N)	2.150	1.991	1.926	2.144	2.232	3.375
d(N–C)	1.484	1.469	1.455	1.484	1.388	1.342
q(Cu)	1.12	1.20	1.25	1.11	1.03	0.82
$q(O_p)$	−0.28	−0.37	−0.45	−0.28	−0.47	−0.64
$q(O_d)$	−0.22	−0.38	−0.45	−0.20	−0.38	−0.47
q(X)	−0.83	−0.77	−0.68	−0.36	−0.23	0.19
q(H)	0.40	0.47	0.50	0.24	0.40	0.53

Starting from the $[(tren^{Me1})CuO_2]^+$ adduct, intramolecular oxidation of the $tren^{Me1}$ ligand necessarily proceeds by attack of the coordinated dioxygen moiety on either a N–H or a C–H bond. Figure 3 presents the two conformers of the starting adduct for initial attack of NH or CH by the distal (O_d) oxygen atom. Attack by the proximal oxygen atom (O_p) was found to be endoenergetic by about 50 kcal mol^{-1} and will not be considered further.

Triplet potential-energy surfaces: A detailed description of the triplet structures can be found in the Supporting Information. The two starting triplet adducts, respectively labeled $\mathbf{A_B}$ and $\mathbf{A_C}$ for initial NH and CH attack, evolve toward homolysis of the corresponding bond. Indeed, in both cases, the natural population analysis (NPA) and spin densities reveal formation of a formal Cu^{II} complex interacting with an N- or C-centered radical (see complexes **B** and **C** in Figures 4 and 6, respectively). The chemical reactions can thus be described as H-abstraction processes. No energetically accessible TS corresponding to any further evolution could be found after this first H abstraction. In any case, as seen in Figures 4 and 6, the initial barriers for the processes on the triplet surface are high (>30 kcal mol^{-1}) and they do not compete with the processes taking place on the singlet surfaces described below.

Singlet pathway for NH cleavage: In the singlet state, the intramolecular redox process starts with conformer $\mathbf{A_D}$ for initial NH attack by distal oxygen atom O_d and ends with the formation of imino complex **E** (Figure 4). The first hydrogen atom transfer (from NH to O_d) leads to intermediate **D**. It proceeds through the transition state $\mathbf{TS(A_D\text{-}D)}$, described in Figure 5 and Table 1. This H transfer is associated with a pronounced shortening (0.224 Å) of the corresponding N–Cu distance. According to a formal electron count, intermediate **D** may be described either as a Cu^{III} center bound to a deprotonated amino group and a hydroperoxo ligand, or as a Cu^{II} center bound to an amino radical. The NPA results for **D** strongly support a Cu^{II}/amino radical structure, since only a slight increase in q(N) between $\mathbf{A_D}$ and **D** is observed (Table 1), which is in disaccord with formation of a deprotonated amino group. Inspecting the SF-TDDFT wave function of **D** shows that the leading determinant (89% of the wave function) corresponds to localization of two electrons in a single molecular orbital exhibiting a major contribution from copper.[44] The second largest determinant (6%) corresponds to an open-shell electronic distribution built from the previous one by exciting one electron to a mainly Cu-centered orbital. This results in an even stronger Cu^{II}/amino radical character.[46] A radical reaction mechanism, very similar to that observed in the triplet state, can thus be proposed for N–H bond attack by the singlet $[CuO_2]^+$ core.

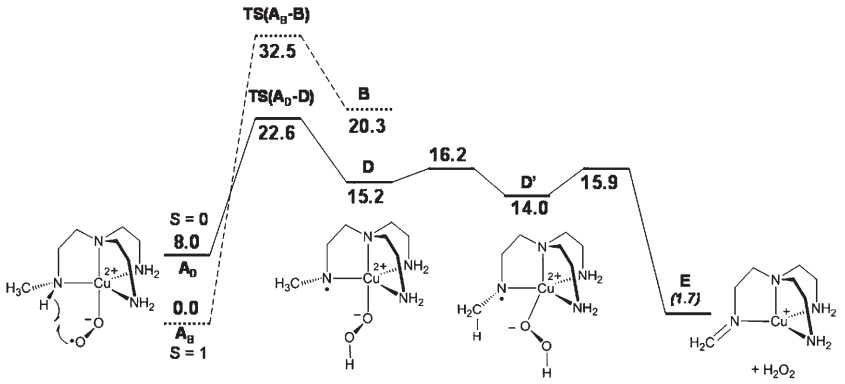


Figure 4. N–H pathways in the triplet (dashed lines) and singlet (full lines) states. SF-B3LYP energies (if not available, B3LYP values are given in parentheses) are in kcal mol^{-1}. Since the "arrow" representation is identical for singlet and triplet paths, only one scheme is given for structures **A**, **B**, and **D**.

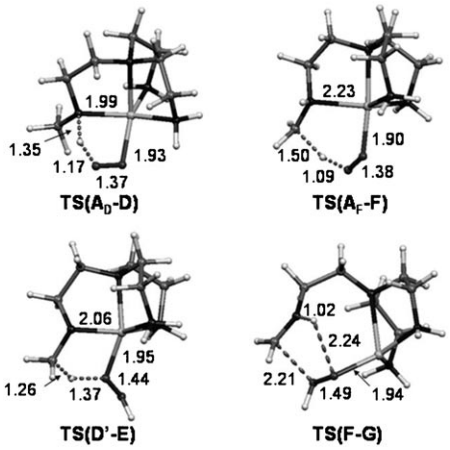


Figure 5. Singlet transition states. Distances are given in Å.

For **D** to evolve to **E** by a second H abstraction, the hydroperoxo ligand must rotate from **D** to **D′**. The geometry evolves from a trigonal bipyramid to a square-based pyramid[47] with the reacting nitrogenous ligand lying in basal position. This is evidenced by the opening of one of the N-Cu-N angles of the bipyramid base (116.5°) to a value closer to 180° (155.1°), whereas the other two angles (103.7 and 137.7°) significantly decrease (101.6 and 98.9°). This rearrangement then allows hydrogen transfer from the terminal CH_3 moiety to the hydroperoxide via **TS(D′-E)** and yields an imino group coordinated to the Cu^I center. Concomitantly, formation and decoordination of H_2O_2 are observed (Figure 4). This second H transfer takes place via a quite early transition state, in line with a reaction facilitated by formation of the C=N bond. The structure of **TS(D′-E)** is similar to that depicting radical H transfer between two carbon atoms. This is shown, for instance, by comparing the C···H···O angle (137°) to the C···H···C angles of about 145° determined in intramolecular H transfers.[48] It is noteworthy that the Cu–N distance in the imino form (2.00 Å) is significantly shorter than that in the starting amino ligands (2.15 Å).

The whole process thus corresponds to oxidation of an amino group to an imino moiety by a singlet $[Cu^{II}(O_2^{\bullet-})]$ core resulting from O_2 binding to a $[(N_4)Cu^I]$ complex. The reaction proceeds through two successive monoelectronic events, initiated by N–H bond homolysis and ultimately releasing a hydrogen peroxide molecule and a Cu^I complex.

Singlet pathway for CH cleavage: The mechanism initiated by C–H attack (Figure 6) has an activation barrier of 13.0 kcal mol^{-1}. This value is in full agreement with the activation barriers reported for similar C–H transfer processes and obtained either experimentally ($\Delta H \approx 13$ kcal mol^{-1} in PHM)[49] or theoretically (about 14 kcal mol^{-1},[13] 20 kcal mol^{-1},[14] 17 kcal mol^{-1},[15] and 23 kcal mol^{-1} [16]). Even though these values appear scattered, they are within the range observed upon variation of the substrates.[32] Starting from $\mathbf{A_F}$, the first step involves transfer from the terminal CH_3 group to distal oxygen atom O_d. The geometric parameters of the C···H···O arrangement in **TS($\mathbf{A_F}$-F)** (Table 1) are within 0.01 Å compared to those of triplet **TS($\mathbf{A_C}$-C)**.[44] Starting from adduct $\mathbf{A_F}$, H transfer requires, as in the triplet

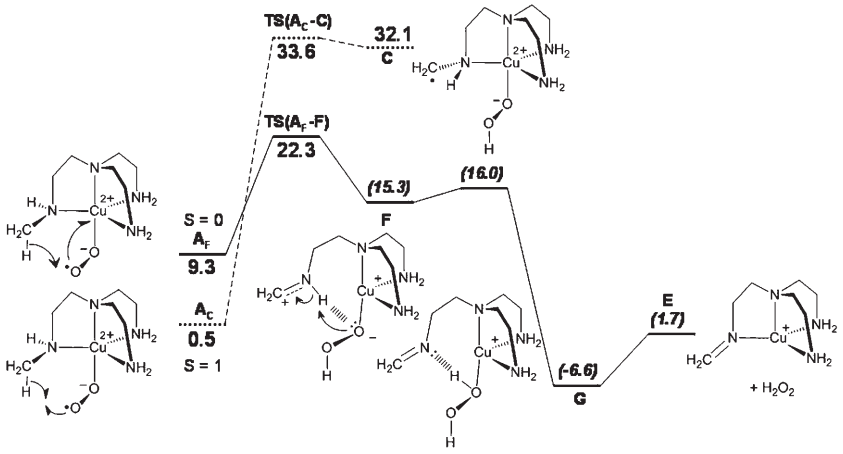


Figure 6. C–H pathways in the triplet (dashed lines) and singlet (full lines) states. SF-B3LYP energies (if not available, B3LYP values are given in parentheses) are in kcal mol^{-1} and relative to $\mathbf{A_B}$.

case, a significant lengthening of the N–Cu distance, from 2.14 Å in $\mathbf{A_F}$ to 2.23 Å in **TS($\mathbf{A_F}$-F)**. However, in contrast to the triplet case, this lengthening process continues beyond the TS and leads to complete decoordination of the amino ligand in **F** (d(Cu–N) = 3.38 Å, Figure 7). This behavior can be rationalized by the electronic description of the reaction.

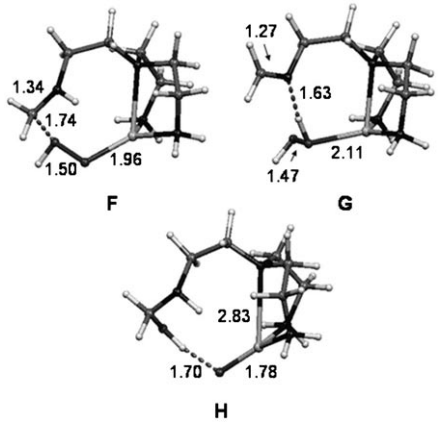


Figure 7. Singlet intermediate structures. Distances are given in Å.

Species **F** is best described as a carbocation/iminium intermediate, as testified by the planarity at the carbon atom and by the C–N distance (1.34 Å), which is significantly shorter than that observed in the triplet carboradical analogue (1.42 Å). For instance, the NPA charge at the reactive carbon atom in **F** is 0.19 (Table 1), whereas a value of −0.18 is obtained for **C**.[44] This stabilization of the carbocation by the nitrogen lone pair (iminium) accounts for the decoordination of the nitrogen atom from the copper atom. The NPA charges on the oxygen atoms, and especially that on O_p, are in favor of a hydroperoxide anion HOO^- coordinated to Cu^I in **F** ($q(O_p) = -0.64$; Table 1) in contrast to $HOO^\bullet$ coordinated to Cu^{II} in triplet species **C** ($q(O_p) = -0.52$).[44] The relatively short distance between O_d and the cationic carbon atom (1.74 Å) suggests an additional stabilizing intramolecular electrostatic interaction. Such stabilization and decoordination of the nitrogen atom have not been observed in the radical triplet intermediate.

Finally, **F** is formally obtained by heterolytic cleavage of the C–H bond and migration of the resulting hydride to the terminal oxygen atom. Formation of the intermediate HOO^- moiety in **F** is thus the result of a two-electron oxidation of the NHCH moiety by O_2, rather than a stepwise bielectronic process leading to a putative carboradical–Cu^{II} intermediate. The $C^+\cdots O_d$ interaction in **F** results in close vicinity between the hydrogen atom located at the nitrogen atom of the carbocation/iminium entity and the oxygen atom linked to the copper center. This provides an ideal arrangement for proton transfer from the carbocation/iminium group to the HOO^- moiety. The negligible energy barrier for this transfer (0.7 kcal mol^{-1}) allows facile formation of **G**, a copper(I) complex of hydrogen peroxide stabilized by an intramolecular H-bond with the imine nitrogen lone pair (Figures 6 and 7).

Discussion

Energetics and competition between different pathways

Comparison of singlet and triplet pathways: Since no evolution of the H-transfer products **B** and **C** could be found on the triplet surface, the only possible reactions for these species are the return to the oxygen adducts **A** or a jump to the singlet surface to evolve towards the imine. The competition between the singlet and triplet reactions is thus restricted to the first H-transfer step. Our efforts to properly reproduce the energetics of the singlet–triplet gaps thus focuses on the O_2 adducts and on the **TS(A-X)** transition states (**X** = **D** or **F**, see Table 1 and Figures 4 and 6). Application of the SF-TDDFT procedure to these Cu–O_2 adducts provides triplet ground states associated with singlet–triplet gaps of only 8.0 kcal mol^{-1}.[43] The triplet transition states are, however, located about 10 kcal mol^{-1} above the singlet transition states. Hence, this rules out any reactivity on the triplet surfaces. The H transfers on the singlet surfaces are kinetically favored, even though the singlet adducts are not the lower-energy reactants. Consequently, H transfer in these $[CuO_2]^+$ systems is proposed to proceed by a two-step mechanism. First, a spin transition from the triplet to the singlet state must take place. Note that, in contrast to the compounds investigated here, copper monooxygenases have been proposed to reach the singlet surface as soon as molecular dioxygen has coordinated the copper center.[50,51] Then, H transfer itself takes place on the singlet surfaces.

Comparison of NH and CH abstraction mechanisms: Quite surprisingly, H-transfer reactions from the N–H and C–H bonds are fully competitive, as the differences between their activation barriers lie within the error bars of the computational methodologies used. However, these paths differ strongly when considering the electronic structures of the implied intermediates and transition states (Figures 4–7): they are thus expected to show different dependences on environmental or appendage modulations. When H transfer first takes place at the NH moiety, a one-electron transfer is observed that gives singlet biradical intermediate **D**. Recovery of the closed-shell structure then occurs upon the second H transfer from **D′**. In strong contrast, instead of giving a carboradical associated with a Cu^{II} center, an initial C–H cleavage leads to carbocationic intermediate **F**, a fully closed shell species, and the second H transfer is simply an acid–base reaction. This means that the two-electron transfer from the NHCH moiety to yield the N=C bond takes place in the first step, as a net hydride transfer from the CH center. Such a bielectronic redox process that transforms $\mathbf{A_F}$ to **F** is fully consistent with the known fact that Cu^{II} centers can oxidize carboradicals.[52–56] The significant charge transfer from copper to dioxygen in $\mathbf{A_F}$ (predominantly

$[Cu^{II}O_2^{\bullet-}]$), indicated by a rather long O–O bond (Table 1), is certainly of great importance for C–H attack. The influence of this initial charge transfer on the energy barrier for this kind of adducts with predominant superoxide character is currently under investigation.[57]

Hence, in contrast to the N–H initial attack, for which the singlet and triplet states lead to similar formal electronic descriptions with N–H bond homolysis, the C–H attack on the singlet surface strongly differs from that on the triplet surface and leads to the C–H bond heterolysis.

Coordination of hydrogen peroxide: The singlet pathways thus open the way to a thermodynamically favored reaction of $[(tren^{Me1})Cu]^+$ with O_2 that yields an imine (oxidized complex **E**) and H_2O_2 (Scheme 1). At this point, coordination of H_2O_2 to **E** must be discussed, since it will decide whether H_2O_2 and **E** can be released into the reaction medium. The computed overall energy for the reaction of $[(tren^{Me1})Cu]^+$ with O_2 to give the imino Cu^+ complex and H_2O_2, as depicted in Scheme 1, is -9.0 kcal mol^{-1}. No coordination of H_2O_2 to copper can occur when the four nitrogen atoms of **E** are coordinated to the Cu^I center. This observation agrees with other results available for tetradentate copper(I) complexes for which the coordination of CO or CH_3CN is observed with binding energies of about 8 kcal mol^{-1},[41] but not the coordination of water.[58] On the other hand, coordination of hydrogen peroxide is achieved in **G** by decoordinating the imine arm and forming a Cu–O bond with the hydrogen-bonded H_2O_2 ligand (Figures 6 and 7). Release of H_2O_2 yielding imino-coordinated product **E** is endothermic by 8.3 kcal mol^{-1}, but becomes exergonic by 3.2 kcal mol^{-1} if entropic contributions are taken into account at $T=298$ K.

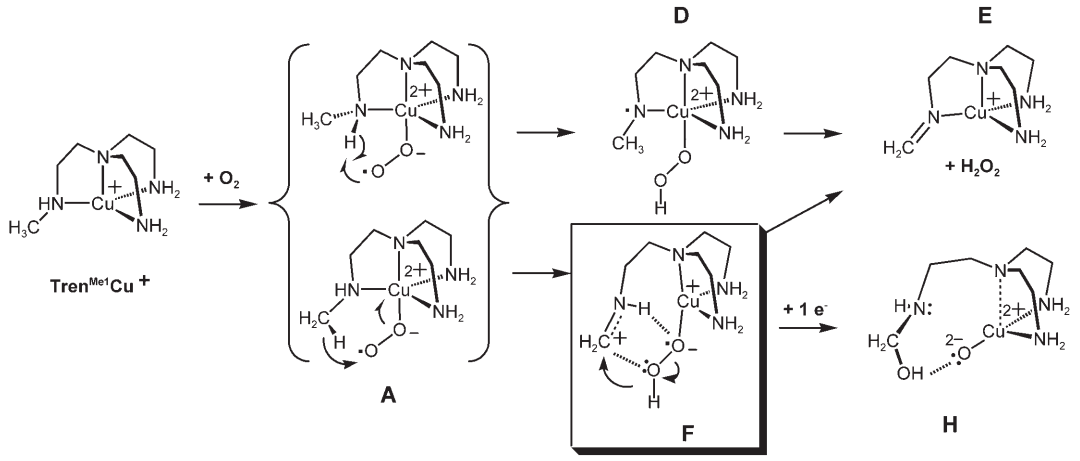
Implications for enzymatic mechanisms

Metalated (N_3N) species are inspired by centers of mononuclear monooxygenases such as PHM or DβH.[8–10] It is thus interesting to compare the above-described reaction schemes initiated by the C–H bond attack to these enzymatic systems. However, only the first H transfer from C–H can be envisioned for such species, since in peptidylglycine, a natural substrate of PHM, the adjacent N–H group is too far from the copper center to allow reactivity at the nitrogen atom. In DβH, no NH group is found at this position. The analogy with our reaction mechanism is thus limited to the $\mathbf{A_F}$-to-**F** path and ends at carbocation **F**.

First, we note that the high energy of the computed triplet transition states is perfectly in line with values derived from QM/MM modeling of the enzyme.[14] It thus appears that the reaction in monooxygenases should take place on the singlet surface with electronic rearrangements similar to those described in the present work. We also recall that the energy barrier of the singlet adduct (13 kcal mol^{-1}) is close to the experimental enthalpies proposed for PHM.

Second, our results for the singlet state also compare rather well with experimental and theoretical data. For instance, for the singlet transition in **TS($\mathbf{A_F}$-F)**, the C–H distance (1.50 Å) is close to that reported for a gas-phase model of the PHM active site (ca. 1.6 Å).[13] Introducing the reaction coordinate ξ_{TS}[59] evaluated at the transition state allows a better comparison to other structural data reported in the literature. The value found in this work ($\xi_{TS}=0.44$ Å) is almost identical to that reported for a biomimetic complex ($\xi_{TS}=0.45$ Å) involving N_2-type ligands,[32] and slightly larger than that derived from a QM/MM modeling of PHM ($\xi_{TS}\approx 0.38$ Å).[14] However, it corresponds to a significantly later TS than those reported in the gas phase ($\xi_{TS}=0.16$ Å)[15] or obtained from QM/MM modeling of the active site of the DβH enzyme ($\xi_{TS}\approx 0.13$ Å).[16]

Relevance of the carbocation intermediate: The cationic character of intermediate **F** is not inconsistent with biochemical studies claiming a radical nature of the H-transfer product for DβH[60–62] or PHM.[63] Indeed, whereas experiments clearly rule out a carbanionic intermediate, the carbocation possibility was discarded solely as "highly unlikely".[62] Additionally, the peculiar O···C interaction observed in **F** can



Scheme 1. Calculated pathways on the singlet surface for the intramolecular oxidation of the $tren^{Me1}$ ligand mediated by the $[CuO_2]^+$ core without (top) and with (bottom) injection of a supplementary electron (the arrows for **F** refer to its transformation into **H**).

be proposed to play an important role in the stereocontrolled formation of the final C–O bond when transposed to the enzymatic mechanism (vide infra).

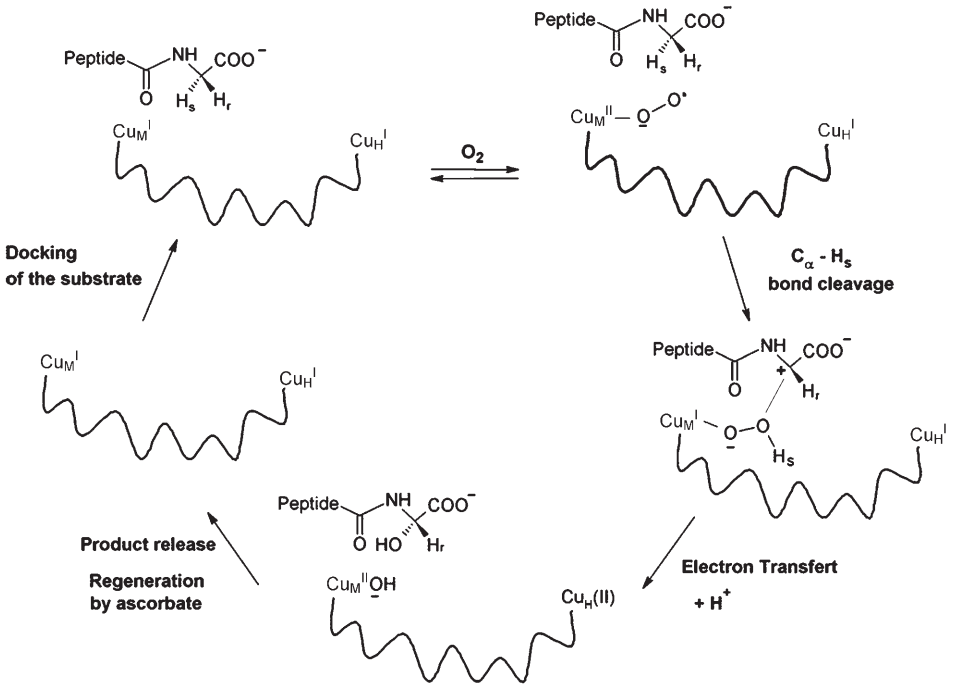
Whereas the electronic behavior can be reasonably extended from the model investigated here to PHM, the energy data are likely to be affected by the exact nature of the copper environment and by the substrate. We have stressed the role played by the amine nitrogen atom in stabilizing the carbocation/iminium intermediate **F**. The presence of a stabilizing chemical group adjacent to C_α should be critical for hydroxylation by PHM and DβH. Indeed, the putative cationic intermediate in PHM and DβH should have a lifetime long enough to fit with experimental data showing that the rate-limiting step occurs after the C–H bond breaking.[64,65] Starting from our $tren^{Me1}$ model it is possible to gain more insight into how the energy of the reaction can be modified when the biological substrate is replaced by the $tren^{Me1}$ model. In this connection, we examined abstraction of H^- from a variety of organic systems by heterolytic cleavage: $RH \rightarrow R^+ + H^-$.

The corresponding reaction energies were computed for various R: a model tren arm ($EtNHCH_2H$, 250 kcal mol^{-1}), a carboxylated tren arm ($EtNHCH(COO^-)H$, 143 kcal mol^{-1}), the terminus of the DβH substrate ($PhCHHCH_2NH_2$, 256 kcal mol^{-1}), and that of the PHM substrate ($CH_3CONHCHHCO_2^-$, 176 kcal mol^{-1}). Compared to the tren arm used in our mechanistic study, abstraction is found to be much less endothermic in the case of the peptidylglycine, whereas similar values are found for the DβH substrate. Since the energy difference between the cationic intermediate and the starting $[CuO_2]^+$ adduct is linked to such a hydride abstraction, it can be proposed that the cationic intermediate in copper monooxygenases is at least as stable as in the $tren^{Me1}$ bioinspired model.

Achieving hydroxylation: For PHM and DβH, the literature indicates that the electronic contribution of two Cu^I centers is required to achieve hydroxylation of substrates (Figure 1). After rupture of the C–H bond with subsequent formation of the stabilized carbocation, it seems reasonable to invoke a long-range electron transfer from the PHM Cu_H site.[25,66] We thus simulated this electron transfer in our $tren^{Me1}$ model by injecting one electron into the optimized intermediate **F.** Optimization of the resulting $\mathbf{F}^-$ species spontaneously leads to O–O bond cleavage and yields hydroxylated species **H** together with a neutral [CuO] entity (Scheme 1 and Figure 7). Product **H** exhibits a trigonal geometry at the copper center and only a weak interaction with the apical nitrogen atom (2.83 Å). The existence of a short hydrogen bond (1.70 Å) between O_p and the hydrogen atom linked to O_d is noteworthy.

In terms of formal electron counts, injection of one electron thus allows reduction of O_2 to proceed towards the formation of a C–OH bond and a $Cu^{II}O^{2-}$ moiety. Product **H** is characterized by the following NPA charges: $q(Cu) = +0.92$, $q(O_p) = -0.98$, and $q(O_d) = -0.87$. Together with the fact that the Cu–O bond length is 1.78 Å, and has thus decreased compared to **F** (1.96 Å), comparison with the charges in **F** ($q(Cu) = +0.82$) indicates that oxidation of copper is only partial, with pronounced electron transfer from the O atom. This injected electron was the only missing element for completing the reaction up to hydroxylation of the substrate.

Proposed mechanism for catalysis of hydroxylation by noncoupled copper monooxygenases: It is thus possible to propose a novel mechanistic scheme for hydroxylation by noncoupled copper monooxygenases (here PHM, which is characterized by two uncoupled Cu^I sites, Cu_M and Cu_H) adapted from the singlet C–H cleavage mechanism obtained with the $[(tren^{Me1})Cu]^+$ complex. It proceeds through a hydride abstraction in the singlet $[Cu_MO_2]$ adduct to yield an **F**-like intermediate, which, on injection of an additional electron issued from Cu_H,[25,66] spontaneously induces hydroxylation in a single step. The resulting $[Cu_M^{II}O^{2-}]$ core is most likely stabilized by protonation in a biological environment, since it is known to be surrounded by water molecules. This revised mechanism is summarized in Scheme 2, and it is com-



Scheme 2. Proposed catalytic cycle for PHM-catalyzed hydroxylation.

pared to the two other mechanisms described in the literature in the Supporting Information section.[11,13,67,68]

A first difference in our proposal stems from the C–H bond-breaking mechanism that proceeds by heterolytic cleavage upon attack by the superoxide species of $[CuO_2]^+$. Indeed, this contrasts with the usually considered homolytic cleavage. An important consequence is that no biradical intermediate is produced, the existence of which was questionable in view of the known capacity of Cu^{2+} to directly oxidize organic radicals.[52–56] A second, possibly major, difference lies in the Cu_H-to-Cu_M electron-transfer step. Here we propose that it occurs after C–H bond cleavage but before oxygen-atom transfer to the substrate. Its key role is thus to direct the chemical pathway towards C–O bond formation thanks to the breaking of the Cu^I-coordinated hydroperoxide O–O bond to liberate a formal hydroxide anion that is trapped by the carbocation. Interestingly, the peculiar $O\cdots C^+$ interaction observed in the carbocationic intermediate may play an important role in the stereocontrol of the final C–O bond formation.

Previous theoretical investigations pointed out that the critical step of breaking the O–O bond of a putative $[Cu^{II}(^-OOH)]$ intermediate, as proposed in previous mechanisms, is a strongly endothermic step. Furthermore, it was difficult in these previous studies to unquestionably rationalize the fact that the electron provided by the Cu_H center will generate the most oxidizing species $Cu^{II}O^\bullet$ *after* C–H bond cleavage, leading to a carboradical that should be easily oxidized by a Cu^{II} center.[52–56] The currently proposed mechanism (Scheme 2) bypasses these problems and exhibits a novel, elegant, and appealing solution to such difficulties.

Conclusions

Reaction mechanisms involving O_2 are often black boxes to chemists due to the lack of simple models that allow the electronic reorganizations to be understood. In this study, we used the prototypical oxidation of the $tren^{Me1}$ ligand by O_2 in the presence of Cu^+ as a benchmark system to examine the reaction paths associated with C–H or N–H cleavage. We have shown that, although the fundamental state of the considered starting $[CuO_2]^+$ adduct is a triplet, the reactivity of interest proceeds on singlet surfaces and that the starting adduct exhibits superoxide character. The use of SF-TDDFT allowed the energetic competition between these singlet pathways to be examined and showed that the C–H and N–H pathways are competitive. Despite the small energy differences between these two processes, they proceed along fully different paths: N–H cleavage occurs by radical hydrogen transfer, whereas a stabilized carbocation product is found in the case of C–H bond breaking, which thus corresponds to a hydride transfer. In both cases, the system evolves to an imine and H_2O_2. However, stabilization of the carbocation along the C–H abstraction pathways allows such a moiety to be proposed as a viable intermediate in enzymes, in full accordance with the most recent proposals gathered from biochemical studies.[25,69] Further evolution of the reaction in monooxygenases was then mimicked by injection of one electron into this cationic system: this spontaneously yields the hydroxylation product in a single step. This set of results leads to the proposal of a revised catalytic scheme for noncoupled copper monooxygenases in which electron transfer plays a key role in inserting oxygen into a C–H bond.

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