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FORUM: Bioinspired Heme, Heme/non-heme Diiron, Heme/copper and Inorganic NO_x Chemistry: ·NO_(g) Oxidation, Peroxynitrite-Metal Chemistry and ·NO_(g) Reductive Coupling

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Abstract

The focus of this Forum review highlights work from our own laboratories and those of others in the area of biochemical and biologically inspired inorganic chemistry dealing with nitric oxide (nitrogen monoxide, ·NO_(g)) and its biological roles and reactions. The latter focus is on (i) oxidation of ·NO_(g) to nitrate by nitric oxide dioxygenases (NOD's), and (ii) reductive coupling of two molecules of ·NO_(g) to give N₂O_(g). In the former case, NOD's are described and the highlighting of possible peroxynitrite-heme intermediates and consequences of this are given by discussion of recent works with myoglobin and a synthetic heme model system for NOD action. Summaries of recent copper complex chemistries with ·NO_(g) and O_{2(g)} leading to peroxynitrite species are given. The coverage of biological reductive coupling of ·NO_(g) deals with bacterial nitric oxide reductases (NOR's) with heme/non-heme diiron active sites, and on heme/Cu oxidases such as cytochrome *c* oxidase which can mediate the same chemistry. Recent designed protein and synthetic model compound (heme/non-heme diiron or heme/copper) as functional mimics are discussed in some detail. We also highlight examples from the chemical literature, not necessarily involving biologically relevant metal ions, which describe the oxidation of ·NO_(g) to nitrate (or nitrite) and possible peroxynitrite intermediates, or reductive coupling of ·NO_(g) to give nitrous oxide.

Introduction

In the Forum review, we wish to overview certain aspects of the biological inorganic chemistry and associated synthetic 'biomimetic' chemistries relevant to transformations carried out on nitrogen monoxide (nitric oxide; ·NO, indicating it is a radical odd-electron species). These are, in order of coverage in this article, (i) the oxidation of ·NO_(g) to nitrate anion (NO₃[−]), eq. 1, (ii) the oxidation of ·NO_(g) to the peroxynitrite anion (O=NOO[−]; oxoperoxonitrate(1−)), eq. 2, or (iii) the reductive coupling of two molecules of ·NO_(g) to give nitrous oxide ('laughing gas'; dinitrogen oxide; N₂O), eq. 3. Thus, ·NO_(g), the reductive derivative N₂O or the gaseous aerobic surroundings oxidation products such as nitrogen dioxide (·NO₂) and nitrous anhydride (dinitrogen trioxide, N₂O₃) occur naturally, but are also important pollutants which have deleterious environmental and atmospheric chemistries. In nature, the reactions described by eqs. 1 and 3 occur enzymatically and are part of the biochemistry of nitric oxide which involve heme proteins. As we will point out, we speculate that the process described by eq. 2 might as well take place as a metal ion (iron, copper or manganese) center mediated reaction.

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Bioinorganic chemists from a variety of sub-disciplines have taken recent interest in such chemical reactivity, given the biological importance as well as because these processes involve very interesting and important metal ion mediated electron-transfer and/or atom-transfer conversions. We will describe here a number of recently described chemical systems which are structural and/or functional models for these transformations, while placing our discussions into the context of the biochemistry which is known. However, first we need to provide some background on $\bullet\text{NO}_{(\text{g})}$.

Nitric oxide can be produced *in vivo* from a variety of sources (Fig. 1) including: (i) nitric oxide synthase (NOS). As members of a class of heme-containing monooxygenases, NOS's specifically catalyze the oxidation of L-arginine to L-citrulline producing $\bullet\text{NO}_{(\text{g})}$.¹⁻³ (ii) nitrite reductase (NiR). In bacteria, nitrite reduction to form nitric oxide is the second step during anaerobic bacterial denitrification, which is catalyzed by two genetically unrelated classes of nitrite reductases: heme NiRs (cytochrome *cd*₁ nitrite reductase, *cd*₁-NiR), or copper nitrite reductase (Cu-NiR),⁴⁻⁵ (iii) reductions of nitrite (NO_2^-) with various metalloproteins,⁶ all of whose main functions are not nitrite reduction, such as hemoglobin/myoglobin (Hb/Mb) (oxygen transport/storage),⁷⁻⁹ xanthine oxidoreductase (XOR),¹⁰⁻¹¹ cytochrome c oxidase (CcO) (O_2 reduction; proton translocation),¹²⁻¹³ and possibly soluble guanylate cyclase (sGC).¹⁴ (iv) reductions of organic nitrates (R-O- NO_2), e.g. nitroglycerin has been widely employed for cardiovascular therapy accredited to its ability to generate $\bullet\text{NO}_{(\text{g})}$, and although the mechanism of nitrate reduction to $\bullet\text{NO}_{(\text{g})}$ is ill-defined, it is thought to be mediated by cytochrome P450 (P450) and cytochrome P450 reductase (CPR) in mammals,¹⁵ (v) nonenzymatic nitrite reduction as well as acidic disproportionation.

The production of $\bullet\text{NO}_{(\text{g})}$ is critical to living systems in a number of biochemical processes that include blood pressure regulation, neurotransmission, and immune response to protect against pathogens.¹⁶⁻¹⁸ Perhaps the most well known function of nitric oxide is the signaling agent role it plays in the critical process of smooth muscle relaxation involved in vasodilation (blood pressure regulation). $\bullet\text{NO}_{(\text{g})}$ is biosynthesized from NOS and is able to act on the enzyme sGC converting guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) which is a secondary messenger in smooth muscle relaxation, and similarly in neurotransmission. Another important role of $\bullet\text{NO}_{(\text{g})}$, or other oxides of it, is assisting in the destruction of invading microorganisms; although the production of $\bullet\text{NO}_{(\text{g})}$ and its oxidation products is used to protect against these pathogens/diseases, local environments of high concentrations can lead to deleterious or inhibitory effects even on the host cells themselves.¹⁹⁻²⁰ Nitric oxide also plays a role regulating (P450) enzymatic activities during inflammation by inhibiting its catalytic activity.²¹⁻²² Inhibition of other enzymes such as terminal oxidases, and catalase has also been suggested. At high enough concentrations *in vivo*, or through the use of $\bullet\text{NO}_{(\text{g})}$ releasing agents it is suggested that nitric oxide can reversibly inhibit the catalytic functions of these enzymes by binding to the heme, or it can irreversibly inhibit the enzyme if nitrogen oxides derived from $\bullet\text{NO}_{(\text{g})}$

oxidation, e.g., peroxynitrite anion ($^-\text{OON}=\text{O}$; PN) or the nitrogen dioxide radical ($\cdot\text{NO}_2$) react with and alter the overall structure of the protein, e.g., via protein tyrosine nitration.^{17,23–24} A recent symposium was held at the Nobel Forum (2009) to discuss advances in the understanding of the roles nitrite and nitrate may play in therapeutics, nutrition and more generally biology.⁶ Since the reduction of nitrate to nitrite and nitrite to $\cdot\text{NO}_{(\text{g})}$ are now known to be catalyzed by numerous enzymatic and non-enzymatic pathways, as discussed above, these species are now quickly becoming increasingly interesting to nutritional researchers as well as possessing possible therapeutic applications regarding various cardiovascular diseases including ischemia-reperfusion and hypertension.²⁵

Nature has developed ways to maintain $\cdot\text{NO}_{(\text{g})}$ homeostasis *in vivo* by both oxidative and reductive pathways of removing $\cdot\text{NO}_{(\text{g})}$. Certain bacterial and mammalian enzymes possess nitric oxide dioxygenase (NOD) activity, the ability to catalyze the reaction of $\cdot\text{NO}_{(\text{g})}$ and O_2 to yield the biologically benign nitrate (NO_3^-). The copper enzyme ceruloplasmin, normally oxidizing iron(II) ion, has recently been shown to oxidize $\cdot\text{NO}_{(\text{g})}$ to nitrite, perhaps to remove excess nitrogen monoxide and “store” it in the nitrite pool.^{26–27} Other enzymes possess nitric oxide reductase (NOR) activity, the ability to reductively couple two molecules of $\cdot\text{NO}_{(\text{g})}$ giving N_2O and water. Both NOD and NOR pathways will be discussed in more detail below, highlighting some recent work in the field done in our lab as well as others.

Nitric Oxide Dioxygenase (NOD)

Given the variety of sources of $\cdot\text{NO}_{(\text{g})}$ production *in vivo*, it is necessary that living systems possess a defense against excess concentrations of $\cdot\text{NO}_{(\text{g})}$ (Fig. 2), possibly analogous to an anti-oxidant defense system protecting against hydrogen peroxide and other reactive oxygen species (ROS). Indeed hemoglobin and myoglobin appear to possess this type of reactivity, and it has been suggested that $\cdot\text{NO}_{(\text{g})}$ detoxification may have been a more ancient function of the two heme proteins.²⁸ Hb and Mb are major sinks of $\cdot\text{NO}_{(\text{g})}$ in mammals and it has been shown that truncated hemoglobins (trHbs) and microbial flavohemoglobins (flavoHbs) exhibit nitric oxide dioxygenase (NOD) activity.^{28–29}

Gardner and co-workers used electrospray mass spectrometry to analyze the stoichiometric product nitrate in the reaction of doubly ^{18}O -labeled red blood cell oxyHb [$\text{Hb}(\text{Fe}^{\text{III}}\text{-}^{18}\text{O}_2\text{-}^-)$], sperm whale oxyMb [$\text{Mb}(\text{Fe}^{\text{III}}\text{-}^{18}\text{O}_2\text{-}^-)$], and *E. coli* oxyflavoHb [$\text{flavoHb}(\text{Fe}^{\text{III}}\text{-}^{18}\text{O}_2\text{-}^-)$] with $\cdot\text{NO}_{(\text{g})}$, seeing greater than 99% incorporation of the labeled dioxygen.³⁰ These data support a mechanism that involves rapid reaction of the oxyheme [$\text{Fe}^{\text{III}}\text{-O}_2\text{-}^-$] with $\cdot\text{NO}_{(\text{g})}$ to form a transient peroxynitrite (PN) species [$\text{Fe}^{\text{III}}\text{-OON}=\text{O}^-$] which then rapidly forms the NO_3^- anion. A more detailed understanding of this final step is being investigated by a number of groups, and the most commonly accepted mechanism (Scheme 1) is thought to involve (after peroxynitrite formation) isomerization via homolytic O-O bond cleavage producing an oxo-ferryl ($\text{Fe}^{\text{IV}}=\text{O}$) species and $\cdot\text{NO}_2$ which within the cage attacks the ferryl O-atom to produce NO_3^- .^{24,31–37} However, recent reactive molecular dynamics simulations on a truncated hemoglobin structure suggest O-O bond cleavage is too slow to likely be involved in the dioxygenation mechanism.³⁸ An intermediate species detected by UV-vis and EPR spectroscopy has been assigned to a peroxynitrite complex, but a recent resonance Raman study clearly supports its reassignment to a heme-nitrate (product) complex.³⁴

A NOD Model Compound with Implication for PN Formation

Recent work from our group using a synthetic oxy-heme which exhibits NOD reactivity has been explored with the hope of adding to the understanding of the above mechanism.³⁹ A reduced heme (F_8) Fe^{II} [F_8 = tetrakis(2,6-difluoro-phenyl)porphyrinate(2-)] reacts

reversibly with dioxygen to give a diamagnetic iron(III)-superoxo species $(S)(F_8)Fe^{III}-(O_2^{\cdot-})$, which is stable in solution below $-40\text{ }^{\circ}\text{C}$ in coordinating but non-aqueous solvents (S).^{40–41} Addition of 1 equiv of $\cdot NO_{(g)}$ (at $-80\text{ }^{\circ}\text{C}$ in tetrahydrofuran) produces the five-coordinate nitrato heme complex $(F_8)Fe^{III}-(NO_3^-)$ in near quantitative yield as is seen in the NOD reactions. To further investigate the possible proposed intermediates in this reaction we sought chemical evidence which might suggest the formation of a peroxynitrite species. In fact, we observed effective nitration chemistry when 2,4-di-*tert*-butylphenol (DTBP) (1 equiv) was added *prior* to addition of 1 equiv of $\cdot NO_{(g)}$ to the superoxo species (Scheme 2). Workup of the reaction solution revealed that the ferric hydroxo product $(F_8)Fe^{III}-OH$ formed ($\sim 85\%$ yield) along with high yields ($> 82\%$) of 2,4-di-*tert*-butyl-6-nitrophenol (NO_2 DTBP). These findings, along with appropriate control experiments indicated that an as yet unobserved heme- NO_x intermediate must have formed and it was able to effect a phenol nitration reaction faster than its own isomerization to the nitrate complex.³⁹

Peroxynitrite Myoglobin Chemistry

Recent work from Groves' lab indicates that a peroxynitrite-heme, generated by reaction of peroxynitrite with oxidized (met) horse heart myoglobin produces nitrate along with other reactive nitrogen species.⁴² Stopped-flow spectrophotometry evidence was presented to show that a ferryl Mb (i.e., a high-valent compound II like $Fe^{IV}=O$ species) and $\cdot NO_2$ are formed in this reaction. In these experiments, a large excess of peroxynitrite relative to oxidized myoglobin allowed for multiple reaction cycles between met Mb and peroxynitrite such that the ferryl Mb species could accumulate to high concentrations and produce unambiguous results. Fluorescein (Fl) was used to trap $\cdot NO_2$ escaping from the protein active-site "cage"; Fl was nitrated by $\cdot NO_2$ to form NO_2Fl , the formation of which was followed by its spectral changes. The ratio of the rates of in-cage rebound to cage escape (k_r/k_e) was approximately ten (Fig. 3). Groves' results are thus also consistent with the primary mechanism mentioned above, that is by the typically described NOD process where oxy-Mb reacts with $\cdot NO_{(g)}$ to give a ferryl and $\cdot NO_2$, however the latter may escape and effect the nitration of a nearby tyrosine (phenol) moiety.

Peroxynitrite, discussed above with respect to NOD chemistry and/or heme-peroxynitrite interactions, is known to form from the near diffusion controlled reaction of superoxide anion ($O_2^{\cdot-}$) with $\cdot NO_{(g)}$ (Fig 4). This highly reactive nitrogen species can form other free radicals such as nitrogen dioxide, hydroxyl radical, and in the presence of CO_2 carbonate radical anion. All of these are strong oxidants, and in the case of $\cdot NO_2$ also a nitrating agent. A variety of biomolecules are oxidized/nitrated by peroxynitrite derived radicals including, tyrosine, thiols, unsaturated-fatty-acid containing lipids, and DNA. Such oxidations/nitrations have been associated with a number of disease states, however peroxynitrite and/or $\cdot NO_2$ derived nitration of protein tyrosine residues may interfere with critical cell signaling functions.^{43–45} Metal ions react with peroxynitrite and can form complexes,^{35,46} and as described above carry out chemistry with hemes. Peroxynitrite interactions and nitration chemistry has also been described with respect to manganese^{47–48} and copper/zinc superoxide dismutases.^{49–51} Other heme enzymes include cytochrome P450 monooxygenases.⁵² For example, in separate experiments the reactions of peroxynitrite with various ferric P450 enzymes led to the generation of an appreciable amount of compound I (i.e., $(P^+)Fe^{IV}=O$; P = porphyrinate), and compound II $((P)Fe^{IV}=O)$ derivatives along with the more stable $Fe^{III}-NO$ species, as determined by UV-vis, Mössbauer and X-ray absorption spectroscopies.^{52–55} These reactions also led to the nitration of approximately three tyrosine residues within the enzyme.⁵²

While $\cdot NO_{(g)}$ oxidations by oxy-heme ($Fe^{III}-(O_2^{\cdot-})$) are discussed with respect to heme NOD reactivity, there exists an inorganic chemistry wherein metal-nitrosyl complexes (derived from metal ion (M^n) complexes plus $\cdot NO_{(g)}$) react with dioxygen to generate either metal-

nitro/nitrito species ($M^{n+1}\text{-NO}_2$), metal nitrates ($M^{n+1}\text{-NO}_3$), or both.³⁷ While not detected previously, these reactions are thought to proceed through peroxynitrite ($M^{n+1}\text{-}(\text{OONO})$) intermediates. The first example of this comes from Clarkson and Basolo, where they described the kinetics of the dioxygen reaction with a Co-nitrosyl complex in the presence of coordinating bases to give the corresponding nitro complex (eq. 4); the reaction mechanism has been suggested to involve an N-coordinated peroxynitrite species as an intermediate, before transfer of one of its oxygen atoms to a second equivalent of Co-nitrosyl.⁵⁷ A similar dioxygen reaction was carried out with an Ir-nitrosyl complex, and formation of an N-coordinated peroxynitrite was also suggested as an intermediate before its isomerization to the Ir-nitrate complex final product (eq. 5).⁵⁸ Additionally and more recently, when a reduced nitroprusside was reacted with dioxygen, the authors suggest its reaction proceeds through an N-coordinated peroxynitrite complex, as suggested from their DFT calculations, before decomposition (eq. 6).^{46,59}

Given the known inorganic chemistry of metal nitrosyl complexes and dioxygen (vide supra) and the abundance of metallo-proteins in biological systems, it would seem possible that biological peroxynitrite generation could occur via other (e.g., Cu, Mn) metal ion mediated pathways, rather than only from $\text{O}_2^{\cdot-} + \cdot\text{NO}_{(\text{g})}$ (Fig. 4) or heme reactivity. For example, does a $\text{M}(\text{O}_2)$ complex ($\text{M} = \text{Mn}$ or Cu) react with $\cdot\text{NO}_{(\text{g})}$ to give peroxynitrite like chemistry; conversely does a $\text{M}(\text{NO})$ complex react with dioxygen giving similar results? This led us to initiate investigations of (ligand) $\text{Cu}^{\text{I}}/\cdot\text{NO}/\text{O}_2$ chemistry.

Copper-Peroxynitrite Generation, Characterization and Transformation

Copper-Peroxynitrite Complexes and Chemistry

Discrete metal-peroxynitrite complexes are rare,³⁵ but as discussed above, they are suggested to form as transients from metal- $\text{NO} + \text{O}_{2(\text{g})}$ or metal- $\text{O}_2 + \cdot\text{NO}_{(\text{g})}$ reactions.^{35,37,46} However, we have recently been able to generate discrete copper(II)-peroxynitrite complexes via two approaches,^{60–61} the reaction of $\cdot\text{NO}_{(\text{g})}$ with the copper superoxide species $[(\text{TMG}_3\text{tren})\text{Cu}^{\text{II}}(\text{O}_2^{\cdot-})]^+$ (eq. 7),^{62–63} or the complementary reaction of O_2 with a copper-nitrosyl species $[\text{Cu}^{\text{I}}(\text{AN})(\text{NO})]^+$ (eq. 8).

Starting with the copper/dioxygen species $[(\text{TMG}_3\text{tren})\text{Cu}^{\text{II}}(\text{O}_2^{\cdot-})]^+$, bubbling $\cdot\text{NO}_{(\text{g})}$ at -80°C results in the formation of a yellowish green complex, formulated as the peroxynitrite

species $[(\text{TMG}_3\text{tren})\text{Cu}^{\text{II}}(\text{OON}=\text{O})]^+$, the first example of such a species. The formulation was in part confirmed by the ESI-MS evidence.⁶⁰ This species' EPR spectrum is distinctly tetragonal, which is consistent with the square pyramidal (SP) geometry predicted by DFT calculations (B3LYP), which also led to the lowest energy structure possessing a cyclic bidentate $\kappa^2\text{-O,O'-OONO}$ moiety (Fig. 5).

Upon warming to RT or just prolonged storage at $-80\text{ }^\circ\text{C}$, $[(\text{TMG}_3\text{tren})\text{Cu}^{\text{II}}(\text{OON}=\text{O})]^+$ transforms to the nitrite complex $[(\text{TMG}_3\text{tren})\text{Cu}^{\text{II}}(\text{ONO})]^+$ in high yield, along with dioxygen in 30–35% yield (50% theoretical; see Eq. 7). The X-ray crystal structure of $[(\text{TMG}_3\text{tren})\text{Cu}^{\text{II}}(\text{ONO})]^+$ shows an $\eta^1\text{-O}$ -nitrito bound to Cu(II) ion in an overall trigonal bipyramidal (TBP) environment, which is in accordance with the reverse axial EPR spectrum. The dramatic spectroscopic differences between the $\text{OON}=\text{O}$ (peroxynitrito) complex and ONO^- (nitrito) complex highlights the distinctly different nature of these species (Fig. 5).

The complementary reaction to that just described involves the complex $[\text{Cu}^{\text{I}}(\text{AN})(\text{NO})]^+$, formed at $-80\text{ }^\circ\text{C}$ by reaction of the precursor copper(I) compound with $\cdot\text{NO}_{(\text{g})}$ (Scheme 3).⁶¹ As is now well appreciated,^{64–68} this is best described as a Cu(I)-($\cdot\text{NO}$ radical) species. Bubbling it with $\text{O}_{2(\text{g})}$ at $-80\text{ }^\circ\text{C}$ results in a light to deep blue colored species formulated as the mononuclear peroxynitrite complex $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}=\text{NOO}^-)]^+$ which possesses a typical tetragonal copper(II) EPR spectrum. DFT calculations suggest that the lowest-energy structure for $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}=\text{NOO}^-)]^+$ favors a cyclic bidentate $\kappa^2\text{-O,O'-O}=\text{NOO}$ moiety (Scheme 3). These computations also indicate that the overall chemistry proceeds via $\cdot\text{NO}_{(\text{g})}$ dissociation from $[\text{Cu}^{\text{I}}(\text{AN})(\text{NO})]^+$, dioxygen interaction to give a transient $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}_2^-)]^+$ species, followed by re-attack by $\cdot\text{NO}_{(\text{g})}$ to give $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}=\text{NOO}^-)]^+$.⁶¹ The same sort of chemistry is thought to occur in protein (heme)Fe(NO) + $\text{O}_{2(\text{g})}$ reaction to give nitrate.^{35,37}

While $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}=\text{NOO}^-)]^+$ is moderately stable in solution at $-80\text{ }^\circ\text{C}$, it also thermally transforms to the Cu-nitrite complex $[\text{Cu}^{\text{II}}(\text{AN})(\text{NO}_2^-)]^+$ as identified by EPR and UV-vis spectroscopies along with X-ray crystallography. Dioxygen is also released as per eq. 8. As an initial homolytic O-O cleavage is most often associated with peroxynitrite chemistry, a plausible mechanism of reaction, as adapted from that proposed by Babich and Gould⁶⁹ in a copper(II) plus peroxynitrite aqueous system is shown in Scheme 4.

Another possibility is a disproportionation reaction involving peroxynitrate, as the latter is a known “decomposition” product of PN, however producing singlet oxygen, eq. 9.^{70–72} Thus, we speculate that the peroxynitrito-copper(II) to nitrito-copper(II) conversion might proceed according to eq. 10.

Possessing peroxynitrite-like oxidative capability, $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}=\text{NOO}^-)]^+$ effects exogenous phenol substrate oxidative coupling chemistry, such as oxidation of 2,4-di-*t*-

butylphenol (DTBP) to form a bis-phenol with the yield of 80%. A small amount (~5%) of *ortho*-nitrated phenol is also observed (Scheme 3). The main copper product is the nitrito complex $[\text{Cu}^{\text{II}}(\text{AN})(\text{NO}_2^-)]^+$, as identified by comparison with authentic material. After addition of chloride (tBu_4NCl) to replace the $\text{O}=\text{NOO}^-$ group on $\text{Cu}(\text{II})$, it is suggested that a free peroxynitrite group or species derived from $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}=\text{NOO}^-)]^+$ is (are) present and this is supported by the observation that added DTBP is now primarily nitrated (Scheme 3). Thus, under the solvent and reaction conditions employed, peroxynitrite copper(II) complex $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}=\text{NOO}^-)]^+$ and free peroxynitrite react differently, but both in a manner consistent with the peroxynitrite formulation for $[\text{Cu}^{\text{II}}(\text{AN})(\text{O}=\text{NOO}^-)]^+$.

With these works, we have accomplished the generation of peroxynitrite via (ligand) $\text{Cu}^{\text{I}}/\cdot\text{NO}/\text{O}_2$ reactivity studies. Clearly, mechanistic insight is lacking for the thermal transformation chemistry of the Cu^{II} -peroxynitrito compounds. It is notable that both systems lead to nitrite plus dioxygen products, and not the isomerization which would give nitrate. We suspect that a change in the ligand environment around the copper ion might alter the peroxynitrite reactivity.⁷³ Further studies will focus on reaction mechanisms, (ligand) $\text{Cu}^{\text{I}}/\cdot\text{NO}/\text{O}_2$ with differing ligands, and examination of how copper-binding affects the inherent reactivity of peroxynitrite anion or peroxynitrous acid. Given the presence of copper ion and copper protein active sites present in biological fluids, we speculate that $\text{Cu}(\text{O}_2)$ or $\text{Cu}(\text{NO})$ species that form transiently may generate peroxynitrite type chemistry.

Bacterial NO Reductases (NORs), Models and Inorganic Chemistry

It has been long known that microorganisms transform nitrogen containing oxyanions to gaseous products.^{74–75} Anaerobic denitrifying bacteria use nitrogen oxides to dump metabolically derived reducing equivalents, starting with nitrate, finally releasing dinitrogen. All of the enzymes in this pathway are metalloenzymes, including molybdenum, iron or copper, (Scheme 5). NOR's effect the third reaction, mediating the reductive coupling of two mole equiv $\cdot\text{NO}_{(\text{g})}$ to $\text{N}_2\text{O}_{(\text{g})}$.^{4,76–78} Via such chemistry, NO reductases also serve to reduce toxic levels of $\cdot\text{NO}_{(\text{g})}$. We should note that in Fungi, NOR enzymes are mononuclear heme-iron of the P-450 type, that is with a cysteinyl sulfur axial ligand.^{4,79–80} Another type of NOR is shown to exist in strict or facultative anaerobic Bacteria, Archaea, and also in some eukaryotes such as microaerobic protozoan parasites.^{77,81} Here, the active sites are non-heme diiron (i.e., no heme); they are flavodiiron proteins (one is a so-called flavorubredoxin)^{77,81–85} which contribute resistance to the potential toxic effects of $\cdot\text{NO}_{(\text{g})}$.⁷⁵

Inorganic/Organometallic $\cdot\text{NO}_{(\text{g})}$ Reductive Coupling

For the purposes of this bioinorganic oriented article, our focus will be on chemical mimics of heme/non-heme diiron or heme/copper enzymes. However, we wish to note some examples from the inorganic-organometallic literature, studies carried out to make fundamental advances concerning $\cdot\text{NO}_{(\text{g})}$ interactions with metal ions, and for potential environmental chemistry applications.⁸⁶ In the 1970's, a stoichiometric iridium-bound NO reduction to form N_2O was reported, accompanied with the oxidation of CO to CO_2 , during which dinitrogen dioxide (N_2O_2) (or perhaps more accurately a $\text{N}_2\text{O}_2^{2-}$ hyponitrite dianion)^{87–89} was detected as an intermediate that transferred an O-atom to CO (eqs 11 & 12).^{86,90–93}

Eisenberg and coworkers showed that in an aqueous solution of HCl and ethanol, the anionic complex $[\text{RhCl}_2(\text{CO})_2]^-$ catalytically mediated the CO reduction of NO. There, it was shown using isotope labeling studies that water played the role of oxygen atom transfer agent.^{91,94–95} It has also been shown by others that chlorides salts of Pd, Cu or Pt, could catalyze NO reductive coupling to N_2O , either using CO, olefins as reducing agents.⁸⁶

In a significant work, Trogler and co-workers⁹⁶ carried out detailed kinetic mechanistic studies of a Pd/Cu-catalyzed reduction of $\bullet\text{NO}_{(\text{g})}$ in aqueous solution where, in a rate limiting step, a cuprous salt reduces the dinitrogen dioxide species $\text{PdCl}_3(\text{N}_2\text{O}_2)^{2-}$ to give PdCl_4^- , N_2O , and water. The proposed mechanism is outlined in Scheme 6 (adapted from ⁹⁶). The remarkable resemblance here to biological $\bullet\text{NO}_{(\text{g})}$ reductive coupling from either diiron bacterial NOR's or that occurring in heme/Cu containing cytochrome c oxidases (CcO's), see detailed discussions below, is that the second metal ion, here copper, does not bind $\bullet\text{NO}_{(\text{g})}$ very strongly and seems primarily to serve as a key reductant; the first metal ion, here palladium (but heme in biological systems) binds the first $\bullet\text{NO}_{(\text{g})}$ ligand.

A very exciting chemical system illustrating N–N coupling from metal-nitrosyl fragments and crystallographic characterization of the $\{\text{N}_2\text{O}_2\}$ intermediate was recently described for a pyrazolylborate bridged diruthenium complex.⁹⁷ A “curious” N–N bond distance observed for the coupled product was 1.861(3) Å, which is shorter than the cis-NO dimer (2.18 Å) but longer than the typical N–N single bond (1.42 Å). Remarkably, reversible oxidation gave the dinitrosyl complex $[\{\text{TpRu}(\text{NO})\}_2(\mu\text{-Cl})(\mu\text{-pz})]^{2+}$ (Scheme 7, adapted from ⁹⁷). Preliminary results showed acidification produced a μ -oxo compound (21% yield) with release of nitrous oxide (5% yield). As will be seen from the further discussion (vide infra), this chemistry bears a remarkable resemblance to what is known for the enzymatic diiron NOR chemistry.

An interesting example of another type is where a terminal osmium nitride complex, (Tp = hydrotris (pyrazolyl)borate) reacts with 2 equiv $\bullet\text{NO}_{(\text{g})}$ first transferring an “N-atom” to generate N_2O and finally (Tp)Os(NO)Cl₂ eq 13.⁹⁸ See a recent review for more inorganic examples of metal complex $\bullet\text{NO}_{(\text{g})}$ reduction, including cases where the N–O bond is cleaved.⁸⁶

Bacterial NOR's.^{4,76–78,99}

In fact, bacterial NOR's possess a binuclear active site with a high-spin heme-*b*₃ and adjacent non-heme iron Fe_B that catalyzes the reduction of nitric oxide to nitrous oxide (Scheme 8). The electron source derives from soluble cytochrome *c*. The Fe_B is coordinated by nitrogen atoms from three histidine ligands. This NorB subunit, which includes the heme/non-heme diiron active site, exhibits weak homology to subunit I of cytochrome *c* oxidase (CcO). CcO and other members of the heme-copper oxidase (HCO) superfamily serve in the mitochondrial electron-transport chain of aerobic organisms to couple the reduction of O₂ to water with membrane proton translocation; the latter is utilized for ATP synthesis, the cell's energy carrier.^{100–101} In fact, the four histidine residues that ligate heme *a*₃ and Cu_B in CcO (Scheme 8) are conserved in NOR's. In essence, the Cu_B site of CcO has been replaced by a non-heme iron (Fe_B) in NOR. Heme-copper oxidases and NOR's are structural and

functional homologs and it is found that some heme-copper oxidases and NOR's reduce both $\text{O}_{2(\text{g})}$ and $\cdot\text{NO}_{(\text{g})}$ (also see below) with different selectivity.

There are as yet no published bacterial NOR X-ray structures. The three conserved His residues clearly serve as Fe_{B} ligands, while an additional O-atom donor from a Glu residue carboxylate has been proposed or discussed. Recent investigations, including a computer model alignment of NorB catalytic subunit from *P. denitrificans* with evolutionarily related heme-copper oxidases, suggest that while a series of conserved glutamic acid residues act as a proton entry point and pathway proceeding to the active site, the nearest Glu is still too far away from Fe_{B} to be ligated.^{102–103}

NOR Reaction Mechanism

Scheme 9 depicts three possible mechanisms which have discussed likely steps in NOR action.^{77,104} In *trans*, binding of two NO's to separate irons leads to N-N coupling and a hyponitrite $\text{N}_2\text{O}_2^{2-}$ intermediate. Supporting this mechanism are the known strong affinity of $\cdot\text{NO}_{(\text{g})}$ for reduced hemes,¹⁰⁵ and observations that *both* Fe's can bind CO ($\nu_{\text{C-O}} = 1972$ & 2068 (Fe_{B}) cm^{-1}) or NO.^{106–107} For *cis-Fe_B*, two Fe_{B} bound NO's couple to give a hyponitrite which eliminates N_2O and H_2O following protonation/N-O cleavage; the heme serves to do electron-transfer and perhaps stabilize the $\text{N}_2\text{O}_2^{2-}$ ligand. This mechanism can be seen as reasonable in that it would not involve a generally highly stable 'dead-end' heme- $\text{Fe}^{\text{II}}\text{-NO}$ species.^{77,108} For *cis-heme-*b*₃*, an initial heme-*b*₃-NO reacts directly with NO to give a hyponitrite radical and then (or simultaneously) is reduced and coordinated by Fe_{B} . Siegbahn's calculations¹⁰⁴ favor this scenario; N-O bond cleavage ($\rightarrow \text{N}_2\text{O}$) is aided by $\text{Fe}^{\text{IV}}_{\text{B}}=\text{O}$ formation. The "timing" of N-O scission, further electron-transfer, protonation (by a nearby Glu?) and possible $\text{N}_2\text{O}_2^{2-}$ isomerization to an O-bound form are events which remain to be defined.⁷⁷ Other mechanisms can be envisioned, for example one that is initiated by a 'mixed-valent' heme^{III}.. $\text{Fe}^{\text{II}}_{\text{B}}$ species.^{77,108} Also, note that there is general agreement that the oxidized form of the enzyme, which might be involved in actual turnover,⁷⁷ is a high-spin heme coupled to Fe_{B} via a μ -oxo (O^{2-}) ligand; here, the heme's proximal His may be dissociated (Scheme 9).

CcO to μ -Oxo-Diferic NOR Models

Our own entry into heme/metal nitrogen oxide chemistry derived from our early efforts in heme-copper oxidase modeling and the design and synthesis of heterobinucleating ligands. For example, the synthesis of ⁵L and introduction of excess FeCl_2 led to our generation and structural characterization of $[(^5\text{L})\text{Fe}^{\text{III}}\text{-O-Fe}^{\text{III}}\text{-Cl}]^+$.¹⁰⁹ The non-heme iron in the tetradentate tris(2-pyridyl)methyl coordination environment can be (reversibly) removed by addition of base and 'rusting', giving $[(^5\text{L})\text{Fe}^{\text{III}}\text{-OH}]$ (Scheme 10). This high-spin complex could be 'reconstituted' with iron or copper, in the latter case to give an interesting heterobinuclear complex with $\text{Fe}^{\text{III}}\text{-O-Cu}^{\text{II}}$ core structure.¹¹⁰

In fact, we were able to generate two other μ -oxo heme/non-heme diiron(III) complexes to compare and contrast (Chart 1).

Both heme and non-heme irons are in their high-spin states, but magnetically strongly antiferromagnetically coupled as shown from Mössbauer, ¹H- or ²H-NMR spectroscopies, and SQUID susceptometry for $[(^6\text{L})\text{Fe-O-Fe-Cl}]^+$ ($J = -115 \text{ cm}^{-1}$, $S = 0$),^{111–112} and the structural parameters are consistent with these findings.¹¹³ This includes that the fact that the heme-iron atoms are well above ($\sim 0.5 \text{ \AA}$) the heme plane.¹¹¹ These complexes are very stable and do not react with pyridine or imidazole bases, in line with the conclusion from enzyme studies that the heme's proximal histidine in the oxidized NOR active site is dissociated.

Thus, these synthetic diiron complexes have been good first generation models for the presumed μ -oxo diiron(III) enzymes (Chart 1). Such μ -oxo complexes may be important in enzyme mechanism and turnover. We speculate that once the diiron active site becomes fully reduced and able to react with $\cdot\text{NO}_{(\text{g})}$ substrate, the timing of active site protonation is such that the enzyme can first employ a driving force associated with a reaction to form the μ -oxo complex, i.e., $2 \text{Fe}^{\text{II}} + 2 \cdot\text{NO}_{(\text{g})} \longrightarrow (\text{heme})\text{Fe}^{\text{III}}\text{-O-Fe}^{\text{III}} + \text{N}_2\text{O}_{(\text{g})}$. Subsequent protonation and reduction would give fully reduced enzyme plus water as byproduct.

Reduced Diiron(II) Complexes and Reactivity

The heme/non-heme diiron(II) complex $[(^6\text{L})\text{Fe}^{\text{II}}\dots\text{Fe}^{\text{II}}\text{-Cl}]^+$ was generated by reduction of $[(^6\text{L})\text{Fe}^{\text{III}}\text{-O-Fe}^{\text{III}}(\text{Cl})]^+$.¹¹⁴ Interestingly, its reaction with the enzyme substrate surrogate dioxygen at -80°C gives a product formulated as $[(^6\text{L})\text{Fe}^{\text{III}}(\text{O}_2)(\text{THF})\dots\text{Fe}^{\text{II}}\text{-Cl}]^+$ (THF = tetrahydrofuran) $\{\lambda_{\text{max}} 418$ (Soret), 536 nm ; $\nu_{\text{O-O}} = 1176 \text{ cm}^{-1}$, $\nu_{\text{Fe-O}} = 574 \text{ cm}^{-1}\}$, i.e., where the non-heme iron does not react with O_2 nor does it participate in bridge bonding to the O_2 -derived fragment. The heme dioxygen adduct, formally an iron(III)-superoxide species, models the spectroscopic and physical properties of oxy-hemoglobins or oxy-myoglobins. In the reaction with carbon monoxide (Scheme 11) a heme-carbonyl complex is formed, $[(^6\text{L})\text{Fe}^{\text{II}}(\text{CO})(\text{THF})\dots\text{Fe}^{\text{II}}\text{-Cl}]^+$. This observation suggests that future generations of model compounds need an altered coordination environment for the non-heme iron atom, perhaps without a chloride ligand and with one less nitrogen donor, to enable this “ Fe_B ” center to bind CO as the enzyme does.^{77,115}

However, reaction of carefully purified nitrogen monoxide with $[(^6\text{L})\text{Fe}^{\text{II}}\dots\text{Fe}^{\text{II}}\text{-Cl}]^+$ leads to the dinitrosyl complex $[(^6\text{L})\text{Fe}(\text{NO})\dots\text{Fe}(\text{NO})\text{-Cl}]^+$ (Scheme 11). This complex is quite stable, and thus far we have not been able to induce it to couple the nitrosyl fragments to produce N_2O and the hoped for μ -oxo complex $[(^6\text{L})\text{Fe}^{\text{III}}\text{-O-Fe}^{\text{III}}(\text{Cl})]^+$. The addition of protons also does not effect the formation of nitrous oxide and with an excess of a base such as 1,5-dicyclohexyl-imidazole, nitrogen monoxide is liberated: $[(^6\text{L})\text{Fe}(\text{NO})\dots\text{Fe}(\text{NO})\text{-Cl}]^+ + \text{excess “base”} \longrightarrow \text{Fe}^{\text{II}}\dots\text{Fe}^{\text{II}} + 2 \cdot\text{NO}_{(\text{g})}$.¹¹⁴

A Functional Model for the Stoichiometric Reaction

On the other hand, Collman’s group has succeeded in this regard. In recent exciting reports,^{116–117} a fully reduced heme- $\text{Fe}_\text{H}^{\text{II}}\dots\text{Fe}_\text{B}^{\text{II}}$ synthetic complex reacts with two equiv. of $\cdot\text{NO}_{(\text{g})}$ to yield one equivalent of $\text{N}_2\text{O}_{(\text{g})}$ and a bis-ferric complex. The similar reduction of $\cdot\text{NO}_{(\text{g})}$ was not observed upon the reaction of a mixed-valent complex heme- $\text{Fe}_\text{H}^{\text{III}}\dots\text{Fe}_\text{B}^{\text{II}}$. The product $\text{N}_2\text{O}_{(\text{g})}$ was quantified by use of the enzyme nitrous oxide reductase, and the bis-ferric species was identified by FT-IR and EPR spectroscopies.¹¹⁶ A follow-up investigation for possible intermediates during this $\cdot\text{NO}_{(\text{g})}$ reductive coupling reaction suggested the existence of two nitrosyl intermediates, in the form of heme- $\text{Fe}_\text{H}^{\text{II}}\dots\text{Fe}_\text{B}^{\text{II}}\text{-NO}$ at -80°C and heme- $\text{Fe}_\text{H}^{\text{II}}\text{-NO}\dots\text{Fe}_\text{B}^{\text{II}}\text{-NO}$ at -40°C , as monitored by EPR, resonance Raman and IR spectroscopies (Scheme 12). These authors suggest that their results support the *trans* mechanism (Scheme 9).¹¹⁷

Carboxylate Containing Ligands for Fe_B in NOR Models

Collman and coworkers,^{101,118} who have in recent years moved to emphasize the use of “faithful” or “accurate” mimics, i.e., use of imidazole ligand donors both proximal and distal (i.e., for a non-heme iron or copper ion), have also now generated binucleating ligands with a distal RCO_2^- group, that is speculated to be an Fe_B ligand (but see above). One should be reminded that it is by definition not possible to deduce enzyme chemistry from model system investigations. This can only be accomplished from studies on the enzyme itself, in part because no model can truly mimic an enzyme’s local environment, i.e., with respect to exact geometry and juxtaposition of groups, local polarity or dielectric (and resulting redox

potential) and due to second coordination sphere modulating effects. Models are not meant to duplicate data but to “sharpen the questions” being asked,¹¹⁹ or if possible to build/create something that works so the system can be truly understood,¹¹⁹ and to carry out systematic investigations where input synthetic modifications are tested for structure, spectroscopic properties and/or function, so as to enhance our fundamental understanding.^{120–122} It will be most interesting to learn of diiron/ $\text{NO}_{(\text{g})}$ reactivity studies which may be carried out on the synthetically sophisticated ligands depicted in Chart 2.

An Engineered Protein Bacterial NOR Model

Using protein design/engineering,¹²³ Yi Lu and co-workers¹²⁴ used sperm whale myoglobin as a scaffold to engineer an Fe_{B} binding site into the distal pocket of the heme showing the resulting heme/non-heme diiron protein to be a structural and *functional* mimic of the nitric oxide reductase (NOR) active site. The non-heme “ Fe_{B} ” atom was inserted and from X-ray crystallography was found to be bound by three histidine’s and a glutamate oxygen atom as may occur in NOR’s. Exposure of excess $\cdot\text{NO}_{(\text{g})}$ to the deoxy ($\text{Fe}^{\text{II}}\text{..Fe}^{\text{II}}$) $\text{Fe}_{\text{B}}\text{Mb}$ protein, formed by addition of two reducing equivalents, yielded $\sim 30\%$ $\text{N}_2\text{O}_{(\text{g})}$, and the process could be repeated step-wise. Most recently,¹²⁵ this group’s protein engineering has been further developed to include *two* glutamates, one new one (I107E; Figure 6) near the active site. A number of NOR protein glutamates are conserved and known to be critical for activity,^{102,126} likely facilitating a hydrogen bonding network and providing proton delivery for NO reduction. For Lu’s engineered protein, introduction of the second glutamate (non-bonding to Fe_{B} ; Figure 6) increased the yield of N_2O by $\sim 100\%$, suggesting the importance of the second coordination sphere as well as the dual roles of the two glutamates. Their finding¹²⁵ that redox active Fe^{2+} or Cu^+ (vs. Zn^{2+}) in the Fe_{B} site is necessary for NO reduction also suggests the importance of electron donation and the mediation of the heme-NO intermediate.

Thus, there now exists heme/non-heme diiron synthetic and engineered protein NOR functional models, which signify considerable accomplishments and advances, providing insights and promise for more in the near future. Notwithstanding all these research efforts from the perspective of microbiology, biophysics, biochemistry and model compound synthetic coordination chemistry, there is still much to learn about bacterial NOR chemistry and their chemical mechanisms. In particular, aspects concerning the coordination chemistry of $\cdot\text{NO}$, CO and even O_2 at this binuclear but unsymmetrical diiron center, the nature of intermediates and their redox states, protonation events and their timing, all of which are related to the important N–N bond forming and N–O bond breaking steps. Fundamental characteristics of such nitrogen oxide chemistries apply to many other subareas, e.g., peroxyxynitrite formation and reactivity,^{127–128} biological nitrite reduction to $\cdot\text{NO}_{(\text{g})}$ (a research area which has undergone explosive growth),^{6,128} N_2O reduction to dinitrogen¹²⁹ and even to fundamental aspects of O_2 -reduction to water or O_2 -utilization for oxidation-oxygenation reactivity.

Nitrogen Monoxide Interactions with Heme/Cu Containing CcO, Including Reductive Coupling

As mentioned, NOR’s and CcO’s are evolutionarily related, both considered as part of a heme/M genetic superfamily. Specifically, certain types of CcO’s such as *ba*₃ and *caa*₃ oxidases from *Thermus thermophilus*, *cbb*₃ oxidase from *Pseudomonas stutzeri*, and *bo*₃ from *Escherichia coli* also carry out nitrogen monoxide reductive coupling, that is, NOR chemistry (Scheme 13), although at reaction rates much lower than for the diiron NOR’s.

However, the study of CcO mediated $\cdot\text{NO}_{(\text{g})}$ reductive coupling has recently received considerable attention from the biophysical-biochemical and computational research

communities.^{34,115,130–132} This interest certainly in part derives from the fact that X-ray structures are available for a good number of HCO's, and the study of these systems can/should provide valuable fundamental insights.

In mammalian systems, the study of cytochrome c oxidase interaction with $\cdot\text{NO}_{(\text{g})}$ interactions has a long history.^{133–136} Nitrogen oxide is thought to be a (reversible) cellular CcO respiration inhibitor and CcO/ $\cdot\text{NO}_{(\text{g})}$ interactions have physiological and pathological consequences, including influencing NO-signaling and cell death.^{135–136} $\cdot\text{NO}_{(\text{g})}$ can react at the heme and/or Cu_{B} ^{131,135} and NO conversions to Fe-nitrosyl and/or nitrite (via Cu_{B} redox chemistry) are known.^{135–137} Recent literature suggests that $\cdot\text{NO}_{(\text{g})}$ reversible inhibition of CcO's and $\cdot\text{NO}_{(\text{g})}$ oxidation to nitrite at CcO active sites are natural functions and occur as a consequence of the available $\text{O}_{2(\text{g})}$ concentrations (i.e., organ or tissue hypoxia vs hyperoxia), all to lead to proper $\cdot\text{NO}_{(\text{g})}$ homeostasis via production of an appropriate nitrite (as source of $\cdot\text{NO}_{(\text{g})}$) pool.^{12–13} Recent papers even suggest that $\cdot\text{NO}_{(\text{g})}$ oxidation to nitrite and not CcO inhibition is a primary CcO/ $\cdot\text{NO}_{(\text{g})}$ interaction.^{138–139} For NO_2^- formation, peroxynitrite ($\text{O}=\text{NOO}^-$) may form as an intermediate,¹⁴⁰ but this has been partially disputed.¹⁴¹ Nitrite dependent $\cdot\text{NO}_{(\text{g})}$ production appears to occur under hypoxic conditions, and this $\cdot\text{NO}_{(\text{g})}$ may be involved in cell signaling via tyrosine nitration.¹²

CcO NO-Reductase Activity—Concerning heme/Cu $\cdot\text{NO}_{(\text{g})}$ reductive coupling (bio)-chemistry, recent biophysical studies and DFT calculations from Varotsis, Kitagawa, Ohta and co-workers^{130–132,142} have led to the suggestion of what is referred to as a *trans* mechanism (Figure 7). This was adopted on the basis of their biophysical and DFT studies, with reference to the literature on the diiron NOR's (see Scheme 9). Rate-limiting addition of $\cdot\text{NO}_{(\text{g})}$ and a proton to a heme-nitrosyl complex leads to a " $\text{N}_2\text{O}_2\text{H}$ " intermediate, one-electron oxidized from a hyponitrite dianion ($\text{N}_2\text{O}_2^{2-}$) and protonated, which possesses a N–N bond. Note that reduced $\text{Cu}_{\text{B}}^{\text{I}}$ is proposed to be present and a DFT calculated structure for this intermediate which is N-bound to both the heme-iron and copper ion has been presented.¹³² These researchers also assert direct detection of the a_3 -nitrosyl and hyponitrite species via resonance Raman spectroscopic interrogation; we note that the latter hyponitrite complex was generated by adding excess $\cdot\text{NO}_{(\text{g})}$ to an oxidized ($\text{Fe}^{\text{III}}.. \text{Cu}^{\text{II}}$) form of the enzyme. Finally, it is suggested that further protonation leads to $\text{N}_2\text{O}_{(\text{g})}$ generation.

The computational studies carried out by Blomberg, Blomberg and Siegbahn¹⁴³ favor a NOR *cis-heme* like mechanism (Fig. 7; Scheme 9). The heme-nitrosyl complex would undergo direct attack by $\cdot\text{NO}_{(\text{g})}$, as the latter is expected to bind the cuprous ion only weakly at best. The hyponitrite complex formed is suggested to be O-bound to $\text{Cu}_{\text{B}}^{\text{II}}$. These latter two suggestions are also in line with recent studies by Moënne-Loccoz, colleagues and co-workers.^{77,115} From studies using FT-IR spectroscopy following photolysis of the heme-nitrosyl moiety, it was demonstrated that NO binds to the Cu_{B} weakly and in an η^2 -side-on or η^1 -O-bound (end-on) form for the CcO from *T. thermophilus*, but no Cu_{B} interaction is observed at all for the CcO bo_3 from *E. coli*. Such observations suggest how a second $\cdot\text{NO}_{(\text{g})}$ molecule might slip in to form an N–N bond with the nitrosyl heme already N-bound to iron, but leave a hyponitrite product O-bound to the Cu_{B} ion.

One of many foci for future studies on the proteins or models (designed protein or synthetic, vide infra) will be on the nature of the putative hyponitrite complex intermediate, its structure and redox or protonation state. There are very few hyponitrite metal complexes known from inorganic chemistry, but they may coordinate or bridge metals in many different ways.^{87,89} A very recent complex from Richter-Addo and co-workers⁸⁹ may be relevant (Scheme 14). They reported on the first structurally characterized heme-hyponitrite complex, a stable bimetallic species from the μ -oxo species $[(\text{OEP})\text{-Fe}]_2(\mu\text{-O})$ (OEP = octaethyl-porphyrinate) complex and hyponitrous acid. It is notable that the $\text{N}_2\text{O}_2^{2-}$ anion is

O-bound to the iron(III) atoms, suggesting this is a stable bonding arrangement. Addition of acid to their complex releases $\text{N}_2\text{O}_{(\text{g})}$ and so it will be interesting to eventually know if the structure here is relevant to NOR and/or heme/Cu oxidase (CcO) mechanisms and intermediate structures proposed (vide supra).

Model Systems, Protein and Synthetic

Yi Lu and coworkers,¹⁴⁴ in a protein based model system, demonstrated CcO type NOR chemistry coming (again) from a modified/engineered myoglobin (Mb) protein from sperm whale. They designed in histidine residues which serve as copper ion ligands (i.e., the Cu_{B}), leading to a binuclear heme/Cu center. With an added external reductant (ascorbate with tetramethylphenylenediamine (TMPD), they demonstrated $\cdot\text{NO}_{(\text{g})}$ to $\text{N}_2\text{O}_{(\text{g})}$ conversion (Scheme 15). From their studies, the presence of Cu (or Fe) was seen to weaken the proximal His interaction with the heme-iron, allowing for the ability to form a 5-coordinate heme-NO species, which was seen as being critical for the chemistry.

In the last two years, efforts have been made toward the study of $\cdot\text{NO}_{(\text{g})}$ interactions with synthetic heme-copper oxidase (e.g., CcO) model compounds. Collman et al.^{100–101,118} have over the years generated numerous such constructs, many as functional models which electrocatalytically reduce dioxygen to water. In a recent study by that group¹⁴⁵ however, a reduced heme-Cu complex was shown to bind $\cdot\text{NO}_{(\text{g})}$, but it did not give reductive coupling chemistry; further O_2 -reaction with the NO-adduct gave an oxidized heme product, and it was suggested that the reaction involved formation of nitrate anion (although this was not directly detected).¹⁴⁵

In the last year, our research group has in fact been able to demonstrate NOR functional modeling, i.e., employment of heme-copper synthetic complexes to effect the coupling of two $\cdot\text{NO}_{(\text{g})}$ molecules to give N_2O . In our initial efforts, we employed a binucleating ligand ${}^6\text{L}$, whose iron and heme-copper derivatives have been previously used in our investigations involving O_2^- chemistry.¹⁴⁶ In the presence of protons, two equivalents $\cdot\text{NO}$ which bind to the heme are reductively coupled to yield one equivalent N_2O (Fig. 8).¹⁴⁷

The starting point was the reductive nitrosylation of $({}^6\text{L})\text{Fe}^{\text{III}}(\text{OH})$ to straightforwardly give the iron(II)-nitrosyl compound $({}^6\text{L})\text{Fe}(\text{NO})$ (Fig. 8, red EPR spectrum), possessing the expected classic three-line hyperfine split EPR spectrum. The affiliated bis-nitrosyl species, $({}^6\text{L})\text{Fe}(\text{NO})_2$, was generated via the addition of $\cdot\text{NO}_{(\text{g})}$ to $({}^6\text{L})\text{Fe}(\text{NO})$. $({}^6\text{L})\text{Fe}(\text{NO})_2$ is EPR silent, as expected, and only stable at low-temperature; warming releases one mol-equiv $\cdot\text{NO}_{(\text{g})}$ and $({}^6\text{L})\text{Fe}(\text{NO})$ is reformed (Fig. 8). $({}^6\text{L})\text{Fe}(\text{NO})_2$ was deemed an ideal starting point, as addition of a copper(I) source would result in this ion's binding to the uncoordinated tris(pyridylmethyl)amine "tether" portion of ${}^6\text{L}$ and facilitate further chemistry. This indeed was the case and with the addition of $[\text{Cu}^{\text{I}}(\text{MeCN})_4]^+$ and two mole-equivalents of acid, $\text{N}_2\text{O}_{(\text{g})}$ is indeed produced in high yield (80 %), as determined from gas-chromatographic analysis. Consistent with this stoichiometry expected and that each metal ion is a source of one reducing equivalent, the oxidized metal complex product is $[({}^6\text{L})\text{Fe}^{\text{III}}\dots\text{Cu}^{\text{II}}(\text{D})]^{3+}$ (D = water or solvent), as demonstrated by UV-vis and EPR spectroscopies (Fig. 8).¹⁴⁷

By contrast, if acid is not added along with the copper(I) source, the chemistry occurring is that of the well known^{4,86} Cu(I)-complex mediated disproportionation of nitrogen monoxide:

As the binding of one $\cdot\text{NO}_{(\text{g})}$ to the heme is very strong, i.e., in $(^6\text{L})\text{Fe}(\text{NO})$, only the other one $\cdot\text{NO}_{(\text{g})}$ equiv is available, and the “upper” copper(I) chelate effects its disproportionation. The amount of $\text{Cu}(\text{II})$ complex present should be at a level of 1/3 of the amount of heme-nitrosyl complex present, see eq. 14 and Fig. 8, thus, the $g = 2.0\text{--}2.3$ region of the EPR spectrum for this reaction mixture (Figure 8) is dominated by the heme-nitrosyl complex signal on top of a typical copper(II) tetragonal EPR signal. The yield of $\text{N}_2\text{O}_{(\text{g})}$ was 90 %, based on this expected stoichiometry, and the presence of nitrite anion was proven using ion chromatography. Further supporting evidence was that a close analogue of just the Cu^{I} -chelate present in the ^6L -containing heme/Cu metal complex does in fact disproportionate $\cdot\text{NO}_{(\text{g})}$ according to eq. 14.¹⁴⁷

More recently,¹⁴⁸ the similar $\cdot\text{NO}_{(\text{g})}$ reductive coupling reaction was shown to occur when the component complexes are employed, $(\text{F}_8)\text{Fe}^{\text{II}}$ and $[(\text{tmpa})\text{Cu}^{\text{I}}(\text{MeCN})]^+$ ($\text{tmpa} = \text{tris}(2\text{-pyridylmethyl})\text{amine}$) as metal complexes and sources of electrons, i.e., one from each. Formation of the heme-dinitrosyl complex $(\text{F}_8)\text{Fe}(\text{NO})_2$ by the $-80\text{ }^\circ\text{C}$ addition of excess $\cdot\text{NO}_{(\text{g})}$ to $(\text{F}_8)\text{Fe}^{\text{II}}$, (vacuum-purge removal of any excess $\cdot\text{NO}_{(\text{g})}$) addition of the copper(I) complex and acid, effect reductive coupling and production of nitrous oxide (Scheme 16). Here more detailed UV-vis, EPR (Fig. 9) and NMR characterization were described, such as for the heme-nitrosyl (pentacoordinate, $\nu_{\text{N-O}} = 1691\text{ cm}^{-1}$ in CH_2Cl_2 ; hexacoordinate with tetrahydrofuran (THF) axial base ligand ($\nu_{\text{N-O}} = 1670\text{ cm}^{-1}$ in THF), and heme-dinitrosyl complexes (EPR silent, Fig. 9). The overall chemistry is essentially the same (Figure 10), as described by Figure 8. Again, without acid present, only disproportionation of one of the two mol-equivalents of $\cdot\text{NO}_{(\text{g})}$ present at the beginning took place.

For both chemical systems described above (Figures 8 and 10), acid alone plus the heme dinitrosyl complexes does not effect $\cdot\text{NO}_{(\text{g})}$ reductive coupling. The copper ion has a critical role in this NOR mimetic chemistry. We suggest possible steps in the chemistry described here. A likely first one would be the attack of the copper(I) complex moiety on the nitrogen monoxide ligand in the heme-dinitrosyl fragment. This may release one $\cdot\text{NO}_{(\text{g})}$ molecule, which now attacks a binuclear heme- $\text{Fe}^{\text{II}}(\text{NO})\cdot\text{Cu}^{\text{I}}$ species to give a hyponitrite-type species. Then, protonation, electron-transfer from the copper ion and N-O cleavage could give $\text{N}_2\text{O}_{(\text{g})}$ and water, and the heme- Fe^{III} and Cu^{II} complexes observed. As yet, we do not possess any direct mechanistic information, nor have we yet been able to detect any intermediates. However, we expect new insights to be made from further low-temperature monitoring and/or kinetic studies, and hopefully these will provide useful information on this fundamentally important nitrogen oxide chemistry, and possibly provide further clues to the mechanistic details associated with the enzymatic reactions.

Conclusions

In this Forum article, we have emphasized recent works, especially from our own labs, pertaining to iron and/or copper mediated chemistry with nitrogen oxides. At the center of the picture is $\cdot\text{NO}_{(\text{g})}$, and although it was not emphasized here, its formation from sources other than Nitric Oxidase Synthases (NOS's), especially nitrite, is a particularly important and right now a ‘hot’ subject. We have focused on bioinspired inorganic chemistry and biochemistry involved with either $\cdot\text{NO}_{(\text{g})}$ oxidation or reduction. For the former, oxidation by heme containing NOD's, including hemoglobin and myoglobin which carry out this function, nitrate is the product. However, peroxynitrite and/or $\cdot\text{NO}_2(\text{g})$ may form as intermediates, and may leak from the center of action, i.e., the enzyme active-sites. As these species have their own chemistry, other types of products might appear, such as nitrite, hydroxyl radical or dioxygen. As concerns the reduction of $\cdot\text{NO}_{(\text{g})}$, we have emphasized its heme/non-heme diiron or heme/copper mediated reductive coupling to give $\text{N}_2\text{O}_{(\text{g})}$, and

inorganic/model systems which give rise to this biologically interesting and important reaction.

There is considerable current interest and activity in the subjects discussed here. From the synthetic bioinorganic perspective, we suggest that a considerable increase in activity will soon take place. There is already a strong history of nitrogen oxide research in the literature, but there are many fundamental aspects to still be uncovered, having chemical, biological, medicinal and environmental implications. As the very exciting biology continues to be unraveled, inorganic chemists, certainly ourselves, will watch with great interest and be inspired to carry out new inorganic chemistry.

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Figure 1.
Chemistry of Nitrogen Monoxide: Sources of Production in Biological Systems, Its Functions and Roles, and Multiple Oxidation and Reduction Pathways Leading to Formation of a Variety of Species Which May Effect a Diverse Range of Biomolecules and Functions. Also, see the Text.

Nitric Oxide, Peroxynitrite (PN), $\bullet\text{NO}_2$ and Hemes

Figure 2. Various Deleterious Effects of $\bullet\text{NO}_{(\text{g})}$ and Formation of Reactive Nitrogen Species (RNS) *in vivo*. Adapted from Gardner.²⁸

Figure 3. Depiction of the Mb active site following reaction of the oxidized heme with peroxynitrite (or which would form from an oxy-heme plus $\cdot\text{NO}_{(\text{g})}$ reaction), which generates a caged radical pair, $[\text{Fe}^{\text{IV}}=\text{O} \cdot\text{NO}_2]$. Nitrogen dioxide can either attack the oxo group leading to nitrate anion, or escape, which may result in Tyrosine nitration. Adapted from Su and Groves,⁴²

Figure 4.

Peroxynitrite chemistry showing formation of nitrogen dioxide radical, hydroxyl radical and carbonate radical anion. These species can effect protein tyrosine nitration, protein oxidation, DNA, lipid and thiol oxidation/nitration, and reactions with metalloproteins including Fe/Mn/Cu. As discussed metal ions may also react with peroxynitrite, for example met-hemes, to give $[\text{Fe}^{\text{IV}}=\text{O} \cdot \text{NO}_2]$ caged radicals. Adapted from ⁵⁶.

Figure 5.

The peroxynitrito complex $[(\text{TMG}_3\text{tren})\text{Cu}^{\text{II}}(^-\text{OON}=\text{O})]^+$ was formed from $\text{Cu}/\text{O}_2/\cdot\text{NO}_{(\text{g})}$ Chemistry and its formulation confirmed by ESI-MS experiments including with ^{18}O Labeling (O is ^{18}O). An X-ray Crystal Structure of the Nitrite Product $[(\text{TMG}_3\text{tren})\text{Cu}^{\text{II}}(^-\text{ONO})]^+$ was obtained. Dramatic Differences in the EPR Spectra of the $^-\text{OON}=\text{O}$ and ^-ONO Species (Square Pyramidal vs. Trigonal Bipyramidal) are observed, and the Calculated Lowest Energy Structure for the Peroxynitrito-copper(II) Complex is Shown.⁶⁰

Figure 6.

Depiction of two X-ray Crystallographically Determined Forms of the Engineered Protein Fe_BMb (Left) and I107E Fe_BMb (Right), both Exhibiting NOR Activity.^{124–125} Both Structures Possess the “Distal” Histidine (H64) from Wild-Type Mb plus the Designed in (i.e., Mutated) New Histidines (H29, H43) and Glutamate Residues (E68) which Serve as Non-heme Iron Ligands. The Right Structure reveals the New Glutamate which is not Bound to the Fe_B (E107), but which Leads to Dramatically Enhanced Protein Activity. See Text for further Discussions.

Figure 7.

Proposed Mechanisms for $\cdot\text{NO(g)}$ Reductive Coupling in Heme/Cu CcO's. Top. Proposal from Varotsis, Kitagawa, Ohta et al, based on Biophysical Studies and DFT Calculations. Bottom. Computationally Derived Mechanism from Blomberg, Blomberg and Siegbahn. See text for further discussions and references.

Figure 8.

Reductive coupling chemistry mediated by the heme/copper complex with binucleating ^6L ligand. Addition of a copper(I) source to the heme dinitrosyl complex, with acid, leads to $\cdot\text{NO}_{(\text{g})}$ reductive coupling to give $\text{N}_2\text{O}_{(\text{g})}$. The EPR spectrum (blue) for the oxidized heme/Cu product $[(^6\text{L})\text{Fe}^{\text{III}} \dots \text{Cu}^{\text{II}}(\text{D})]^{3+}$ shows the presence of a $g = 6$ signal for high-spin iron(III) and a typical Cu(II) spectrum in the $g = 2.0$ to 2.3 region. See text for further explanations.¹⁴⁷

Figure 9. EPR Spectra of (F₈)Fe(NO) (ligand ($g_1 = 2.113$, $g_2 = 2.078$, $g_3 = 2.025$))(blue) and the dinitrosyl species (F₈)Fe(NO)₂ (red; EPR silent) at the same concentration in acetone, recorded at 77 K.¹⁴⁸

Figure 10.

Outline of the $\cdot\text{NO}_{(g)}$ reductive coupling chemistry mediated by the component system, starting with 1:1 mixtures of heme and copper complexes. $(\text{F}_8)\text{Fe}(\text{NO})$ and $[(\text{tmpa})\text{Cu}^{\text{I}}(\text{MeCN})]^+$. When the dinitrosyl species $(\text{F}_8)\text{Fe}(\text{NO})_2$ is reacted with $[(\text{tmpa})\text{Cu}^{\text{I}}(\text{MeCN})]^+$ and acid the products obtained are shown in panel **A**, associated with EPR spectrum **A** (blue). The trigonal bipyramidal nature of $[(\text{tmpa})\text{Cu}^{\text{I}}(\text{MeCN})]^{2+}$ leads to the expected “reverse” axial spectrum, while the $g = 6$ feature is associated with a high-spin $[(\text{F}_8)\text{Fe}^{\text{III}}]^+$. Spectrum **A'** is a made-up 1:1 mixture of authentic compounds showing a match with spectrum **A**. EPR spectrum **B** is a mixture ascribed to that product of reaction without acid, panel **B**. See text for further explanation.¹⁴⁸

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

- I. NAR: Nitrate Reductase (Mo-oxotransferase)
 II. NIR: Nitrite Reductase (heme cd_1 or multicopper)
 III. NOR: Nitric Oxide Reductase (heme/non-heme diiron)
 IV. N_2OR : Nitrous Oxide Reductase (multicopper sulfide)

Zumft, W. G., *Microbiol. Mol. Biol. Rev.* **1997**, 61, 533.

Scheme 5.

Scheme 6.

Scheme 7.

Scheme 8.

Scheme 9.

Scheme 10.

Scheme 11.

Scheme 12.

Scheme 13.

Scheme 14.

Scheme 15.

Scheme 16.

Chart 1.

Chart 2.