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Dioxygen Activation by Mononuclear Copper Enzymes: Insights from a Tripodal Ligand Mimicking Their Cu_M Coordination Sphere

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A mononuclear cuprous complex is proposed as a novel in silico model for the Cu_M active site of noncoupled copper monooxygenases. To the best of our knowledge, it is one of the first biomimicking models that allows one to recover the intimate structural features of the enzymatic oxygenated adducts and to gain clear-cut insights relevant to dioxygen activation by these enzymes.

The way enzymes make use of the oxidative power of dioxygen (O_2) relies on a broad range of chemical strategies. Most of the biological systems activating O_2 are iron- and/or copper-dependent enzymes.[1] The family of uncoupled copper monooxygenases, namely, dopamine β-monooxygenase, tyramine β-monooxygenase, and peptidylglycine-α-hydroxylating monooxygenase (PHM), represents a striking case. Each member contains two copper sites, Cu_M and Cu_H, separated by a solvent cleft of about 11 Å width.[2] Numerous experimental and theoretical (QM/MM) studies have been devoted to these systems to address the exact nature of the chemical intermediate reponsible for the C−H bond breaking:[3] Various proposals have been made (e.g., $[Cu_MO^\bullet]^+$ or $[Cu_MO]^{2+}$), but recent biochemical experiments support reactivity through a copper/superoxo $[Cu_M^{II}/O_2^{\bullet -}]$ adduct.[4]

Recently, a theoretical model of the "H" transfer from a C−H bond has been reported within a bioinspired $[Cu^{II}/O_2^{\bullet -}]$ adduct.[5a] This study confirmed the intrinsic oxidative properties of the system but only when singlet spin-state pathways are considered; the triplet-state reactions have been found to be especially unfavorable. It has also been shown that only copper/superoxo forms result in low activation barriers for the C−H bond breaking (~13 kcal·mol^{-1}), in contrast to both the oxygen $[Cu^I/O_2]$ and peroxo $[Cu^{III}/O_2^{2-}]$ forms.[5b] Fine control of the charge transfer from copper to dioxygen is thus essential to tune the reactive properties of the Cu/O_2 adducts. The very short O−O bond length (1.23 Å) observed in the precatalytic structure of PHM thus raises puzzling questions about the capability of this adduct to achieve its catalytic role.[6] Biomimetic approaches provide a powerful strategy to complement studies performed on enzymatic systems, but despite the accelerating pace of research on the development of bioinspired models,[7] the reproduction of the Cu_M active-site features is still a challenge. We here propose a new tripodal complex that accurately reproduces the enzymatic crystallographic Cu_M/O_2 coordination and allows one to derive fresh conclusions related to dioxygen activation at mononuclear copper centers.

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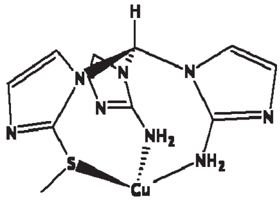
Chart 1



The new supporting neutral ligand (Mim^{N_2S}) is depicted in Chart 1. It is characterized by two nitrogen and one sulfur atoms coordinated to copper as encountered in the enzymatic Cu$_M$ coordination sphere (two histidine and one methionine residues). In a previous study involving a set of Cu/O$_2$ adducts, we highlighted the decisive role of the valence angles at the copper center in directing O$_2$ coordination toward *end-on* or *side-on* modes.[5c] Here, ligand Mim^{N_2S} was thus built in order to reproduce the Cu$_M$ valence angles as closely as possible.[6] On the other hand, the ligand electronic effects also have to be taken into account, with the aim of favoring superoxide-like adducts. To this end, a neutral tripodal ligand, lacking any apical coordinating atom, seems appropriate. This point is crucial to avoid any push−pull effect that would enhance charge transfer toward O$_2$.[5c] The new mixed N/S ligand contrasts with older ligands based on tetradendate tris(2-aminoethyl)amine or borate architectures.[5,7]

Several initial [Cu(Mim^{N_2S})O]$^+$ structures have been optimized to characterize the various binding modes of O$_2$ (*side-on* or *end-on*, singlet or triplet). Isomers **A**−**C** are singlet species, whereas **D**−**F** are triplets (Figure 1). In order to account for the biradical character (open-shell singlets)[8] of these adducts, a two-step strategy has been followed. First the complexes were fully optimized at the density functional theory (DFT) level of computation (B3LYP functional). The relative energies of structures **A**−**F** (see below) were then evaluated within the spin-flip time-dependent DFT formalism. A previous study has shown the reliability of this procedure by means of systematic comparisons of spin-flip DFT results with CASMP2 computations.[5d] More details about the computational protocol (e.g., basis set, functionals, etc.) are given in the Supporting Information.

Whatever the spin state, *side-on* adducts **A**, **B**, **D**, and **E** exhibit similar geometrical features, namely, a trigonal-pyramidal structure in which the O$_2$ moiety occupies a basal position and the axial position is occupied either by S (**A** and **D**) or N (**B** and **E**). The distance between the copper and the coordinating atom experiences a significant increase when going from the basal to the apical position. This is most probably connected to the presence of the π-accepting O$_2$ ligand in one of the basal positions. This elongation is particularly noticeable where sulfur is concerned. The Cu−S distance increases from ca. 2.35 Å (**B** or **E**) to 2.71 Å [**A** $^1(\eta^2)$] or 2.55 Å [**D** $^3(\eta^2)$]. It is worth noting that the position of the sulfur atom (basal or apical) has no effect on the charge transfer toward *side-on* O$_2$ for a given spin state. For both **A** and **B**, the O−O bond length remains about 1.33 Å, and it is about 1.27 Å in **D** and **E**. As testified by the interoxygen distances (about 1.3 Å), these complexes are typical of [Cu^{II}/O$_2^{\bullet -}$] cores.[5d]

For *end-on* adducts **C** and **F**, different geometries at the metallic center are found, depending on the spin state. Whereas the coordination angles of the CuN$_{(1\ \mathrm{or}\ 2)}$ S moiety are almost similar, two different coordinations are observed for O$_2$. In the singlet complex **C**, the O$_1$CuN$_2$ angle is quite large (148°) and a low-symmetry structure with no obvious parentage is obtained. Actually, this particular geometrical arrangement has already been reported with various Cu/O$_2$ models built on mono- or polydentate ligands.[5c,9] The O−O bond length (1.27 Å) indicates a moderate charge transfer toward O$_2$ and, consequently, a moderate superoxide character ([Cu^{II}/O$_2^{\bullet -}$] form).

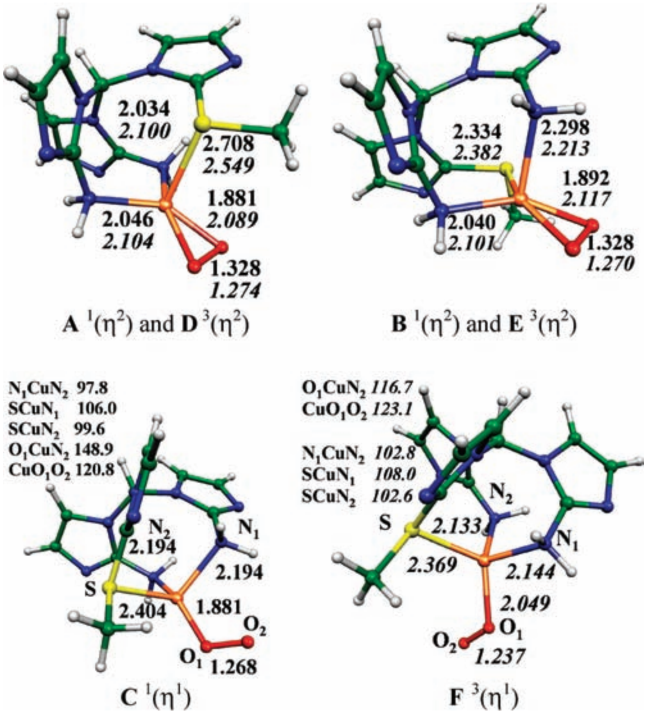


Figure 1. DFT-optimized isomers of the [Cu(Mim^{N_2S})O$_2$]$^+$ adducts. The notation $^x(\eta^y)$ stands for a complex in which x is the spin multiplicity and y is the hapticity of O$_2$. Distances are in angstroms and angles in degrees (italics for triplets).

On the other hand, the triplet adduct is found to be tetrahedral, with all valence angles at Cu between 102 and 117°. A very weak superoxide character is deduced from the short O−O bond length (1.24 Å). Hence, this adduct is best described as a [Cu^I/O$_2$] entity. Such a weak charge transfer was partly anticipated, first because of the *end-on* binding mode,[5c] and second, as was already mentioned, because of the lack of a binding atom in the trans position to O$_2$.

The geometrical features of these oxygenated adducts are now compared to the structural data derived from the X-ray experiments for the PHM enzyme, The Cu$_M$/O$_2$ precatalytic adduct has been shown to exibit a tetrahedral *end-on* coordination (Figure 2),[6] but its spin state remains unknown. The close match between the triplet structure **F** and the precatalytic adduct is remarkable from the viewpoint of both the O−O bond length and the NCuO angles. Besides the tetrahedral geometry encountered in both cases, the amount of charge transfer toward O$_2$ is perfectly in line, as testified by the O−O bond lengths (1.24 vs. 1.23 Å). In contrast, the singlet adduct **D** reproduces neither such a geometric arrangement nor the very small superoxide character of the

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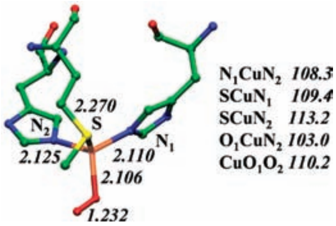

Figure 2. X-ray structure of the $[Cu/O_2]$ precatalytic adduct of PHM.[6] Distances are in angstroms and angles in degrees.

precatalytic adduct. These features thus suggest that PHM was experimentally trapped in a triplet precatalytic state of its Cu_MO_2 core.

Adduct **F** is the first bioinspired adduct to reflect so closely the geometrical features of the enzymatic adduct. Hence, we take advantage of this new Mim^{N_2S} ligand to provide some preliminary hints addressing the question of O_2 activation at the Cu_M metallic center. We will especially focus on the relaxation of the triplet primary adduct toward the singlet species, which are generally considered as the reactive entities[4,5a] The former transformation may arise by means of various mechanisms, the theoretical treatment of which remains challenging.[10] Here we first consider the relative stability of the different isomers (Table 1).

Compound **F** does not appear as the absolute minimum of all possible species because *side-on* complexes **A**, **B**, **D**, and **E** are favored by about 4 $kcal\cdot mol^{-1}$. Only the singlet *end-on* structure is found to be higher in energy ($+5$ $kcal\cdot mol^{-1}$). We note that a similar ordering has been reported by Cramer et al. using a CASMP2 approach to model the galactose oxidase active site.[9]

The energetic similarity for **A** and **B**, on the one hand, and for **D** and **E**, on the other hand, shows that the position of the sulfur atom in the coordination sphere does not influence the energetical properties of the adducts. We also evaluated the energetic effect of the substitution of the SMe group by a NH_2 group ("N_2N" columns in Table 1). Similar structural and energetic conclusions can be drawn for the N_2N series, supporting the absence of any specific role of the thioether group in the relative energies of the adducts.

Alternatively, one might suspect a possible role of sulfur in enhancing the probability of the triplet–singlet transition in the case of a spin-forbidden transition as an effective mechanism to reach a singlet Cu_M/O_2 species in PHM. The spin–orbit (SO) coupling might then play a critical role in the kinetics of this spin transition: it is well-known that heavy atoms favor such couplings. Furthermore, the sulfur atom can be proposed to allow discrimination between different isomers and direct O_2 activation toward a unique species, for example, **A** rather than **B**. We investigate here such hypotheses by estimating the SO coupling constants (H_{SO}) between the triplet and singlet states at the triplet geometries. The H_{SO} values have been computed within the DFT linear response theory (see the Supporting Information).

Table 1. Relative Energies ΔE and Cartesian Components of the SO Coupling Constants H_{SO}

		N_2S						N_2N			
		A	**B**	**C**	**D**	**E**	**F**	**A/B**	**C**	**D/E**	**F**
ΔE^a ($kcal\cdot mol^{-1}$)		−4.0	−4.4	4.7	−3.8	−2.4	0.0	−4.9	5.5	−4.4	0.0
$\|H_{SO}\|$ (cm^{-1})	x				44	156	70			84	18
	y				2	7	131			15	59
	z				14	29	83			25	40

[a] The ΔE values correspond to the adducts of Figure 1, taking **F** as the energetic reference. The $|H_{SO}|$ values have been computed at the DFT level on triplet geometries (see the Supporting Information for details).

The H_{SO} values range from a few tens of cm^{-1} to about 160 cm^{-1}, depending on the complex studied. No dramatic differences are found between the complexes, especially within the *per*-nitrogenous series. Nevertheless, all H_{SO} values are certainly high enough to allow spin transitions.[10] These constants are found to be somewhat larger for **E** and **F**. This suggests a putative role for the sulfur atom in increasing the rate of the triplet–singlet spin transition for these two forms. This theoretical observation should stimulate future experimental work to explore the role of a sulfur atom at the Cu_M site of the PHM enzyme.

This communication has reported a new model able to mimic the Cu_M active site of monomolecular copper monooxygenases. It has been possible to recover the structure of the experimentally characterized precatalytic Cu/O_2 adduct of PHM. This species led us to identify the enzymatic entity as a triplet *end-on* adduct. Numerous ligands exibiting close similarity with the Mim^{N_2S} ligand have been synthesized in the past,[7g] suggesting that it is certainly amenable to synthesis, as such or grafted on supramolecular devices.[11] This novel tripodal ligand will also be used in future computational studies, in particular to address more deeply the role of the thioether group.[12] Such investigations are in progress and will be reported in due course.

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Supporting Information Available: Computational approaches and Cartesian coordinates of the *per*-nitrogenous complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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