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Review

Naturally and artificially generating nitric oxide

Mary Grace I. Galinato* and Robert S. Fogle III
School of Science, Penn State Erie, The Behrend College, Erie, PA 16563, USA

ABSTRACT

Since its discovery as the endothelium-derived relaxing factor, nitric oxide (NO) has garnered immense interest for a molecule so chemically simple but so functionally diverse. The complexity of its effects is demonstrated in a broad range of physiological functions and anti-pathogenic effects on one end, and a host of diseases and dysfunctions on the other. The beneficial and detrimental nature of NO is a function of concentration: sub-nM amounts are crucial for proper physiological functions, while its overproduction (μM concentration) causes damaging effects. Researchers therefore strive to understand how NO is either generated or expended. The first half of this review summarizes the different biological pathways for the generation and transportation of NO, starting with the classic conversion of L-arginine (L-Arg) via nitric oxide synthases (NOS). Other mechanisms such as the nitrite reductase activities of various enzymes, anhydrase activities of myoglobin (Mb) and hemoglobin (Hb), as well as the S-nitroso (SNO) formation of these globins will also be presented. The second part of this review focuses on synthetic metal complexes that donate NO upon UV-visible light exposure and have pharmacological potential. These select synthetic complexes contain metal centers such as iron (Fe), ruthenium (Ru), manganese (Mn), and chromium (Cr). This review highlights recent work that focuses on the natural (enzymes) and artificial (metal complexes) production of NO and their implications in human physiology and pharmacological potential.

*Corresponding author
mig11@psu.edu

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INTRODUCTION

Nitric oxide (NO), a signaling molecule, performs a wide range of functions in humans such as dilation of blood vessels, inhibition of platelet aggregation, and neurotransmission [1]. However, the highly toxic nature of this chemical messenger emerges when a malfunction of its regulating network occurs, where NO is then implicated in different diseases and dysfunctions such as septic shock, atherogenesis, sporadic Parkinson's disease, and the initiation of cancer [1]. The functional behavior of NO depends on its concentration *in vivo*: sub-nM amounts are important for proper physiological performance, while μM concentrations generate damaging effects. The delicate balance between beneficial and detrimental therefore requires the tight regulation of NO.

Several mechanistic pathways are available for the degradation of NO to a less toxic form when this diatomic molecule is present in excess. These degradation processes are commonly mediated by enzymes that contain a heme (a porphyrin that contains a Fe center). For example, oxyHb, oxyMb, and bacterial flavoHb individually reacts with NO in a bimolecular reaction commonly known as NO dioxygenation, forming nitrate and the "met" form of the iron [2-4]. "Oxy" refers to a heme-bound O_2 to the iron center, and "met" refers to iron in the ferric form, Fe(III). Heme-bound NO (Fe(II)-NO) can also react with O_2 to produce Fe(III) and nitrate in a very slow process

called oxidative denitrosylation [5]. In addition, nitric oxide also directly reacts with the Fe(II) and Fe(III) forms of Hb, with the latter form being ~1000 times faster [6, 7].

In conditions where NO is lacking or required, several sources of this diatomic molecule become available for its production, depending on where NO is most needed. For example, L-Arg, a major source of this diatomic molecule, is converted to citrulline through nitric oxide synthase (NOS). Another source of NO is plasma nitrite (NO_2^-), most of which is derived from endothelium NOS (eNOS) [8]. Nitrite is catalytically reduced to NO through various enzymes such as deoxyHb and deoxyMb ("deoxy" pertains to the Fe(II) form) [9, 10], xanthine oxidoreductase [11], carbonic anhydrase [12], NOS [13], mitochondrial enzymes [14], and cytochrome P450 [15]. A non-enzymatic system involving ascorbic acid also reduces NO_2^- to NO [16, 17]. Because NO is thought to remain intact in the blood vessel for a longer period of time than initially assumed, more distal and sustained effects are observed. However, some studies suggest that NO must be transported as a different form due to its short half-life. N_2O_3 [18] and nitrosylated cysteine (SNO) in Hb [19, 20] have been theorized and explored as possible intermediates for delivering NO. In this review article, the pathways proposed for the generation of bioactive NO are discussed. In addition, model compounds that serve as artificial sources of NO will also be presented in light of potential applications in treating diseases and maintaining the physiological functions of the body.

Nitric oxide production (biological)

A. Classic NO production

The most common enzyme that generates nitric oxide in mammals is NOS. This enzyme exists as (i) three major isoforms depending on their specific location and function (endothelial (eNOS), neuronal (nNOS) or inducible NOS (iNOS)) [21, 22], and (ii) a few splice variants [23, 24]. Structurally, each NOS isoform is a homodimer, where each monomer is comprised of two functional units: an N-terminal oxygenase and a C-terminal reductase domain. The oxygenase domain contains an Fe-protoporphyrin thiolate heme, (6R)-5,6,7,8-tetrahydro-L-biopterin (BH_4), and a distinct binding site for the substrate L-Arg. The substrates nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN) are associated with the reductase domain. It has been shown that the FMN domain docking to the heme domain modulates reactivity and structure of the heme active site, thus regulating NOS catalysis [25, 26]. Particularly for iNOS, the FMN domain influences the interaction between L-Arg and a ligand of the heme center [27]. In addition to the two functional units, a calmodulin binding domain (CaM) exists between the oxygenase and reductase domain. This is required for initiating NO synthesis in nNOS and eNOS [28, 29].

Nitric oxide is generated by NOS enzymes through a two-step catalytic process: (1) the hydroxylation of L-Arg to N^G-hydroxy-L-Arg (NOHA), followed by (2) the oxidation of NOHA to form citrulline and NO (Fig. 1a) [30, 31]. Prior to the oxidation of L-Arg, electron transfer occurs in the reductase domain. Here, NADPH transfers electrons to FAD, which upon H^+ addition forms $FADH_2$. This product disproportionates and generates FMNH and FADH, which then reduces the heme from Fe(III) to Fe(II) in the oxygenase domain [32, 33]. Different scenarios of proton and electron transfer have been proposed for the *first* catalytic step [34-39], but the most commonly accepted model is described as follows (Fig. 1b): O_2 readily binds to Fe(II) porphyrin (*vide supra*) *trans* to the bound cysteine, forming a short-lived Fe(II)-O_2 species that quickly converts to a Fe(III)-peroxo species via an electron transfer from BH_4. A proton transfer then occurs generating a Fe(III)-hydroperoxo intermediate that subsequently undergoes O-O heterolytic bond cleavage. This results in the formation of a high-valent Fe(IV) species, Fe(IV)=O ($P^{\bullet+}$), where P is the porphyrin macrocycle. This reactive compound is able to abstract a hydrogen atom, presumably from the guanidine nitrogen of L-Arg. The hydroxyl radical formed from the previous step is ultimately transferred to L-Arg then generating NOHA [30].

The *second* catalytic step, which was suggested to rely on the formation of a Fe(III)-hydroperoxo intermediate as the reactive species of NOHA oxidation [40-42], is now shown to be dependent on a ferric-peroxide species instead [43, 44].

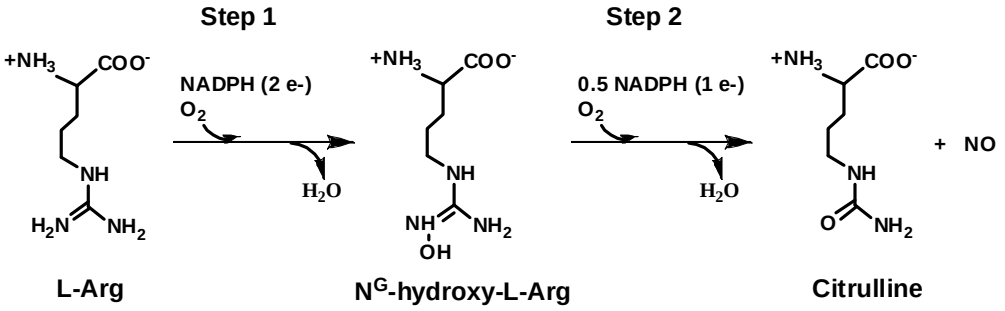

Fig. 1a. The two-step hydroxylation/oxidation process in the generation of NO via NOS. L-Arg is hydroxylated to form the intermediate NOHA (N^G-hydroxy-L-Arg) which is then further oxidized to yield citrulline and NO.

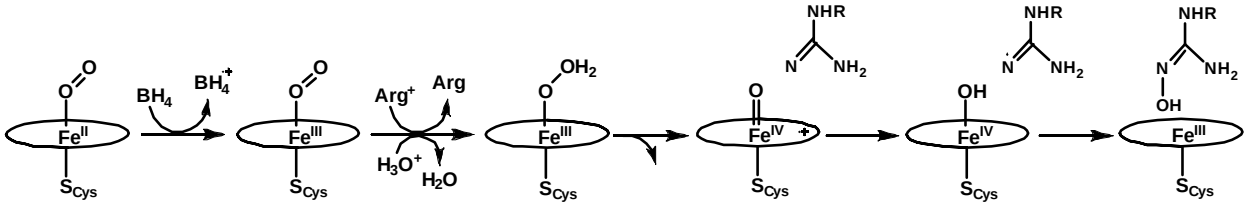

Fig. 1b. Proposed mechanism for the first hydroxylation step in the generation of NO through NOS, starting with the initial intermediate (ferrous-oxo species). The high-valent Fe(IV) species, Fe(IV)=O ($P^{\bullet+}$), where P is the porphyrin macrocycle is known as Compound I. This reactive species abstracts a hydrogen atom where the hydroxyl radical is transferred to L-Arg then generating NOHA. Figure adapted from [45].

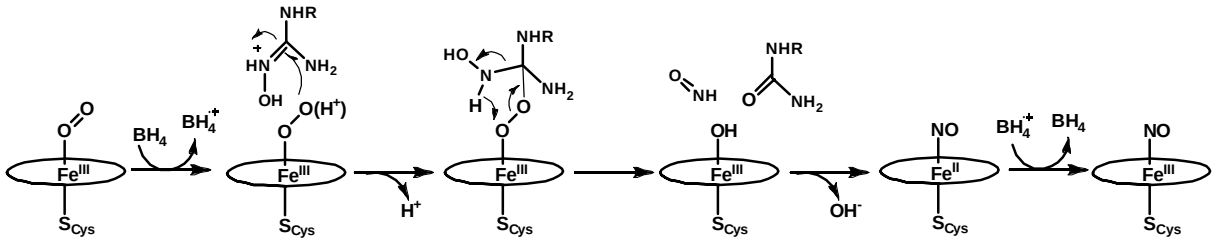

Fig. 1c. Proposed mechanism for the second oxidation step of NOHA. The BH_4 cofactor might act only as an electron donor (shown) or a coupled electron and proton donor (in which case BH_4 would convert to $BH_3^{\bullet}$ in the first step). Unlike Step 1 (Fig. 1b), Step 2 may utilize a ferric hydroxo species as the reactive oxidant. The last species shown in the mechanism, Fe(III)-NO, has a Fe-N bond that is intrinsically labile and readily releases NO forming the Fe(III) resting state, ready for another catalytic cycle. Figure adapted from [45].

Although the mechanism is still unclear, the following model is postulated as a route to NOHA oxidation (Fig. 1c) [45]: the ferric-peroxide intermediate forms a tetrahedral complex with the NOHA guanidinium moiety, which then rearranges and involves the cleavage of the peroxide O-O bond and the guanidinium C-NHOH bond. This process leads to the formation of citrulline, hydroxide anion bound to Fe(III) heme, and a protonated nitroxyl molecule. The latter rebinds to the heme iron (upon release of OH^-) forming a Fe(II)-NO species that is oxidized to Fe(III)-NO through the BH_4 radical. The intrinsically labile nature of the ferric heme nitrosyl then allows for the efficient release of nitric oxide, and forms the resting Fe(III) state back, ready for another catalytic cycle.

B. Nitrite reductase activities

Nitrite has emerged to be the largest physiological repository of NO, with as much as 70% originating from eNOS [8]. Aside from eNOS, nitrite is also generated from bacteria in the digestive system during nitrate reduction [46, 47]. An exogenous source of this anion comes from food such as that present in processed meat in which NO_2^- is used to prevent botulism [46]. Available in nanomolar amounts, NO_2^- can be converted to bioactive NO under an O_2 and pH gradient, eliciting several physiological responses that include hypoxic vasodilation [48, 49], mitochondrial respiratory inhibition [9], protein and gene expression regulation [50], and cytoprotection following ischemia/ repurfusion [51-54]. The different pathways for NO generation via NO_2^- reduction are discussed below.

I. Nitrite reduction by deoxyheme-globins

The first comprehensive study on the reaction of nitrite with deoxyHb is proposed to follow a two-step process [55]:

$$NO_2^- + Fe(II) + H^+ \rightarrow NO + Fe(III) + OH^- \quad \text{(Rxn I.1)}$$

$$NO + Fe(II) \rightarrow Fe(II)\text{-}NO \quad \text{(Rxn I.2)}$$

The NO produced from Rxn. I.1 binds quickly (1.8×10^7 M^{-1} s^{-1}) to vacant hemes of deoxyHb to form the nitrosylated species in Rxn. I.2. Interestingly, this seemingly simple second-order reaction holds unique features that point towards a mechanism more complex than initially thought [9]. First, the reaction exhibits zero-order kinetics when deoxyHb is reacted with excess nitrite under a strictly oxygen-free environment. Second, the reaction rate dramatically increases in the presence of an oxygen leak, particularly when Hb is ~50% saturated with O_2. Last, this reaction is allosterically catalyzed, with the bimolecular rate constant for R-state Hb being greater than that of the T-state. The variation in bimolecular rate constants between the two conformational states of Hb may be either be due to differences in heme redox potential or second sphere coordination [56]. The mechanism of nitrite reduction by deoxyHb is primarily attributed to the allosteric nature of this enzyme [9], which will be the focal point of the succeeding discussion.

Nitrite reduction by deoxyHb follows a mechanism similar to bacterial heme cd_1 nitrite reductase [57], but is instead allosterically catalyzed [58]. Here, nitrite binds to Fe(II) in the R-state tetrameric form of deoxyHb (k_{on} ~6 M^{-1} s^{-1}) producing water and Fe(III)-NO after protonation. The labile nature of the Fe(III)-NO bond allows for NO release, which is then scavenged by another vacant heme within the tetramer, forming a stable Fe(II)-NO complex. The binding of NO, like other heme ligands such as O_2, increases the ligand affinity of vacant hemes within the tetramer, permitting the reaction to proceed at a faster rate. Autocatalysis is therefore attained when the reaction of nitrite with deoxyHb produces two R-state stabilizing heme conformations, which are the Fe(III) and Fe(II)-NO species. The loss of available hemes for nitrite binding, which dissipates the process, is offset by the fast reaction upon formation of these stabilizing species [59]. This reaction is optimally catalyzed at ~50% oxygen saturation of Hb, for the same reasons described above.

Although the nitrite reductase activity of deoxyHb has been accepted and reflects that of bacterial nitrite reductases, one major challenge of this mechanism still exists. Since nitric oxide is easily scavenged by deoxyHb (k ~1.8×10^7 M^{-1} s^{-1}) [7], or reacts readily with oxyHb to form nitrate (k ~$6\text{-}9 \times 10^7$ M^{-1} s^{-1}) [4], the bioavailability of NO upon its generation from nitrite has been questioned. To compensate for this, the intermediate N_2O_3 is proposed to form, which is a viable species that can diffuse through the red blood cells and decompose to NO or a species with NO-like activity [60, 61]. The formation of N_2O_3 upon reacting nitrite with deoxyHb has recently been discovered, pointing to a new function of this enzyme as being a nitrite anhydrase [18]. The proposed reactions for the nitrite anhydrase activity of Hb are shown below:

$$NO_2^- + Fe(II) + H^+ \rightarrow NO + Fe(III) + OH^- \quad \text{(Rxn I.1)}$$

$$Fe(III) + NO_2^- \rightarrow Fe(III)\text{-}NO_2^- \quad \text{(Rxn I.2b)}$$

$$Fe(III)\text{-}NO_2^- + NO \rightarrow Fe(III)\text{-}N_2O_3 \quad \text{(Rxn I.3)}$$

$$Fe(III)\text{-}N_2O_3 \rightarrow Fe(II) + N_2O_3 \quad \text{(Rxn I.4)}$$

The crystal structure of the R-state nitrite-bound Hb at 1.80 Å shows that nitrite binds to Fe(III) through the O-atom (as opposed to the N-atom) forming an *O*-nitrito species [62]. Density functional theory (DFT) calculations that affirm the feasibility of the nitrite anhydrase function of Hb demonstrate that this binding mode is key to the formation of N_2O_3 [63, 64]. Lehnert and co-workers [63] show that the formation and release of N_2O_3 is energetically feasible by 1-3 kcal/mol when the active site of Hb is modeled with the distal histidine residue (H67), which is substantially lower compared to a model without the distal histidine residue [64]. This underscores the significance of the second coordination sphere in controlling the nitrite reductase activity of Hb. It should be pointed out that a "Fe(III)-NO + NO_2^-" pathway to the formation of N_2O_3 may also exist [64]. Although this has not been experimentally verified, this reaction is energetically reasonable and cannot be discounted. Despite the feasibility of the nitrite reductase/anhydrase mechanism, this reaction has been criticized for its presumed involvement in hypoxic vasodilation. Schwab *et al.* [65] re-examined the effective dissociation constant of nitrite from Fe(III) in Hb and obtained a value of 1.8 $\pm$ 0.6 mM. This signifies that the formation of the Fe(III)-NO_2^- complex, and hence the generation of N_2O_3 via this mechanism, is unlikely under physiological conditions. The generation of NO via the nitrite reductase/anhydrase reaction in Hb remains contested. Another reaction that delivers NO to cells involves the formation of SNO-Hb, a pathway that is discussed in Section C.

Aside from Hb, deoxyMb is also characterized as a nitrite reductase, catalyzing the reduction of nitrite 36 times faster than deoxyHb due to its lower heme redox potential [10]. This reaction is optimal at O_2 concentrations around its P_{50} value (3.1 µM), upon which Mb is then deoxygenated [10]. Like Hb, nitrite binds to Fe(III) Mb forming an *O*-nitrito species that flips to the *N*-nitro conformation upon mutation of the distal H64 with valine [66]. Replacing the distal histidine in sperm whale Mb with alanine (H64A) and leucine (H64L) decreased the bimolecular rate constants from 5.6 $M^{-1}s^{-1}$ (wild-type) to 1.8 $M^{-1}s^{-1}$ (H64A) and ~0.5 $M^{-1}s^{-1}$ (H64L) [67]. The slower rates in the mutants may be attributed to the decreased stability of the nitrite-bound heme as a result of hydrogen bonding loss. The similarity in the nitrite-binding mode between Hb and Mb suggests that the latter follows a nitrite reduction pathway analogous to the former.

More recently, other members in the globin family have also exhibited nitrite reductase activity such as neuroglobin (Ngb) [67, 68], plant Hb [69], and cyanobacterial Hb [69]. The six-coordinate Ngb has a rate constant comparable to Mb (5.1 $M^{-1}s^{-1}$) [68] that increases ~50 fold upon mutation of the distal histidine to form a stable five-coordinate geometry [67]. Replacement of the surface thiols (Cys55 and Cys46) stabilizes the six-coordinate geometry and slows nitrite reduction. Unlike Ngb, the hexacoordinate cytoglobin (Cygb) does not show any reaction with nitrite [68], suggesting that the sixth ligand in Cygb is not as loosely bound to iron as that in Ngb. Unlike mammalian Hb, six-coordinate plant and cyanobacterial Hbs have rate constants ten times more than their mammalian counterpart (Fig. 2) [69]. Given that plants and cyanobacteria are exposed to much higher nitrite concentrations, it appears that these species evolved a nitrite reductase mechanism to compensate for the increased availability of nitrite. The fact that nitrite reduction can be catalyzed by several members of the globin family seems to implicate a vital functionality among this class of enzymes. Table 1 lists the rate constants for the reduction of nitrite to NO by different globin enzymes.

II. Nitrite reduction by xanthine oxidoreductase

Xanthine oxidoreductase (XOR) is a 290 kD dimeric protein, with each subunit containing a FAD, two spectroscopically distinct [2Fe-2S] centers, and one molybdopterin cofactor [70]. This enzyme exists as two interconvertible forms in mammals: xanthine dehydrogenase (XDH), which reduces NAD to NADH; and xanthine oxidase (XO), which catalyzes the oxidative hydroxylation for a wide range of aldehydes and heterocyclic compounds [71]. Although XO has long been known to catalyze nitrate reduction [72], the reduction of nitrite to NO has only been reported over the past decade [11, 54, 73-75] and its mechanism is not yet generally understood.

Electron paramagnetic resonance (EPR) spectroscopy and NO-selective electrochemistry confirm NO formation *only* when XO, nitrite, and a reducing substrate (e.g. an aldehyde, xanthine, NADH or

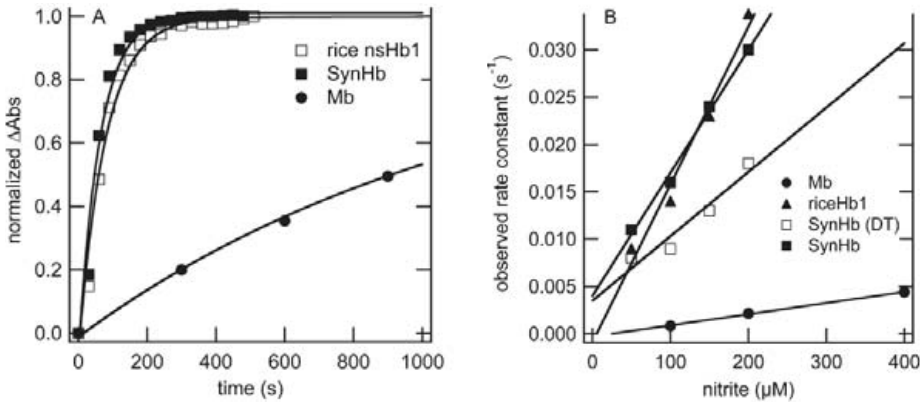

Fig. 2. Panel (A): Time courses for the reaction with each Hb and Mb at 100 μM nitrite. The absorbance change is normalized to that associated with the transition from each deoxyferrous Hb to its end point spectrum. Panel (B): Observed reaction rates for each Hb as a function of nitrite concentration. Linear fits of these plots yield observed bimolecular rate constants of 166, 130, and 11 M^{-1} s^{-1}. Also included are the reaction rates for *Synechocystis* Hb (SynHb) in the presence of sodium dithionite (DT), demonstrating that the slope is half that observed without DT. (Reprinted with permission from Sturms, R., DiSpirito, A. A., and Hargrove, M. S. Biochem., 50, 3873. Copyright (2011) American Chemical Society).

Table 1. Bimolecular rate constants for the reduction of nitrite to NO by different globin enzymes.

Enzyme[a]	Rate constant ($M^{-1}s^{-1}$)[b]	Ref.
R-state hemoglobin	12	[9, 162]
R-state hemoglobin (HbS, solution phase)	20 (pH 7.4, 37°C)	[163]
T-state hemoglobin	0.2	[9, 162]
T-state hemoglobin (HbS, solution phase)	0.2 (pH 7.4, 37°C)	[163]
hh Myoglobin	12.4 (pH 7.4, 37°C)	[10]
sw Myoglobin (sperm whale)	5.6 ± 0.6	[67]
sw Myoglobin H64A	1.8 ± 0.3	[67]
sw Myoglobin H64L	Extremely slow[c]	[67]
Neuroglobin	5.1	[68]
Neuroglobin H64L	259 ± 8	[67]
Neuroglobin H64Q	267 ± 8	[67]
Neuroglobin SH	0.062 ± 0.005	[67]
Neuroglobin SS	0.12 ± 0.02	[67]
Plant hemoglobin	166 (pH 7.0, RT)	[69]
Cyanobacterial hemoglobin	130 (pH 7.0, RT)	[69]

[a]HbS = unpolymerized sickle hemoglobin, hh = horse heart, sw = sperm whale; [b]Unless otherwise specified, kinetics were measured at pH 7.4 and 25°C; [c]The reaction is more than ten-fold slower than wild-type.

sodium dithionite) are simultaneously present in the reaction mixture. Although [2Fe-2S] centers are present in the enzyme, it is the molybdenum center that is the site of nitrite reduction [76]. This is supported by studies demonstrating the complete inhibition of NO formation in the presence of allopurinol, a compound that tightly binds to the reduced molybdenum center, blocking it from XO

hydroxylase activity. Steady-state kinetics of nitrite reduction are suggested to occur through the formation of a "carboxylate (aldehyde)-enzyme-nitrite" ternary complex, with the aldehyde binding first, and NO being the last product to leave the enzyme [76].

In order for XO to act as an effective nitrite reductase, the O_2 concentration must be minimal or negligible because O_2 reduction is much faster (k = 35 s^{-1} [77]) than nitrite reduction (0.693 s^{-1} [76]), and it inhibits NO formation by 90%. Two factors influence the competition between nitrite and molecular oxygen: the type of reducing substrate and pH levels. For example, the reducing substrate NADH binds to flavin and presumably blocks the access of oxygen, which might account for the fact that NO formation is maintained at 70% of the anaerobic levels in the presence of NADH [11]. Low cellular pH levels, as in the case of ischemic conditions (pH 5.5-6.5), also contribute to faster nitrite reduction [78].

Spectroscopic and kinetic studies [76] support the following mechanism of NO_2^- reduction via XO, as shown in Fig. 3 [76]. First, aldehyde binds to the Mo(VI) center and reduces it to Mo(IV). Nitrite then binds to Mo(IV) and displaces the carboxylate (which is formed upon aldehyde oxidation) through the formation of the "carboxylate (aldehyde)-enzyme-nitrite" complex that favors the subsequent release of NO. The one electron reduction of nitrite leaves a Mo(V)-OH state. A proton is suggested to then bind in order to generate a better leaving group. Another molecule of NO_2^- displaces the water molecule and undergoes the same series of steps until the second NO is produced, generating Mo(VI) back. In the presence of other reducing substrates (e.g. sodium dithionite or NADH), an additional branching pathway is implicated. In this extra step, the reducing substrate converts the Mo(VI) center to Mo(IV)-OH_2, where the water molecule is displaced by NO_2^-. Nitric oxide formation then follows the series of steps describe above, completing the catalytic cycle. Interestingly, another member of the XO family, aldehyde oxidoreductase (AOR), also catalyzes the reduction of NO_2^- to NO [73, 79]. However, the specificity constant of AOR for NO_2^- is lower

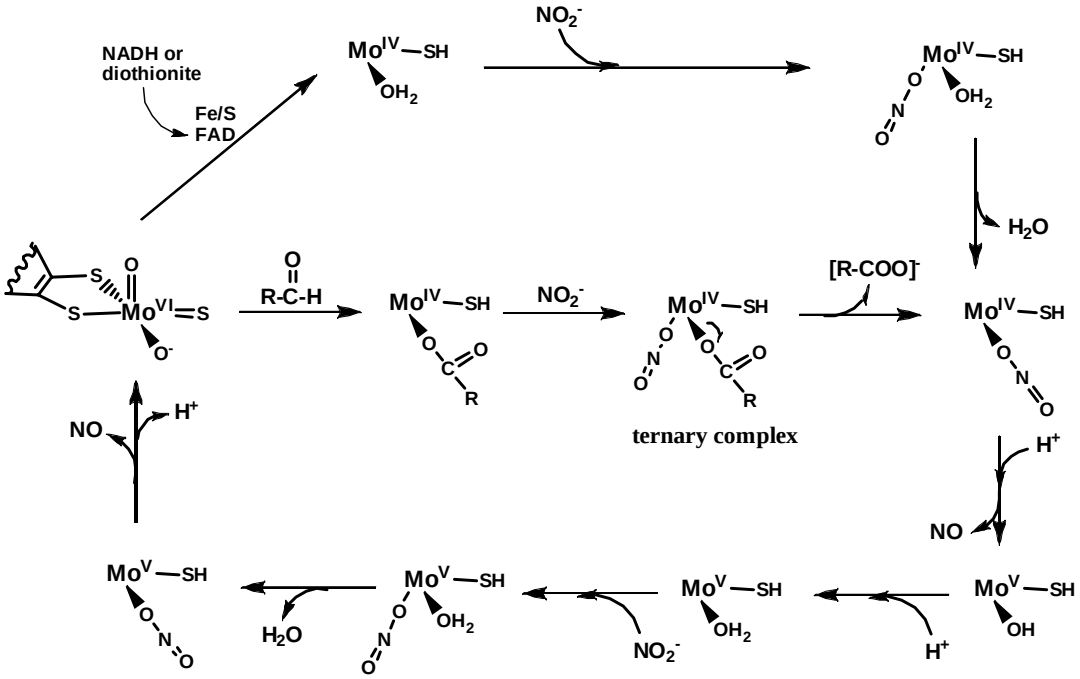

Fig. 3. Proposed mechanism of nitrite reduction by xanthine oxidase. The formation of the carboxylate-enzyme-nitrite ternary complex favors the subsequent release of NO. Another pathway that involves reduction by NADH or dithionite shown in the top branch of the schematic diagram. Figure adapted from [76].

($0.0588\ s^{-1}\ mM^{-1}$) than that of XO ($1.18\ s^{-1}\ mM^{-1}$) [76]. Nonetheless, the rate of NO formation by AOR could achieve similar rates as those of XO as long as the nitrite concentration is large enough.

III. Nitrite reduction by carbonic anhydrase

Carbonic anhydrase (CA) is a ubiquitous enzyme found in bacteria and mammals. In mammals, CA exists as 16 various isoforms (CA I-VII), all of which exists in different intracellular locations and tissue distributions [80]. Although these isoforms differ in several select regions, they all share a remarkable similarity. All isoforms have the same tertiary fold with the active site surrounded by 10 strands of anti-parallel β-sheets and two α-helices on the outer edges of the enzyme (Fig. 4) [81]. The active site includes three His residues that coordinate to a Zn(II) ion and a fourth ligand being a water molecule/hydroxide ion that acts as a strong nucleophile and attacks CO_2 according to the following reaction: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$ [80]. While the main function of CA is to generate carbonic acid, it is also known to produce NO from NO_2^- [12]. Carbonic anhydrase most likely acts as a nitrite anhydrase assuming

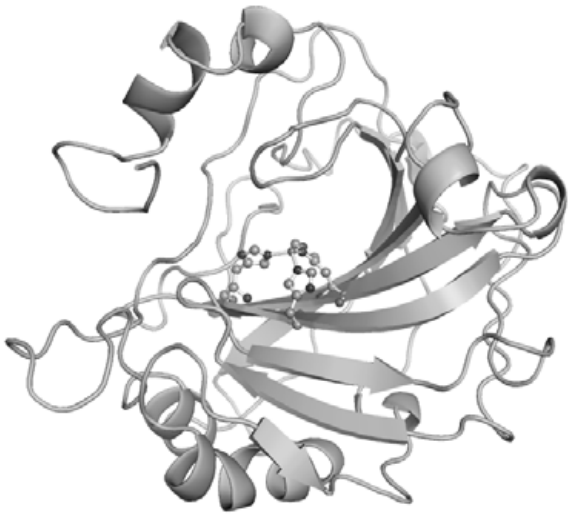

Fig. 4. Structure of the human form of carbonic anhydrase (PDB code: 1CA2) showing the 10 anti-parallel β-sheets and two α-helices on the outer edges of the enzyme. The active site is comprised of three His residues that coordinate to a Zn(II) ion. The fourth ligand is typically a water/hydroxide ligand (not shown in structure).

that Zn(II) does not support any redox activity. The stepwise reactions for the production of NO via NO_2^- in CA are given as follows [12]:

$$2NO_2^- + 2H^+ \leftrightarrow 2HNO_2 \leftrightarrow H_2O + N_2O_3 \quad \text{(Rxn III.1)}$$

$$N_2O_3 \leftrightarrow NO + NO_2 \quad \text{(Rxn III.2)}$$

Two observations support these reactions [12]. First, more NO is generated at low pH, pushing the equilibrium to the right. Second, the reaction rate becomes *faster* at high pH implying that nitrite participates more efficiently in the catalytic process. The reaction mechanism proposed above is similar to the acidic disproportionation process in non-enzymatic systems (Section VI).

One of the several ways to study the catalytic mechanism of CA is through the use of sulfonamide inhibitors, specificially dorzolamide and acetazolamide. These inhibitors work by forming a tetrahedral structure with the Zn(II) atom in hydrophobic CA's active site where CO_2 typically binds [12]. Dorzolamide inhibits hydration of CO_2 but not the dehydration of NO_2^- suggesting that these two ligands bind to different areas within the active site [12]. Curiously, Cu(II) bacterial nitrite reductase has an active site that is surprisingly alike to mammalian CA, alluding to the idea that similar amino acids might be implicated in the two enzymes despite them catalyzing different reactions.

A better understanding of the interaction between CA and NO is already yielding promising medical prospects. The CA inhibition by NO-donating dorzolamide derivatives is a newer treatment being investigated for open-angle glaucoma [82]. This is just one way that a better understanding of CA-NO interactions are beneficial. The generation of physiological NO by CA may not play a massive role compared to other biosynthetic pathways, but it is certainly important at a local, cellular level.

IV. Nitrite reduction by nitric oxide synthase

In the presence of O_2, NOSs catalyze the synthesis of NO from L-Arg (*vide supra*). However, in limited O_2 conditions (e.g. < 4-10 μM), the L-Arg pathway is ineffective due to the K_m values of eNOS, iNOS, and nNOS which are 4-10, 130, and 350 μM, respectively [83]. Interestingly, this does not

preclude NOSs from releasing NO when starved of oxygen. Slama-Schwok and co-workers [84] showed that at physiological NO_2^- concentrations (15 μM) under low O_2 tension, only the eNOS isoform produced NO implicating a critical role for the endothelium in releasing "emergency" NO. At 10 μM extracellular NO_2^-, the rates of NO formation through eNOS were largely enhanced 3.6 fold under hypoxia (2 ppm O_2) and 8.3 fold under anoxia (no O_2) relative to normoxic conditions (22 ppm O_2) [84].

In order to understand and account for the discrepancies observed in NO production among the three NOS isoforms under low O_2 levels, the following assumptions were tested: i) structural differences of the heme pockets; ii) difference in NO release efficiency from the heme; and iii) change in redox potential upon NO_2^- binding [84]. In the first assumption, no evidence was found showing direct coordination of NO_2^- at the reduced heme, unlike other enzymes known to bind NO_2^- such as Mb and Hb (*vide supra*). This implies that NO_2^- instead binds close to the heme as demonstrated through geminate recombination studies [84]. Between eNOS and iNOS, the former demonstrates NO rebinding in the ps timescale which corresponds to short distances of 3-5 Å from the heme, while the latter shows rebinding in the ns range, consistent with a 7-10 Å distance. In addition, results confirm that two nitrites are required per one NO produced [84]. These findings collectively suggest the participation of another site, one of which might be the distal Arg252 located close to the heme [85]. The presence of two nearby catalytic sites could explain how excess NO_2^- obstructs Arg252 and hampers NO release from eNOS [86, 87]. Nevertheless, the possibility of different binding modes to the heme (*N*- versus *O*-bound) in the NOS isoforms cannot be discounted, since different NO_2^- binding modes to the heme may generate various products [88]. The thermodynamics of *N*- versus *O*-binding in nitrite to heme is discussed in Section B.I.

The second assumption involves the NO release efficiency from the heme, which in part, is also influenced by heme pocket size. Among the three isoforms of NOS, NO dissociates from the heme most quickly in eNOS, both in the Fe(II) and Fe(III) forms [86]. On the other hand, nNOS shows enhanced nitrosylation, forming a stable Fe(II)-NO complex with very minimal NO dissociation. The last isoform, iNOS, has a smaller distal heme pocket compared to eNOS. The structural constraint in iNOS may prevent NO_2^- from binding to the catalytic site. As a result, NO_2^- reductase activity is not observed in this isoform. The third and last assumption entails a change in redox potential of the heme upon nitrite binding. Results on NO_2^- binding to the oxygenase domains of eNOS and nNOS reveal a smaller difference in NO yield between the two isoforms, compared to that observed between the full-length enzymes. This justifies the observation that the heme redox potential for the three NOS isoforms are similar without nitrite [89], but may change upon NO_2^- binding. In addition, the tuning of the heme redox potential by substrate binding may also affect the yield of NO [89]. Although the mechanism of NO_2^- reduction by NOS is still unknown, the set of findings described above is a step forward in the direction of formulating a plausible pathway for NO generation under limited O_2 levels.

V. Nitrite reduction by mitochondrial enzymes

A series of studies demonstrate two key aspects with regard to the generation of NO in mitochondria: (1) mitochondrial enzymes produce enhanced levels of nitric oxide under hypoxia [90, 91]; and (2) the synthesis of some amounts of NO is not associated with NOS catalysis [92-94]. The production of NO via NO_2^- reduction by cytochrome *c* oxidase, an electron-donor enzyme found in the mitochondria, was first suggested by Reutov and Sorokina [95] and later confirmed by Poyton and co-workers [14]. Under low O_2 levels and pH, both yeast cells and rat liver mitochondria produce NO from NO_2^-. Interestingly, the intracellular pH of yeast cells decrease when they are exposed to anoxic conditions [96, 97], a trigger that might be required in order for NO to be generated. Aside from cytochrome *c* oxidase, the oxidant site of ubiquinol at the cytochrome bc_1 complex of the respiratory chain (Complex III in particular) in mitochondria also showed nitrite reductase activity [98], implicating the semiquinones as electron donors for nitrite reduction. The interplay among the mitochondrial enzymes is complex and hence limits our knowledge on the overall mechanism of nitrite reduction by these systems.

The amount of NO generated by mitochondrial enzymes can be detrimental or beneficial to the cell. Nitric oxide readily binds to the heme iron of cytochrome *c* oxidase, forming an iron-nitrosyl complex [99]. The formation of this diatomic molecule competes with O_2, and may trigger the generation of superoxide radicals and hinder mitochondrial respiration [100-103]. Despite this drawback, the reversible or partial inhibition of respiration is essential because it is suggested to allow O_2 to diffuse better to more distant parts of a tissue [104], pointing to a useful role of NO in the mitochondria. In addition to this, the NO-associated events behave as activators by which the mitochondrion regulates reactions involved in defense mechanisms and adaptive responses such as hypoxia [105]. Nitric oxide also increases mitochondrial function and ATP formation by actuating mitochondrial biogenesis *in vitro* and *in vivo* [106]. These examples point to the dual nature of NO, and therefore necessitate more studies on how this diatomic molecule induces physiological or pathophysiological effects.

VI. Nitrite reduction by acidic disproportionation

Nitric oxide can also be produced from NO_2^- via nonenzymatic processes, in particular, through acidic disproportionation. In low pH conditions, NO_2^- is converted to nitrous acid, which subsequently decomposes to nitrogen oxides (including NO) according to the following reactions [107]:

$$NO_2^- + H^+ \leftrightarrow HNO_2 \quad \text{(Rxn VI.1)}$$

$$2HNO_2 \leftrightarrow N_2O_3 + H_2O \quad \text{(Rxn VI.2)}$$

$$N_2O_3 \leftrightarrow NO + NO_2 \quad \text{(Rxn VI.3)}$$

$$2NO + O_2 \leftrightarrow 2\ NO_2 \quad \text{(Rxn VI.4)}$$

The rate of non-enzymatic NO formation is driven by various factors such as pH, oxygen tension (pO_2), thiol concentrations, proximity to heme enzymes, redox state, and the presence of reducing agents (e.g. ascorbic acid) [107]. The interplay among these dynamically changing elements *in vivo* no doubt contributes to the complexity of a seemingly simple chemical reaction. One factor that has primarily been of interest is the effect of the reducing agent ascorbic acid [16]. In its presence, NO is generated over a wide pH range with the simultaneous production of dehydroascorbic acid [108]. This antioxidant, known to enhance NO-mediated vasorelaxation, is suggested to elicit its NO-forming action by scavenging superoxide (which decomposes NO) [109-111], or stimulating eNOS activity [112]. In mammals, the nonenzymatic production of NO from nitrite occurs in the stomach, oral cavity, heart, skin, and urine [16].

C. S-nitrosothiol (SNO) formation

Another way of possibly transporting or storing NO through extracellular fluids and cells is by the nitrite-catalyzed formation of a bioactive species, S-nitrosothiols (SNO) [19, 20]. This is presumably formed when NO is oxidatively transferred (as NO^+) from Fe(III)-NO to cysteine residues, as in the case of the S-nitrosylation of Hb (SNO-Hb) in the presence of O_2. Although this has been thought of as the main mechanism behind SNO-Hb formation, new insight into this mechanism has been suggested [113], as discussed later. The crystal structure of SNO-Hb reveals the presence of a heme-bound NO and a nitrosylated β-Cys93(Cys-S-N=O) located in the heme pocket next to the proximal ligand His92 [114]. However, succeeding analyses on the crystal structure of SNO-Hb at 1.8 Å resolution by the same group revealed a sulfhydryl radical (Cys-S-N$^\bullet$-OH) instead of Cys-S-N=O [115]. The formation of Cys-S-N=O is thought to be due to the presence of electron acceptor species, including O_2 [115]. Without O_2, Cys-S-N$^\bullet$-OH is formed instead. The difference between the initially assumed and current crystal structure of SNO-Hb has important implications in the formation of such bioactive species.

Although the mechanism of SNO formation in Hb and Mb is not without controversy, a pathway for such formation has been proposed based on results from literature (Fig. 5). Here, deoxyHb initially reacts with NO_2^- to yield Fe(III)-NO. Because the Fe(III)-NO is labile [116], NO is readily released and reacts with β-Cys93 in Hb forming the unstable intermediate Cys-S-N$^\bullet$-OH. In the presence of an electron acceptor species such as O_2, the radical is oxidized, which then finally produces SNO-Fe(III). Another possible electron acceptor is ferric heme, where an electron

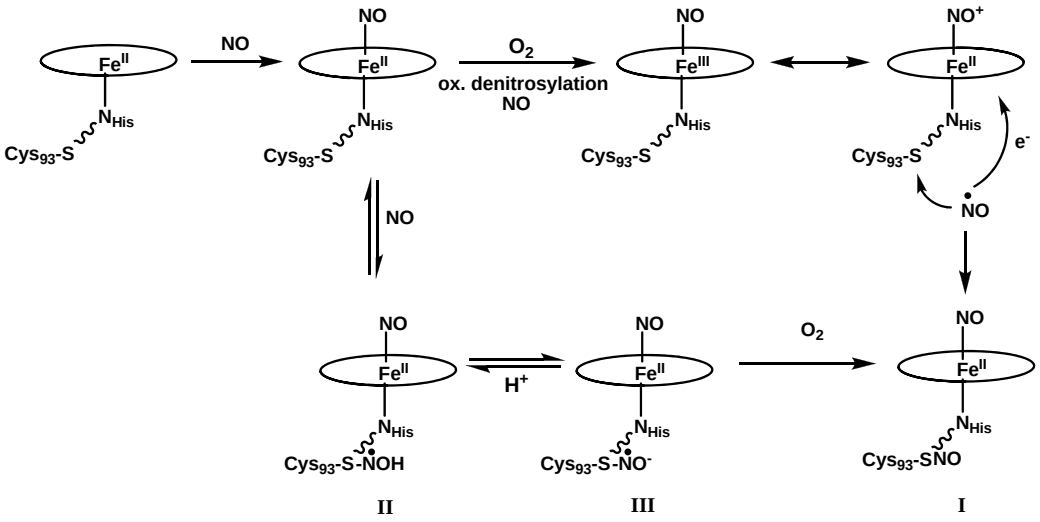

Fig. 5. Proposed mechanism for SNO formation in Hb (Species I). The crystal structural data shows a Cys93-SN•OH radical (Species II) that forms a thionitroxide radical (Species III) upon deprotonation.

may be transferred through the Cys93-His92 linkage reducing the iron ultimately forming SNO-Fe(II). Long-range electron transfer over an amino acid backbone is not uncommon, as it has been observed in other enzymes [117]. The eventual release of NO from SNO-Hb occurs through the "capture" of this diatomic molecule by cysteines of other polypeptides and enzymes such as glutathione and the red blood cell (RBC) protein Anion Exchanger 1 (AE1) [118]. This capture initiates an ATP-dependent pathway that exports nitrosylated glutathione from red blood cells or transports nitrosylated AE1 across the cell membrane [119]. The SNO mechanism is nicely positioned to justify the fact that free NO (formed from nitrite reductase activites of Hb and Mb) is not generated because it is readily scavenged in the RBC. However, a few research groups have suggested that SNO-Hb is not required for hypoxic vasodilation [120].

Nitric oxide generation (artificial)

The diverse physiological and anti-pathological uses of NO (*vide supra*) have prompted researchers in the field to develop drugs designed to effectively deliver NO at biological targets. Several methods for incorporating NO-releasing compounds in polymer matrices have been explored, including wound dressings and patches [121]. More recently, approaches involving NO delivery to selected targets "on demand" have been vastly explored. One strategy is to employ a compound that exhibits low thermal reactivity but releases NO upon controlled light exposure. In this section, different nitrosyl-metal complexes that have the potential to act in drug-delivery will be presented. Because of the wealth of information on inorganic and model complexes that bind and release NO, emphasis will be given on complexes that have historical and biological relevance, with particular importance on nitric oxide photo-release. These synthetic compounds include iron, ruthenium, manganese, and chromium complexes, which are among the more common ones studied.

A. Fe complexes

The significance of heme enzymes in the generation and detoxification of NO is readily apparent (*vide supra*). This is primarily why researchers in the field have attempted to synthesize and structurally and spectroscopically characterize heme model complexes in different oxidation states and coordination number [116, 122-128]. In addition to the simple iron heme compounds, non-heme mimics [129, 130] and more complicated model systems have been synthesized which include tailed [131], picket-fence [132], co-facial [133], and covalently-linked-to-non-heme porphyrins [134].

I. Heme models

One of the most common physiological targets of NO for bioregulation is soluble guanylate cyclase (sGC). The formation of a five-coordinate (5C) heme Fe(II)-NO in this biomolecule results in conformational changes that activate the 3,5-cyclic guanosine monophosphate (cGMP) and induces diverse physiological responses. The interest in this chemistry has led to the synthesis of a host of Fe(II)-NO model complexes such as those listed in Table 2. The crystal structure geometries, vibrational frequencies, and reactions leading to the formation ("on" reaction) and decay ("off" reaction) of these iron-nitrosyl compounds have intensely been studied for many of these systems. In general, 5C Fe(II)-NO heme compounds are extremely stable due to the high association constants (K_{eq}) of NO for ferrous heme, which range in the order of 10^{10}-10^{12} [113]. These complexes therefore make them ideal in studying the inherent stability of Fe(II)-NO in different biomolecules. However, care must be taken when comparing the stabilities of Fe(II)-NO in model complexes versus enzymes since the NO binding constant of the latter is influenced by second sphere coordination effects such as interactions with amino acid side chains, hydrogen bonding, and redox potential of the heme [113]. Aside from ferrous heme nitrosyl complexes, analogous compounds in the Fe(III) form have also been synthesized [125]. Compared to Fe(II)-NO, ferric heme nitrosyl compounds lose NO more readily due to its large dissociation rate constant as a result of the low thermodynamic stability of the Fe(III)-NO bond [116]. The difference in electronic structure between the ferrous and ferric forms of heme iron-nitrosyl compounds allow for the modulation of the Fe-NO bond stability. Table 2 shows the vibrational and kinetic parameters of select ferrous and ferric heme nitrosyl model complexes.

Aside from 5C iron-nitrosyl complexes, a number of six-coordinate (6C) iron nitrosyl compounds with an axial N-donor coordination have been synthesized and spectroscopically characterized. Examples of N-donor ligands that have been used to model 6C NO-bound heme enzymes such as myoglobin, hemoglobin, sGC and bacterial nitrite reductase are 1-methylimidazole [135], 4-methylpiperidine [136], and 4-(dimethylamino) pyridine [137]. While 6C iron nitrosyl complexes with N-coordination are commonly and routinely synthesized, 6C iron-nitrosyls with thiolate coordination, particularly in the ferrous form, are rare. Vibrational and electronic structural information on these types of complexes are normally derived from enzymes such as Cyt P450cam [138]. A more detailed summary of the crystal structure geometries, vibrational and

Table 2. Vibrational frequencies and binding constants of NO in select five-coordinate [$Fe^{II/III}$(Porphyrin)(NO)] and six-coordinate [$Fe^{II/III}$(Porphyrin)(L)(NO)] (L = proximal ligand) iron-nitrosyl complexes.

Model complexes[a]	Vibrational frequencies (cm^{-1})			Binding constants			Ref.
	ν(N-O)	ν(Fe-NO)	δ_{ip} (Fe-N-O)[b]	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	T (°C)	
[Fe^{II}(OEP)(NO)]	1671	522	388				[122]
[Fe^{II}(TPP)(NO)]	1697	371	340	5.2×10^9		RT	[123, 124]
[Fe^{II}(TPPS)(NO)]				1.5×10^9	6.4×10^{-4}	25	[125]
[Fe^{III}(TPPS)(NO)]				0.5×10^6	0.5×10^3	25	[125]
[Fe^{III}(TMPS)(NO)]				2.8×10^6	0.9×10^3	25	[125]
[Fe^{II}(TPP)(MI)(NO)]	1630	437	563				[126]
[Fe^{II}(PPDME)(MI)(NO)]	1618						[127]
[Fe^{III}(OEP)(Pz)(NO)]$^+$	1894						[128]
[Fe^{III}(TPP)(MI)(NO)](BF_4)	1896	580	587				[116]

[a]OEP = octaethylporphyrin^{2-}, TPP = tetraphenylporphyrin^{2-}, TPPS = tetrakis(4-sulfonatophenyl)porphyrin^{2-}, TMPS = tetrakis(3,5-disulfonatomesityl)porphyrin^{2-}, MI = 1-methylimidazole, PPDME = protoporphyrin IX dimethylester^{2-}, Pz = pyrazole; [b]δ_{ip} = in-plane bending mode.

electronic properties of different 6C iron nitrosyl complexes is presented in Ref. [113].

II. Non-heme models

Iron-containing model complexes that are devoid of any porphyrin backbone in its structure have also been synthesized and utilized as a source to photochemically delivery NO to biological targets. For example, Roussin's black ($Fe_4S_3(NO)_7^-$) and red ($Fe_4S_3(NO)_4^{2-}$) salts are highly colored complexes that absorb in the red spectral region, making them appealing for use in biological tissues (*vide infra*) [139]. Upon photolysis of the black salt in buffer solutions, NO is released [140]. The propensity of the black salt to release NO has also been tested in vascular and brain tissues [141]. The red salt, on the other hand, is primarily used as a synthetic precursor to form more functional products, such as the so-called "red salt esters", $Fe_2(\mu\text{-}SR)_2(NO)_4$ [142]. A more in-depth discussion on the photochemical and photophysical properties of Roussin's salts and their derivatives are available in Ref. [139].

While iron-containing inorganic compounds such as Roussin's salt are recognized to photochemically deliver NO, non-heme complexes that model the active site of enzymes that are known to naturally bind NO (and release it upon photolysis) have also been studied. Iron nitrile hydratase, for example, is an enzyme that is modulated by NO. Its inactive NO-bound form is activated when exposed to light, wherein NO is released from the iron center. While researchers have made mimics of the NO-bound active site of nitrile hydratase [143, 144], only Mascharak and co-workers [129] successfully synthesized a compound from a "precursor", $(Et_4N)[(Cl_2PhPepS)Fe(NO)(DMAP)]$ ($Cl_2PhPepSH_4$ = 4,5-dichloro-*N*,*N*'-phenylenebis (*o*-mercaptobenzamide, DMAP = *N*,*N*-dimethylaminopyridine), that nicely mimics its active site, to one that releases NO upon illumination of visible light, $(Et_4N)[(Cl_2PhPep\{SO_2\}_2)Fe(NO)(DMAP)]$. Density functional studies indicate that the propensity of NO photo-release is related to the oxygenation of S in the ligand [129]. Future work on related mimics may well need to take this key "chemical switch" feature into consideration when employing photochemical techniques.

B. Ru complexes

The major benefit of utilizing ruthenium (Ru) complexes as an NO donor is that they are more stable than other metal nitrosyl compounds, yet they exhibit a degree of NO photolability upon exposure to UV-visible light, making them a prime class of compounds for photodynamic therapy (PDT) [145]. In addition, Ru-NO complexes can be embedded in polymeric material due to their inherent thermal stability [146]. The first several synthesized Ru-NO compounds are shown to release NO upon UV light exposure [147, 148]. However, more efforts towards constructing complexes that are photosensitive by visible light have been attempted in order to avoid the harmful effects of UV light on biological tissues. The key electronic transition that causes NO photolability is $d_\pi(Ru) \rightarrow \pi^*(NO)$, which can be modulated by either altering the ligand structural frame or directly ligating a chromophore to the ruthenium center. Years of research in the field have led to synthetic compounds that exhibit greater sensitization in the visible region when the light-harvesting chromophore is *directly* coordinated to the ruthenium center, as opposed to it being appended via alkyl chains [149]. Of particular interest is the complex $[(Me_2bpb)Ru(NO)Resf]$ (bpb = *N*,*N*'-bis (pyridine-2-carboxamido)-1,2-diaminobenzene) (Fig. 6) that induces apoptosis in human breast cancer cells upon NO release under the influence

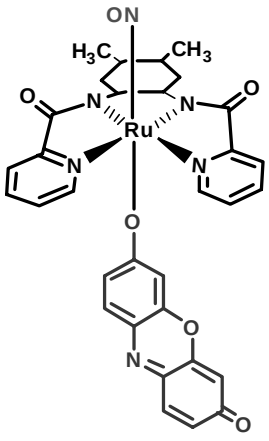

Fig. 6. Structure of the Ru-NO complex $[(Me_2bpb)Ru(NO)Resf]$ (bpb = *N*,*N*'-bis(pyridine-2-carboxamido)-1,2-diaminobenzene), showing the resofurin (Resf) ligand bound trans to NO.

of visible light [149]. Select ruthenium complexes that are photosensitive in the visible region are presented in Table 3.

The pharmacological potential of Ru complexes goes beyond fine-tuning the $d_{\pi}(Ru) \rightarrow \pi^*(NO)$ transition in solution. To effectively and efficiently deliver NO through PDT, the Ru-NO complexes must be stable in the absence of light but readily released in its presence while being incorporated in non-toxic polymer matrix that may serve as a patch, wound dressing, or coatings. The different materials that have been used to entrap or immobilize Ru-NO compounds while demonstrating NO release upon UV/visible light exposure are silica-based xerogel [150], silica matrix with functionalized silanes [151], and covalent attachment of an Ru-NO complex through copolymerization via the ligand [152].

C. Mn complexes

Manganese-nitrosyl complexes are equally interesting compounds to study since the Mn-NO bond in some compounds is readily photolabile and can therefore potentially deliver NO under controlled conditions to specific targets. The interest in Mn-containing porphyrins and its potential for NO delivery was first initiated by Suslick and Watson [153]. They demonstrate that photolysis of the *O*-nitrite bound Mn^{III}(TPP)(ONO) complex (TPP = tetraphenylporphyrin^{2-}) led to high-valent Mn^{IV}(TPP)=O (quantum yield: 5.30×10^{-4}) and NO formation. Their results are further strengthened by photolysis studies on Cr^{III}(TPP)(ONO) that produced Cr^{IV}(TPP)=O and NO [154] offering an alternative pathway for NO production via these classes of metal complexes according to this generic reaction: M-O-NO $\rightarrow$ M=O + NO where M represents a metal center. Interestingly, Ru nitrito complexes of similar nature does not follow this route, but instead display cleavage of the Ru-ONO bond to generate nitrogen dioxide [155]. Ensuing flash photolysis studies on Mn^{III}(TPP)(ONO) show that instead of forming the highly oxidizing Mn^{IV}(TPP)=O species, the prevailing products are Mn^{II}(TPP) (quantum yield: 0.045) and NO_2 [156].

Aside from Mn-containing porphyrins, non-heme Mn-nitrosyl complexes have also been synthesized and photochemically studied. Two Mn-NO complexes which will be the focus of the remaining section are $[Mn(PaPy_3)(NO)]ClO_4$ ($PaPy_3^-$ = *N*,*N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide) and $[Mn(PaPy_2Q)(NO)]ClO_4$ ($PaPy_2Q^-$ = *N*,*N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-quinoline-2-carboxamide) whose optimized structures and absorption spectra are shown in Fig. 7 [157]. These compounds are particularly attractive because they demonstrate remarkable Mn-NO photolability with low energy visible

Table 3. Quantum yields (ϕ) and visible absorption spectra (λ_{max}) of different Ru-NO [149] and Mn-NO complexes [157].

Complex[a]	Quantum yield[b] (λ_{max}, nm)	λ_{max}, nm (ε, M^{-1} cm^{-1})
$[(Me_2bpb)Ru(NO)(Resf)$	0.052 ± 0.008 (500)	500 (11,920)
$[(Me_2bQb)Ru(NO)(Resf)$	0.102 ± 0.009 (500)	510 (12,300)
$[((OMe_2)bQb)Ru(NO)(Resf)$	0.206 ± 0.008 (500)	500 (27,100)
$[(Me_2bpb)Ru(NO)(OH)$	0.0008 ± 0.0002 (500)	390 (5,100)
$[(Me_2bQb)Ru(NO)(OH)$	0.010 ± 0.003 (500)	445 (3,130)
$[((OMe_2)bQb)Ru(NO)(OH)$	0.025 ± 0.003 (500)	490 (4,060)
$[Mn(PaPy_3)(NO)]ClO_4$	0.326 ± 0.010 (550)	550
$[Mn(PaPy_2Q)(NO)]ClO_4$	0.579 ± 0.010 (550)	550

[a]Me = methyl, OMe = methoxy, byb = *N*,*N*'-bis(*y*-2-carboxamido)-1,2-diaminobenzene where '*y*' may be "p" (pyridine) or "Q" (quinoline), Resf = 7-hydroxy-3H-phenoxazin-3-one, $PaPy_3^-$ = *N*,*N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide), $PaPy_2Q^-$ = *N*,*N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-quinoline-2-carboxamide); [b]quantum yield measurements were taken in dimethylformamide for the ruthenium complexes and in water for the manganese complexes.

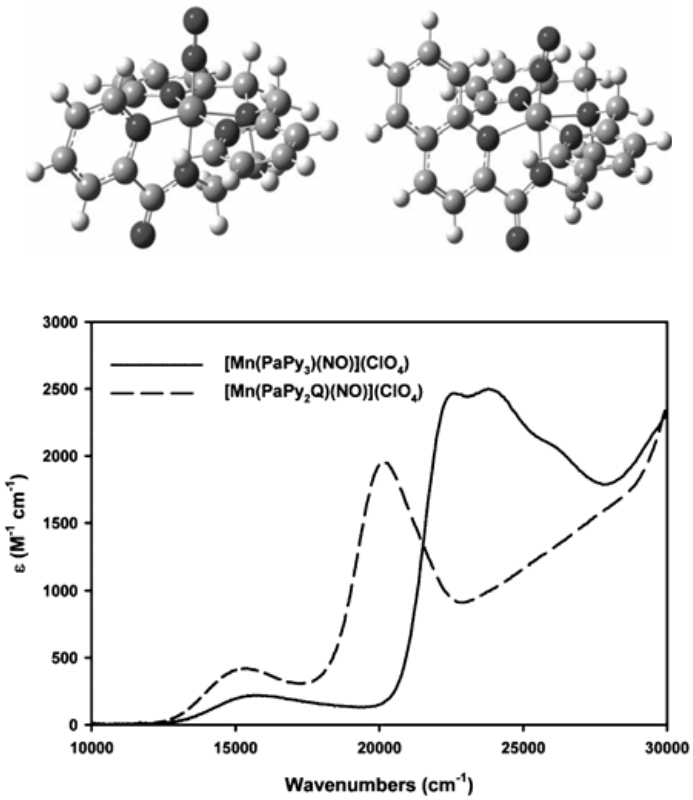

Fig. 7. Top figure: Fully optimized structures of $[Mn(PaPy_3)(NO)]^+$ (top left) and $[Mn(PaPy_2Q)(NO)]^+$ (top right) obtained with basis set and functional BP86/TZVP. Bottom plot: Electron absorption spectra of the Mn complexes obtained in acetonitrile at room temperature. (Reprinted with permission from Merkle, A. C., Fry, N. L., Mascharak, P. K., and Lehnert, N. Inorg. Chem., 50, 12192 Copyright (2011) American Chemical Society).

($[Mn(PaPy_3)(NO)]ClO_4$) and near-IR light ($[Mn(PaPy_2Q)(NO)]ClO_4$), avoiding the harmful effects of UV radiation on biological tissues. Spectroscopic studies (IR, FT Raman and UV-visible spectroscopies) and theoretical computations provide insight into the photolability of these Mn-nitrosyl complexes at low energy [158]. Upon irradiation in the vis-NIR region, the $d_{xy} \rightarrow L(Py/Q\_\pi^*)$ charge transfer (CT) transition between Mn and the co-ligand is populated, followed by the interconversion into the $d_{xy} \rightarrow \pi^*\_d_\pi$ singlet states. The $d_{xy} \rightarrow \pi^*\_d_\pi$ triplet states, that are found in very low energy, show strong spin-orbit coupling with the equivalent singlet state, thereby promoting intersystem crossing. Since these states are Mn-NO antibonding in nature, NO is easily dissociated upon exposure to low energy light. These results provide insight into tuning the structural properties of metal-nitrosyl complexes, in particular the co-ligand, in order to release NO when exposed to visible-NIR light.

Beyond the photolability of these Mn-nitrosyl complexes in solution is its applicability to effectively deliver proper doses of NO in sites of bacterial infection. For this purpose, $[Mn(PaPy_3)(NO)]ClO_4$ has been incorporated in biocompatible matrices that readily release NO when exposed to visible light [159]. The most recent material that has been used to incorporate this complex and provides the long-term stability and ability to release NO is made from bio-grade polyurethane blended with powdered silica xero-gel [159]. This exhibits antibiotic effects against a broad range of bacteria, including *P. aureginosa*, *A baumannii*, *E. coli*, and *S. aureus*. A significant advantage of this particular material over similar systems is that NO can be released with proper control of light intensity or duration.

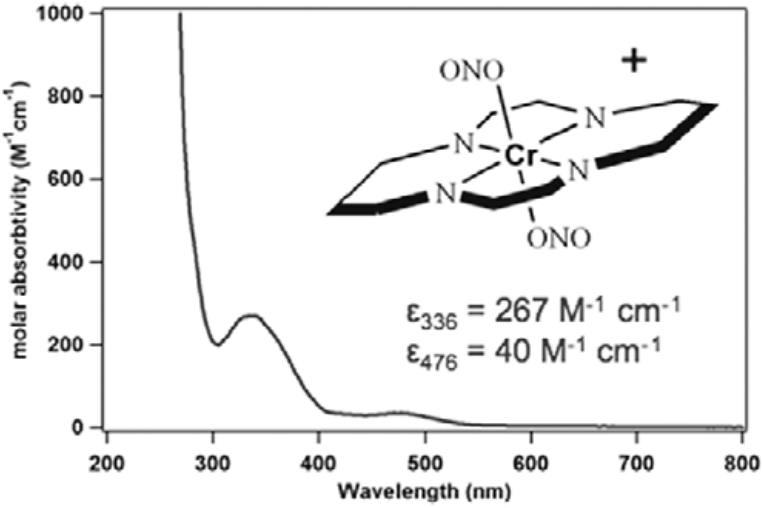

Fig. 8. UV-visible absorption spectrum of *trans*-Cr(cyclam)$(ONO)_2[BF_4]$ in aqueous solution. (Reprinted with permission from Ostrowski, A. D., Absalonson, R. O., De Leo, M., Wu, G., Pavlovich, J. G., Adamson, J., Azhar, B., Iretskii, A. V., Megson, I. L., and Ford, P. C. Inorg. Chem., 50, 4453. Copyright (2011) American Chemical Society).

D. Cr complexes

Chromium metal is unique in a sense that it is considered an essential micronutrient, yet no human enzyme with this metal co-factor is known. This has not prevented researchers from synthesizing chromium complexes and utilizing their desirable features for biomedical applications. For example, Cr(III)TPP-ONO (TPP = tetraphenylporphyrin^{2-} and ONO = nitrite) is shown to be a photochemical NO precursor when nitrite is *O*-coordinated to the metal [156]. Chromium complexes beyond the metalloporphyrin backbone have also been investigated, one of which is the Cr(III) macrocyclic polyamine complex *trans*-Cr(cyclam)$(ONO)_2^+$ (cyclam = 1,4,8,11-tetraazacyclotetradecane) [160, 161]. The UV-visible absorption spectrum of this complex is shown in Fig. 8. Interestingly, irradiation of aerated aqueous solutions of this compound at various wavelengths (365 to 546 nm) produce Cr(IV)(cyclam)$(O)(ONO)^+$ upon which NO is generated. The phototoxicity of this compound was tested against human monocytic tumor cell lines. However, the NO photogenerated from the chromium complex did not induce significant toxicity, despite using high concentrations of the compound (1000 μM) [161]. Ongoing studies on this area involve preparing chromium complexes with strongly absorbing chromophores that sensitize the release of NO at longer wavelengths.

CONCLUSION

Several pathways for nitric oxide production are physiologically accessible. In addition to the major route of NO generation via NOS and L-Arg, mechanistic pathways such as nitrite reduction and SNO formation are available. The preferential method of NO production lies in the location where this diatomic molecule is most needed, as well as the immediate external conditions in which the cell is subject to. Nitrite, an abundant repository of NO, can undergo reduction via enzymatic systems such as myoglobin, hemoglobin, nitric oxide synthases, xanthine oxidase, carbonic anhydrase, and mitochondrial enzymes; or non-enzymatically such as the case in acidic disproportionation. Although the complete mechanistic pathway of some of these systems has yet to be elucidated, the method of how NO is generated largely depends on external factors such as pH, O_2 levels, amino acid residues in the active site, and oxidation of the active metal center, among others. In addition to the levels of NO produced by each system, the bioavailability of NO is also critical because this diatomic molecule can readily be quenched by heme enzymes even before it reaches essential cellular regions. Mechanisms such as nitrite anhydrase and/or SNO formation in Hb have been proposed as plausible pathways for effectively delivering NO, however, these processes are not without controversies.

The active site of enzymes that generate NO has been used as a template for constructing model complexes that deliver this diatomic molecule in focused areas upon controlled light exposure. Biomimetic compounds presented in this review include complexes that contain Fe, Ru, Mn, and Cr as the metal center and catalytic site. Research work in this area has meticulously gone from synthesizing compounds that release NO in the UV-visible region to those that effectively deliver NO in the visible-NIR range, an important feat when considering future applications in drug delivery. Beyond the photolability of solution metal-NO complexes in the visible-NIR region is their applicability in biocompatible matrices. Materials that can incorporate photolabile metal-NO complexes should provide long-term stability while simultaneously being able to release NO "on demand" and have antibiotic effects against a range of bacteria and microbes. A few of these applicable matrices include polyurethane blended with powdered silica xero-gel. Future work in this field will involve employing safe, commercially-available and cheap exogenous NO donors in ointments or patches that can be topically administered to an infected area.

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