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Reaction of a Copper−Dioxygen Complex with Nitrogen Monoxide (•NO) Leads to a Copper(II)−Peroxynitrite Species

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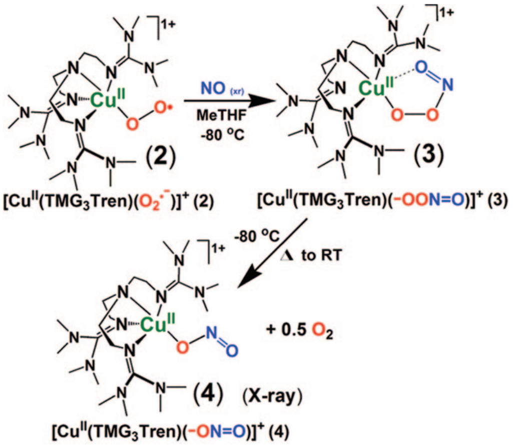
Peroxynitrite (oxoperoxonitrate (1−), $^{-}$OON=O) is a reactive agent generated by the near diffusion controlled combination of •NO (often called nitric oxide) and the superoxide anion ($O_2^{\bullet -}$) and is considered as a likely mediator of nitric oxide biochemistry and oxidative/nitrative stress injury.[1,2] Metal ions in biological systems may be important in $^{-}$OON=O generation, stabilization, thermal transformation reactions (e.g., isomerization to nitrate (NO_3^-) or production of nitrite (+1/2O_2)), or activation toward substrate oxidation/nitration.[2–4] Heme proteins have been recently well-studied with respect to their mediation of peroxynitrite formation and subsequent transformation to nitrate;[1a,2,5] NO dioxygenases convert NO to nitrate using O_2, putatively via peroxynitrite intermediates.[6] Metal complexes with Fe, heme, Mn, and Cu act as peroxynitrite decomposition (i.e., to nitrate) catalysts and may possess therapeutic applications.[1,7] Discrete metal−peroxynitrite complexes are rare[2] but are suggested to form as transients from metal−NO + $O_{2(g)}$ or metal−O_2 + •$NO_{(g)}$ reactions.[3,8]

Following our interest in Cu oxidative chemistries, we note that the literature solution chemistry of copper ion with peroxynitrite is limited;[4,9] no discrete copper−peroxynitrite species have been described. Here, we report the reaction of •$NO_{(g)}$ with a Cu^I/O_2 adduct, [(TMG$_3$tren)Cu^{II}($O_2^{\bullet -}$)]$^+$ (**2**), with end-on bound superoxo ligand, ∠Cu−O−O = 123.5°, O−O = 1.280 Å.[10] The product is a discrete peroxynitrite−Cu^{II} complex, formulated as [(TMG$_3$tren)Cu^{II}($^{-}$OON=O)]$^+$ (**3**) (Scheme 1). This undergoes a thermal transformation to give a nitrite complex, [(TMG$_3$tren)-Cu^{II}($^{-}$ONO)]$^+$ (**4**), plus dioxygen. The results suggest the viability of biological Cu^I/O_2/(•NO) peroxynitrite formation, that is, *not* coming from free superoxide plus •NO reaction (vide supra) and as perhaps already observed for CuZn superoxide dismutase (SOD)[11] and cytochrome *c* oxidase.[12] Peroxynitrite has been discussed with respect to CuZn−SOD, a vector for disease states; might mutant SODs not abrogate $^{-}$OONO toxicity (i.e., downstream oxidative or nitrative stress) or even produce peroxynitrite?[11,13]

Bubbling $O_{2(g)}$ through a colorless solution of [(TMG$_3$tren)−Cu^I]B(C_6F_5)$_4$ (**1**) in 2-methyltetrahydrofuran (MeTHF) at −80 °C leads to the EPR silent light green colored complex [(TMG$_3$tren)Cu^{II}($O_2^{\bullet -}$)]B(C_6F_5)$_4$ (**2**) [λ_{max} = 447, 680, 780 nm (Figure S1)].[14,15] With excess O_2 removed by vacuum/purging$_{(Ar)}$, bubbling **2** with •$NO_{(g)}$ (−80 °C; subsequent excess $NO_{(g)}$ removed) gives a yellowish green complex, formulated as the peroxynitrite species [(TMG$_3$tren)Cu^{II}($^{-}$OON=O)]B(C_6F_5)$_4$ (**3**) (Figure S1, λ_{max} = 314 nm, ε = 6900 M^{-1} cm^{-1}).[15] Direct evidence for the formation of **3** comes from electrospray ionization mass spectrometry (ESI-MS).[15] Injection of −80 °C MeTHF solutions of **3** gives rise to a parent peak cluster with *m*/*z* = 565.15 and expected ^{63,65}Cu pattern for the [(TMG$_3$tren)Cu^{II}($^{-}$OON=O)]$^+$ cation (Figure 1a).[15] When $^{18}O_{2(g)}$ is used for the generation of **2**, subsequent addition of •$NO_{(g)}$ reveals that the peroxynitrite−Cu^{II} peak shifted (by 4 mass units) to *m*/*z* 569.34 [Figure 1b; 65% incorporation (also with appropriate ^{63,65}Cu isotope pattern)], indicating that [(TMG$_3$tren)Cu^{II}($^{-18}O^{18}$ON=O)]$^+$ has formed;[15] the adjacent peroxo oxygen atoms are derived from O_2 (Scheme 1).[16]

Scheme 1



As mentioned, X-ray structures are not known for peroxynitrite−metal species.[2] For the one known isolated complex from Koppenol and co-workers, a k^1-*O*-OONO binding in [(NC)$_5$Co($^{-}$OONO)]$^{3-}$ is expected.[2,17] O- and N-bound $^{-}$OONO ligation in various geometries has been considered, especially for iron porphyrinate adducts.[5b,18] DFT calculations, using B3LYP and a mixed triple-ζ basis set, suggest two possible structures for [(TMG$_3$tren)Cu^{II}($^{-}$OON=O)]$^+$ (**3**), a lower energy (by 9.3 kcal/mol in THF) form with monodentate k^1-*O*-OONO ligation in an overall trigonal bipyramidal (TBP) coordination (d_{z^2} ground state), and a more square pyramidal (SP) form ($d_{x^2-y^2}$ ground

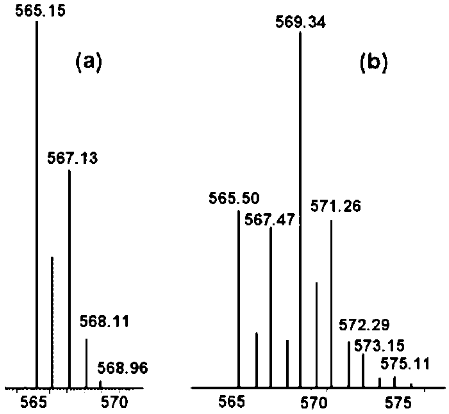


Figure 1. ESI-MS spectra: (a) [Cu^{II}(TMG$_3$tren)($^{-}$OON=O)]$^+$ (**3**) at *m*/*z* = 565.15; (b) [Cu^{II}(TMG$_3$tren)($^{-18}O^{18}$ONO)]$^+$ (**3**), *m*/*z* = 569.34.

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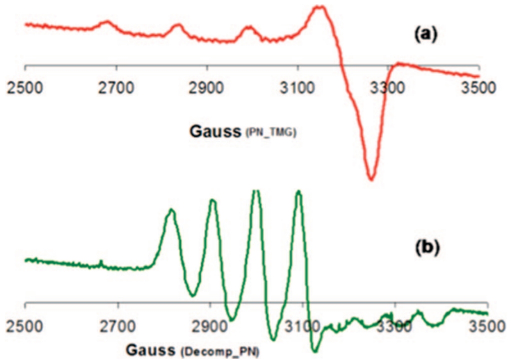

Figure 2. EPR spectrum of (a) **3** (red) and (b) **4** (green).

state) possessing a cyclic bidentate k^2-*O,O′*-OONO peroxynitrite moiety, with one short equatorial and one long axial Cu−O distance (1.95 and 2.58 Å, respectively).[15] One (TMG$_3$tren) guanidine arm functionality in the SP form is dangling, that is, not ligated, and this arm is replaced by the long Cu−O in the axial position. See Supporting Information for structural diagrams.[15] The EPR spectrum of **3** is distinctly tetragonal with a $d_{x^2-y^2}$ ground state (Figure 2a), thus consistent with the latter (SP) and not the former (TBP) structure. In support of a pentacoordinate SP-type structure for **3** are a number of literature observations: (i) displacement of one arm of the tris(2-aminoethyl)amine (tren) derivative five-coordinate Cu(II) complexes in solution is well-established;[19] (ii) the solid-state structure of [(TMG$_3$tren)−Mo(CO)$_3$] reveals one ligand arm to be uncoordinated;[20] and (iii) SP geometries for many pentacoordinate Cu(II) with tridentate analogues of tren (i.e., $[(N_3)Cu^{II}X_2]^{n+}$) and bidentate analogues of TMG$_3$tren are documented via X-ray crystallography.[21]

The thermal transformation properties of $[(TMG_3tren)Cu^{II}(^{-}OON{=}O)]^+$ (**3**) support its formulation and provide insights into the observed peroxynitrite chemistry. Prolonged storage of −80 °C solutions of **3**, or warming to room temperature leads to >90% yields of the green Cu^{II}−nitrite (NO_2^-) complex $[(TMG_3tren)Cu^{II}(^{-}ONO)]B(C_6F_5)_4$ (**4**) [λ_{max} = 350 (sh) (3200), 610 (700) nm, Figure S1], accompanied by the evolution of dioxygen (30−35% yield, 50% theoretical), as determined by trapping with a known Cu-based O$_2$ carrier.[15] The structure of **4** was determined by X-ray crystallography,[15] revealing an η^1-*O*-nitrito bound to Cu(II) ion in an overall trigonal bipyramidal environment. DFT calculations[15] on this structure show that it possesses a d_{z^2} ground state (Figure S12), and an EPR spectrum of **4** (Figure 2b) reveals the reverse axial spectrum expected.[22] The dramatic EPR spectroscopic differences between $^{-}$OONO (peroxynitrito) complex **3** and ONO$^-$ (nitrito) compound **4** (Figure 2) highlight the distinctly different nature of these species.

The identification of nitrite complex **4** and O$_2$, along with their yields (vide supra), further confirms the Scheme 1 stoichiometry and formulation of peroxynitrite complex **3**. We find no evidence for Cu(II)−nitrate (NO_3^-) formation, that is, peroxynitrite isomerization.[15,23]

For **4**, *m*/*z* = 549.45,[15] but when the reaction sequence (Scheme 1) is carried out with $^{18}O_{2(g)}$, this shifts to *m*/*z* = 551.12 (78% incorporation, based on an expectation of one of the two O$_2$-derived atoms being incorporated),[15] indicating that $[(TMG_3tren)Cu^{II}(^{-18}ONO)]^+$ (**4**) has formed. Clearly, an O−O cleavage reaction has occurred. An extensive literature[1a,24,25] known for HOONO conversion to nitrite and O$_2$ may apply; further studies are needed. In fact, related copper(aq) chemistry has been described.[4,9c]

In summary, we have described here the formation, spectroscopic features, and thermal transformation chemistry of the first discrete Cu(II)−peroxynitrite complex. Further studies will focus on the reactivity of this peroxynitrite complex, likely involving peroxynitrite O−O cleavage chemistry. The work described here suggests that copper ion in biological media may facilitate Cu/O$_2$/•NO and thus peroxynitrite chemistry, that is, oxidation and/or nitration.

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Supporting Information Available: Details of synthesis; reactivity, product analyses, ESI-MS findings, DFT calculations, and CIF files. This material is available free of charge via the Internet at http://pubs.acs.org.

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