

Peroxynitrite chemistry derived from nitric oxide reaction with a Cu(II)–OOH species and a new copper mediated NO reductive coupling reaction†

Cite this: DOI: 10.1039/c3cc47942k

Received 15th October 2013,
Accepted 27th November 2013

DOI: 10.1039/c3cc47942k

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New peroxynitrite–copper chemistry ensues via addition of nitric oxide ($\bullet\text{NO}_{(\text{g})}$) to a Cu^{II} –hydroperoxy species. In characterizing the system, the ligand–Cu(I) complex was shown to effect $\bullet\text{NO}_{(\text{g})}$ reductive coupling, a new reaction type. Biological implications are discussed.

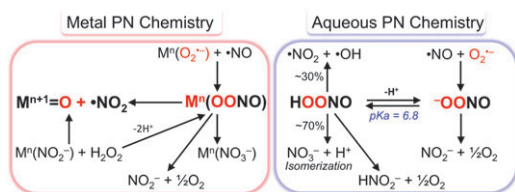
Reactive nitrogen species (RNS) have been of great interest due to their important role in biomolecule oxidation such as in aging and disease development.¹ It is RNS which induce tyrosine nitration in proteins, leading to damage and enzyme inactivation; a signaling role has been also discussed.¹ Tyrosine nitration occurs in the presence of either peroxynitrite ($\text{OON}=\text{O}$ or its conjugate acid; here, PN is defined as either) or nitrogen dioxide ($\bullet\text{NO}_2$). PN is presumed to be generated *in vivo* by the diffusion controlled combination of nitric oxide ($\bullet\text{NO}$) and superoxide anion ($\text{O}_2^{\bullet-}$),² or from nitrite (NO_2^-) oxidation by peroxidase enzymes (Scheme 1). Nitrogen dioxide forms from the homolytic O–O cleavage of PN, however most aqueous PN isomerizes to nitrate (NO_3^-).^{1,3} Metal ion PN chemistry^{3k,4} has developed greatly in just the last few years, and Scheme 1 also outlines recently established M–PN transformations.

Peroxynitrite generation is implicated from studies on the reactions of oxy-heme (formally $\text{Fe}^{\text{III}}\text{–O}_2^{\bullet-}$) proteins with $\bullet\text{NO}_{(\text{g})}$,^{4d,e,5} and superoxo–iron^{4f} or –cobalt^{4k} porphyrinates plus

nitric oxide. Recent reports also describe peroxynitrite intermediates forming from $\bullet\text{NO}_{(\text{g})}$ reaction with Cr–superoxo or peroxy species,^{4l,m} or for a Cu–superoxo complex.^{4g} Cu^{II} –PN complexes may decay to NO_2^- plus $\text{O}_2_{(\text{g})}$ (Scheme 1).^{4g,h}

As indicated, the importance of peroxynitrite generation and chemistry is in its biological role as a RNS. Given the chemistry (just mentioned) implicating $\text{M–O}_2(\equiv\text{M}^{\text{III}}(\text{O}_2^{\bullet-})) + \bullet\text{NO}_{(\text{g})}$ or $\text{M–NO} + \text{O}_2_{(\text{g})}$ in peroxynitrite generation and peroxynitrite-like reactivity, we have previously suggested that such reactivity may occur in biological systems, for example at the active sites of the superoxide dismutases of manganese⁶ or copper.⁷ Here, we describe the reaction of a Cu^{II} –hydroperoxy species with $\bullet\text{NO}_{(\text{g})}$, a new but complementary chemical transformation in the generation of PN chemistry. We suggest that this is quite possibly a biologically relevant reaction.

The copper–hydroperoxy complex $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**) (BA, a tetradentate N_4 ligand possessing a pendant $-\text{N}(\text{H})\text{CH}_2\text{C}_6\text{H}_5$ group), which is stabilized by H-bonding from the ligand side-arm N–H group to the proximal oxygen of the Cu^{II} –OOH moiety (Fig. 1),⁸ reacts with excess $\bullet\text{NO}_{(\text{g})}$ in acetone at -90°C , resulting in a color change from bright green to a pale greenish blue. The accompanying UV-vis bands attributed to **2** (393, 672 nm) decrease in intensity, while a new feature at $\lambda_{\text{max}} = 824\text{ nm}$ ($\epsilon = 315$) develops (Fig. 2).



Scheme 1

Department of Chemistry, The Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218, USA. E-mail: karlin@jhu.edu; Fax: +1 410-516-8420; Tel: +1 410-516-8027

† Electronic supplementary information (ESI) available: Details of synthesis, reaction conditions and spectroscopic monitoring, crystallographic data for **3**. CCDC 965635. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc47942k

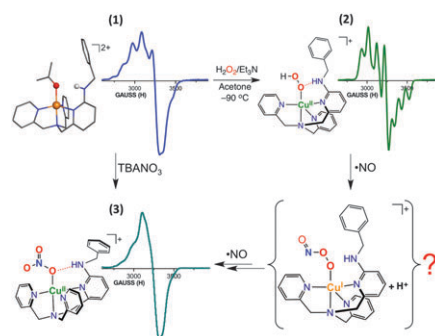


Fig. 1 $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**), formed from precursor **1**, reacts with $\bullet\text{NO}$ leading to the nitrate compound $[(\text{BA})\text{Cu}^{\text{II}}(\text{NO}_3^-)]^+$ (**3**) which can also be generated by the reaction of **1** with TBANO_3 . EPR data of **1**, **2** and **3** are shown (X-band, $\nu = 9.186\text{ GHz}$; acetone at 70 K, also, see ESI†).

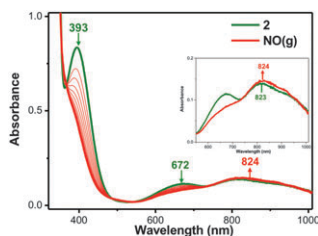


Fig. 2 UV-vis spectra of the reaction of $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**) with $\bullet\text{NO}$ at $-90\text{ }^\circ\text{C}$ in acetone under Ar.

From this solution a complex **3** was isolated (60% yield), identified as a nitrate complex: (i) electrospray ionization mass spectrometry (ESI-MS) reveals a prominent ion peak at $m/z = 520.11$, whose mass and isotope distribution pattern are consistent with the chemical formula $[(\text{BA})\text{Cu}^{\text{II}}(\text{NO}_3)]^+$ (calcd m/z 520.13) (ESI †). (ii) Single crystals of **3** as a perchlorate salt could be isolated, $[(\text{BA})\text{Cu}(\text{NO}_3)](\text{ClO}_4)$ (**3**); X-ray structure determination confirms the formulation and reveals for this complex a slightly distorted square pyramidal (SP) coordination ($\tau = 0.29$; $\tau = 0$ for idealized SP geometries).⁹

This supports a change in geometry, from the trigonal bipyramidal (TBP) coordination geometry observed for the precursor complexes $[(\text{BA})\text{Cu}^{\text{II}}(\text{CH}_3\text{COCH}_3)]^{2+}$ (**1**) and $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**) (EPR criteria).⁸ The structural/coordination changes result in a weaker H-bond between the ligand side-arm N-H to the Cu(II) bound $\text{O}_{\text{nitrito}}$ atom, $\text{H}-\text{O}_{\text{nitrito}} = 2.23(2)$, $\text{N}-\text{O}_{\text{nitrito}} = 2.963(3)$ Å, $\angle \text{N}-\text{H}-\text{O} = 146(2)^\circ$ as compared to that in $[(\text{BA})\text{Cu}^{\text{II}}(\text{CH}_3\text{COCH}_3)]^{2+}$ (**1**), $\text{H}-\text{O}_{\text{acetone}} = 2.10(2)/2.15(3)$, $\text{N}-\text{O}_{\text{acetone}} = 2.886(3)/2.981(3)$ Å, $\angle \text{N}-\text{H}-\text{O} = 161(3)/163(3)^\circ$ (also see the ESI †).

(iii) The EPR spectrum of $[(\text{BA})\text{Cu}^{\text{II}}(\text{NO}_3)]^+$ (**3**) is that of an axial type ($g_\perp \sim 2.10$);¹⁰ a higher resolution spectrum could not be obtained. (iv) Further proof that the product of $\text{NO}_{(\text{g})}$ reaction with hydroperoxo complex $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**) is the nitrate compound **3** derives from the fact that addition of tetrabutylammonium nitrate (TBANO₃) to a solution of $[(\text{BA})\text{Cu}^{\text{II}}(\text{CH}_3\text{COCH}_3)]^{2+}$ (**1**) gives a product with identical spectroscopic (UV-vis, EPR) features (Fig. 3).

The isolation of Cu(II)-nitrate complex **3** supports the intermediacy of a $^-\text{OON}=\text{O}$ species in the **2** + $\text{NO}_{(\text{g})}$ reaction (Fig. 1). It is well known that nitrate is the most common decomposition (as an isomerization) product of PN (Scheme 1). The peroxyxynitrite intermediate would develop from the nucleophilic attack of the hydroperoxo ligand within $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**) on nitric oxide. This would result in release of a proton along with formation of Cu(I) (eqn (1)). Note that PN normally is said to derive from $\text{O}_2^{\bullet-} + \bullet\text{NO}$, so if starting with peroxide

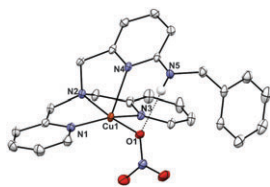
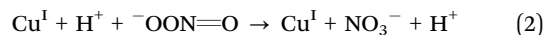
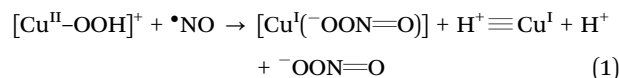


Fig. 3 Displacement ellipsoid plot (50% probability level) of $[(\text{BA})\text{Cu}^{\text{II}}(\text{NO}_3)](\text{ClO}_4)$, (**3**). The perchlorate counterion and the H atoms (except for that attached to N5) were removed for clarity.

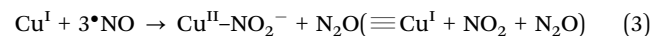
(in **2**) + $\bullet\text{NO}$, there is an extra electron, resulting in cupric ion reduction to Cu(I) (eqn (1)).



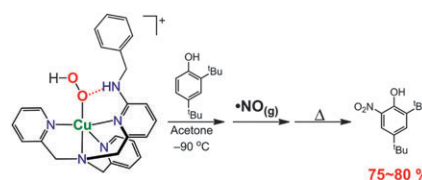
Peroxyxynitrite could then isomerize to nitrate as observed (eqn (2)). Precedent for this metal ion reduction in the formation of peroxyxynitrite comes from the work of Mondal and coworkers.¹¹ In an alternative approach but yet overall isoelectronic with our present chemistry, they performed a Cu(II)-nitrosyl (derived from a ligand Cu(II) compound plus $\bullet\text{NO}_{(\text{g})}$) and added H_2O_2 , giving a Cu(I)-nitrate complex, which was structurally characterized.

To provide further evidence that a PN moiety must be present during the reaction, we carried out a reactivity study employing 2,4-di-*tert*-butylphenol (2,4-DTBP) as a potential substrate and tyrosine mimic, see discussion and references above. When 2,4-DTBP (1 equiv.) is added to a $-90\text{ }^\circ\text{C}$ solution of $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**), prior to $\bullet\text{NO}_{(\text{g})}$ and it is allowed to warm to RT, workup leads to a 75–80% yield of nitrated phenol (2,4-di-*tert*-butyl-6-nitrophenol) and a copper(II) complex (Scheme 2) (ESI †). Phenol (*e.g.*, tyrosine) nitration is normally considered to occur *via* peroxyxynitrite homolytic O–O cleavage to give $\bullet\text{OH} + \bullet\text{NO}_2$; the former (as a Cu ion adduct?) would abstract a phenolic hydrogen-atom and the latter would then add to the resulting phenoxyl radical to give the nitrated product. A control reaction indicates that the combination of $[(\text{BA})\text{Cu}^{\text{II}}(\text{CH}_3\text{COCH}_3)]^{2+}$ (**1**) with 2,4-DTBP plus $\bullet\text{NO}_{(\text{g})}$ leads to less than a 20% yield of 2,4-di-*tert*-butyl-6-nitrophenol (ESI †).

As mentioned, in the original reaction of $[(\text{BA})\text{Cu}^{\text{II}}(\text{CH}_3\text{COCH}_3)]^{2+}$ (**1**) + $\bullet\text{NO}_{(\text{g})}$ where PN isomerization to nitrate occurred, Cu(I) should be formed. This is also the case for the phenol nitration chemistry, Scheme 2. Yet, in both cases, we instead observed that the final solution mixture contained Cu(II), the nitrate adduct for the former case and **1** in the phenol nitration chemistry. To explain this, we further interrogated the product solutions. Since small excesses of $\bullet\text{NO}_{(\text{g})}$ were normally employed, we surmised that Cu(I) which formed then mediated a $\bullet\text{NO}_{(\text{g})}$ disproportionation reaction (eqn (3)). This well-known reaction would lead to nitrous oxide ($\text{N}_2\text{O}_{(\text{g})}$) formation along with a $\text{Cu}^{\text{II}}-\text{NO}_2^-$ complex, for our case (eqn (3)).¹² In fact, for the $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**) + $\bullet\text{NO}_{(\text{g})}$ reaction, we detected significant amounts of $\text{N}_2\text{O}_{(\text{g})}$ (GC analyses). However, only a trace of nitrite could be detected, and as described above, NO_3^- was formed in high yield (eqn (2)).

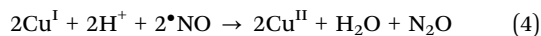


We can now explain these observations as follows. Under acidic conditions, where a proton derives from the hydroperoxo



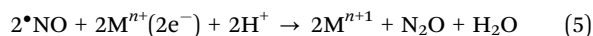
Scheme 2

1 moiety in 2 (eqn (2)), classic disproportionation (eqn (3)) does not occur, but rather $\bullet\text{NO}$ reductive coupling takes place (eqn (4)). This reaction is rare in copper nitrogen-oxide (bio)chemistry.¹³

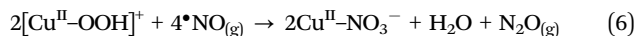


We further proved that this chemistry occurs by demonstrating that a Cu(I) derivative of the BA ligand, $[(\text{BA})\text{Cu}^{\text{I}}]^+$, reacts with $\bullet\text{NO}_{(\text{g})}$ in the presence of 1 equiv. HClO_4 to give the Cu(II) complex **1** and $\text{N}_2\text{O}_{(\text{g})}$. However, in the absence of the proton source only a nitrite complex $[(\text{BA})\text{Cu}(\text{NO}_2)]^+$ is generated *i.e.*, the classic disproportionation reaction (eqn (3)) (see ESI†).

Notably, bacterial Nitric Oxide Reductases (NOR's) effect this same transformation just described (eqn (4)), at a heme-non-heme diiron active site. Further flavo-diiron enzymes (with two proximate non-heme iron active site ions) and fungal P-450nor enzymes possess this same (detoxification) function (eqn (5)).¹⁴



Thus, the newly discovered chemistry involving copper complex NOR activity in the presence of acid (eqn (4)) explains the finding mentioned above that Cu(II) and not Cu(I) is observed as the final product in the reactions described herein. Thus, the overall chemistry that occurs in the $[(\text{BA})\text{Cu}^{\text{II}}(\text{OOH})]^+$ (**2**) + $\bullet\text{NO}_{(\text{g})}$ reaction is described by eqn (6); two processes take place, the generation of peroxyxynitrite *via* $\text{Cu}^{\text{II}}(\text{OOH}) + \text{NO}_{(\text{g})}$, and the Cu(I) therein produced effects NOR chemistry with the excess nitric oxide.



To summarize, the importance of peroxyxynitrite chemistry in biological systems, in terms of PN as destructive oxidant or nitrating agent, calls for further investigation of relevant metal ion chemistry. What reactivity promotes PN formation and/or reactivity and/or its own destruction, the latter in terms of health related metal-based PN decomposition catalysis?¹⁵ From biological or chemical studies, PN or PN associated chemistry can occur starting in the presence of hemes, copper, cobalt, manganese and chromium, and may result from $\text{M-O}_2 + \bullet\text{NO}_{(\text{g})}$, $\text{M-NO}_2^- + \text{H}_2\text{O}_2$, $\text{M-NO} + \text{O}_2$, or $\text{M-NO} + \text{H}_2\text{O}_2$ chemistry. Here, we have described a new means to promote PN chemistry, the reaction of a discrete Cu^{II} -hydroperoxo complex with $\bullet\text{NO}_{(\text{g})}$. Evidence strongly supporting the intermediacy of a Cu-PN complex includes the finding the (i) nitrate ion forms in high yield and (ii) if a phenol as substrate is added, efficient nitration occurs. In the course of the investigation, we also discovered a new reaction in Cu/nitrogen-oxide chemistry, $\bullet\text{NO}_{(\text{g})}$ reductive coupling, not unlike what occurs for NOR's. The nature of intermediates formed and mechanism of the reaction remain to be elucidated.

We believe that the specific reaction type studied here, $[\text{M-OOH}]^+ + \bullet\text{NO}_{(\text{g})}$, is relevant to biological chemistry. How is it that phenol tyrosine nitration occurs at a position close to the active site inside Mn-superoxide dismutase (MnSOD)?^{6b,c} *In vitro* exposure of the protein to an excess of PN effects the indicated reaction.^{6c} Is it not more likely that in the natural environment, a Mn-OOH intermediate, that known to form in the enzyme reaction, reacts with endogenous $\bullet\text{NO}_{(\text{g})}$, and as described here, leads to tyrosine nitration? CuZnSOD is also known to promote Tyr nitration^{7c,16} and based on the chemistry presented herein, we suggest that a protein

Cu^{II} -hydroperoxo intermediate may react with $\bullet\text{NO}_{(\text{g})}$ leading to PN mediated chemistry. (Note, for either MnSOD or CuZnSOD, a M-superoxo enzyme intermediate could react with endogenous $\bullet\text{NO}_{(\text{g})}$ to give PN.) Further consideration of metal ion mediated PN generation and reactivity, like that outlined in Scheme 1, is required.

We are grateful to the USA National Institutes of Health (GM-28962) for support of this research.

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