Published in final edited form as:

Inorg Chem. 2010 July 19; 49(14): 6253–6266. doi:10.1021/ic902423v.

Linkage Isomerization in Heme–NO_x Compounds: Understanding NO, Nitrite, and Hyponitrite Interactions with Iron Porphyrins

Nan Xu, Jun Yi, and George B. Richter-Addo

Department of Chemistry and Biochemistry, University of Oklahoma, 620 Parrington Oval, Norman, OK 73019

George B. Richter-Addo: grichteraddo@ou.edu

Abstract

Nitric oxide (NO) and its derivatives such as nitrite and hyponitrite are biologically important species of relevance to human health. Much of their physiological relevance stems from their interactions with the iron centers in heme proteins. The chemical reactivities displayed by the heme–NO_x species (NO_x = NO, nitrite, hyponitrite) are a function of the binding modes of the NO_x ligands. Hence, an understanding of the types of binding modes extant in heme–NO_x compounds is important if we are to unravel the inherent chemical properties of these NO_x metabolites. In this Forum article, the experimentally characterized linkage isomers of heme–NO_x models and proteins are presented and reviewed. Nitrosyl linkage isomers of synthetic Fe and Ru porphyrins have been generated by photolysis at low temperatures and characterized by spectroscopy and DFT calculations. Nitrite linkage isomers in synthetic metalloporphyrin derivatives have been generated from photolysis experiments and in low-temperature matrices. In the case of nitrite adducts of heme proteins, both *N*-binding and *O*-binding have been determined crystallographically, and the role of the distal Hbonding residue in myoglobin in directing the *O*-binding mode of nitrite has been explored using mutagenesis. To date, only one synthetic metalloporphyrin complex containing a hyponitrite ligand (displaying an *O*-binding mode) has been characterized by crystallography. This is contrasted with other hyponitrite binding modes experimentally determined for coordination compounds and computationally for NO reductase enzymes. Although linkage isomerism in heme–NO_x derivatives is still in its infancy, opportunities now exist for a detailed exploration of the existence and stabilities of the metastable states in both heme models and heme proteins.

Introduction

Nitric oxide (NO) is a well-known biological signaling agent. The wellcharacterized biological receptor for NO is the heme-containing enzyme soluble guanylyl cyclase. NO is now known to bind to many heme proteins; some these interactions are physiologically relevant, and some are not.¹ NO may be oxidized to nitrite under some physiological aerobic conditions, and nitrite can be reduced to NO under some conditions. The hyponitrite dianion

is formed during detoxification of NO by some bimetallic metalloenzymes. Indeed, it is becoming more widely accepted that various forms of nitrogen oxides (NO_x) may coexist under normal physiological conditions. It is reasonable to assume that the chemical reactions of various heme-NO_x species (NO_x = NO, nitrite, hyponitrite) are directly related to the NO_x binding modes in the reactive species. In this Forum article, we present and review the evidence for the existence of metastable linkage isomers and stable alternate coordination geometries of these heme- NO_x species.

Nitrosyl Linkage Isomers

The statistical approach of NO to iron porphyrins could, in principle, involve many forms, as NO can rotate as it approaches the metal center. Three limiting forms are sketched in Figure 1. The approach of NO using its N-atom at the time of attachment to the metal should, in principle, generate the metal–NO (i.e., “nitrosyl”) moiety, and this could either display linear or bent metal–N–O geometries (top of Figure 2). These nitrosyl forms are generally regarded as the ground-state forms of most transition metal–NO compounds.² An approach of NO using its O-atom at the time of attachment to the metal (middle of Figure 1) should generate the metal–ON (i.e., “isonitrosyl”) moiety as shown at the bottom left of Figure 2. Coppens and coworkers have provided unambiguous crystal structural data for the existence of such isonitrosyl species formed during excitation of metal–NO coordination compounds.^{3–5} Third, a side-on “belly flop” approach by NO should, in principle, generate the metal-(*ee*gr;²-NO) moiety displaying a side-on attachment of the NO group to the metal center as shown on the bottom right of Figure 2. Such a binding mode was demonstrated by Coppens for a metastable state of sodium nitroprusside³ and for a metastable state of CpNi(NO) (Cp = *ee*gr;⁵-cyclopentadienyl anion).⁶ This side-on NO geometry has also been determined in X-ray crystal structures of the NO adducts of some copper nitrite reductases.^{7–9} In this article, we will use the Enemark-Feltham {MNO}ⁿ notation to help describe and classify the metal–NO compounds.^{10,11}

Our interest in the linkage isomerization in metal–NO compounds began through a discussion between one of us (GBRA) and Philip Coppens at a Poster Session at the 215th National Meeting of the American Chemical Society in Dallas, Texas, in April 1998. As a result of this discussion on the possible existence of nitrosyl linkage isomers in nitrosyl metalloporphyrins, we embarked on a three-way collaboration together with Kimberly Bagley (the team is referred to as the RA-C-B team in this section) to explore the possible generation of such metal–NO linkage isomers in heme models. Our approach was essentially the counterpart to Figure 1, namely the photoexcitation of stable metal–NO (i.e., nitrosyl) porphyrins to generate alternate binding modes of the NO ligand.

Ruthenium Porphyrins

Our team’s first attempts to generate nitrosyl linkage isomers in metalloporphyrin derivatives involved the use of ruthenium porphyrins.¹² The reasons for choosing Ru were two-fold. First, we needed to prepare stable and pure (as judged by elemental analysis) six-coordinate mononitrosyl porphyrins. Second, the {RuNO}⁶ class of compounds was a good choice for modeling related group 8 and biologically relevant {FeNO}⁶ congeners. With these in mind, our RA-C-P team examined four {RuNO}⁶ derivatives that differed in *trans*

axial ligation, namely (OEP)Ru(NO)(O-*i*-C₅H₁₁), (OEP)Ru(NO)(SCH₂CF₃), (OEP)Ru(NO)Cl, and [(OEP)Ru(NO)(py)]⁺ (OEP = octaethylporphyrinato dianion). Correlated photolysis-IR spectroscopy experiments were then performed.

Irradiation of these compounds (330 < λ < 460 nm; Xe lamp) as KBr pellets at 20 K for 15 min produces changes in IR absorptions discernable from analysis of the difference IR spectra. For example, the difference IR spectra obtained after 15 min of photolysis of (OEP)Ru(NO)(O-*i*-C₅H₁₁) is shown in Figure 3 (bottom trace); the initial ν NO band at 1791 cm⁻¹ decreases and new bands at 1645 cm⁻¹ and 1497 cm⁻¹ become evident.¹² The IR difference spectra for the ¹⁵NO-labeled analogue (OEP)Ru(¹⁵NO)(O-*i*-C₅H₁₁) was also obtained (top trace of Figure 3).

The IR data for both the alkoxide (OEP)Ru(NO)(O-*i*-C₅H₁₁) and the thiolate (OEP)Ru(NO)(SCH₂CF₃) compounds are collected in Table 1. Analysis of the IR spectral changes suggested that photolysis of these compounds generated metastable η^1 -O and η^2 -NO linkage isomers as shown in Figure 4 in low observed yields (1–1.5 %).¹² This conclusion of photoinduced isomerization of the RuNO fragment to the linkage isomers was supported by the following: (i) photolysis at 20 K did not produce free NO (ν NO 1880 cm⁻¹), (ii) the photogenerated bands were 15N-shifted in the Ru-¹⁵NO derivatives and thus associated with ν NO, (iii) warming the photolyzed samples back to room temperature restored the parent nitrosyl bands, indicating that the photoinduced reaction was thermally reversible and that the photoproducts were indeed metastable species, (iv) the ν NO spectral shifts were similar to those recorded for other metastable nitrosyl linkage isomers in coordination compounds of Fe, Ru, Os, and Ni.

Further support for the side-on η^2 -NO linkage isomer in the seven-coordinate Ru porphyrin was provided by a DFT calculation by Ghosh (using the PW91 exchange-correlation functional and triple- ζ plus polarization Slater-type basis sets) on an unsubstituted porphine model for the experimentally observed¹² isomer formed during photolysis of (OEP)Ru(NO)Cl. He determined that the Ru(η^2 -NO) linkage is unsymmetrical (Figure 5)¹³ and that this isomer lies 1.23 eV (28.4 kcal/mol) in energy above the ground state.¹⁴ The DFT calculations revealed a HOMO-4 orbital that involves efficient π -bonding between the Ru d_{xz} orbital and the η^2 -NO π^* orbital (if the xz plane is defined as the Ru(η^2 -NO) plane).

In addition, however, the HOMO-3 orbital revealed a preferential π -bonding interaction between the Ru d_{yz} orbital with the N end of the NO π^* -orbital, leading to the rather unsymmetrical Ru(η^2 -NO) geometry.

Importantly, the observed downshifts (by 3–5 cm⁻¹) in some porphyrin skeletal absorption bands upon photolysis of these {RuNO}⁶ porphyrins indicated a reduction of π -acid character of the NO ligand in the metastable states.¹² We will return to this variation in π -acidity of the NO ligand later.

Iron Porphyrins

Given our success at generating metastable nitrosyl linkage isomers of {RuNO}⁶ porphyrins, our RA-C-B team explored the possibility that such linkage isomers may exist

for the biologically relevant FeNO systems. We focused initially on the known {FeNO}⁷ five-coordinate (por)Fe(NO) class of compounds for proof of concept, as (i) many of these were known to be thermally stable in the ground state, and (ii) these compounds could be obtained in elementally pure form.¹

Photolysis of the (TTP)Fe(NO) compound (TTP = tetratolylporphyrinato dianion) as a KBr pellet ($350 < \lambda < 550$ nm; Xe lamp) at 25 K for 5–10 min results in the generation of a new band in the IR spectra assigned to the metastable η^1 -O isonitrosyl derivative (Figure 6).¹⁵ Isotope labeling with ¹⁵NO and ¹⁵N¹⁸O confirmed that the new band was due to ν_{NO} .

Similar IR spectral shifts were observed with (OEP)Fe(NO), although in this latter case a second photoproduct was also generated. As with the Ru case, these new bands generated upon photolysis disappeared upon warming and the original ν_{NO} bands due to the ground state compounds were restored, although the yields of the photoproducts (2–3 % for (TTP)Fe(NO) and 6–10 % for (OEP)Fe(NO)) were higher than those obtained from photolysis of the six-coordinate Ru compounds described earlier. Additional evidence for the generation of the metastable isonitrosyl (TTP)Fe(η^1 -ON) was provided by DFT calculations on the model (porphine)Fe(NO) compound.¹⁵ The calculations on the ground-state (porphine)Fe(NO) compound reproduced the structural distortions of the N₄Fe(NO) core that had been demonstrated crystallographically by Scheidt and coworkers for (OEP)Fe(NO).^{16,17} For example, our DFT calculations showed that the Fe–N(O) vector was tilted towards the O-atom by 7.8° from the z-axis defined by the normal to the porphyrin N₄-plane (a similar tilt of 6–8° was determined experimentally for (OEP)Fe(NO)^{16,17}), and the NO group was staggered with respect to the Fe–N(por) bonds. Further, the pair of Fe–N(por) bonds closest to the bent FeNO moiety were calculated to be ~0.025 Å shorter than the other pair directed away from the bent FeNO group.

The DFT calculations revealed the existence of the metastable (porphine)Fe(η^1 -ON) linkage isomer that was 1.59 eV (36.7 kcal/mol) in energy above the ground state nitrosyl.¹⁵ The calculated geometries for the ground state (porphine)Fe(NO) and metastable (porphine)Fe(η^1 -ON) linkage isomers are shown in Figure 7, and selected structural data collected in Table 2.

As seen in Figure 7, the calculated structure of the metastable (porphine)Fe(η^1 -ON) isonitrosyl (bottom of the figure) closely resembles that of the ground state compound (top of the figure).¹⁵ Thus, the axial Fe–O(N) tilt and the non-equivalence of the two pairs of Fe–N(por) bond distances are properties of the isonitrosyl as well. The calculated axial Fe–ON tilt of 10.8° is larger than the 7.8° determined for the nitrosyl, and the apical displacement of the Fe atom from the porphine-N₄ plane is smaller. The relevance of the latter structural feature to the π -acid property of the isonitrosyl ligand may be related to an intrinsic reduction in π -acidity of NO when the FeNO moiety converts to the FeON isonitrosyl (see later).

Related DFT calculations (PW91 exchange-correlation functional) on (por)Fe(NO) and other nitrosyl metalloporphyrins of Co, Mn, and Rh have been reported by Ghosh and coworkers who also explored the existence and stabilities of isonitrosyl derivatives.^{14,18}

Ghosh^{13,18} and Coppens¹⁹ have provided additional insight into the axial Fe–N/O tilts in the ground state (porphine)Fe(NO) and metastable (porphine)Fe(η^1 -ON) compounds, respectively. In both cases, increased interaction between the NO π^* -orbital and a “tilted” Fe d_{z^2} orbital are favored when the axial Fe–N/O vector is tilted. Such “tilting” of the Fe d_{z^2} orbital has been shown to be particularly pronounced for related six-coordinate iron nitrosyl porphyrins.²⁰

Our RA-C-B team then explored the possibility that metastable isonitrosyl compounds could be generated in six-coordinate {FeNO}⁶ systems. For this, we chose to use Yoshimura’s nitrosyl-nitro compound (TPP)Fe(NO)(NO₂) (TPP = tetraphenylporphyrinato dianion) that could be obtained in an elementally pure state.²¹ The ground-state structure of (TPP)Fe(NO)(NO₂) possesses mutually trans η^1 -NO and η^1 -NO₂ ligands, as initially demonstrated by spectroscopy by Yoshimura²¹ and Fanning,²² and later by the crystallographic characterization of several (por)Fe(NO)(NO₂) derivatives by Scheidt and coworkers.²³ The crystal structure of the target compound (TPP)Fe(NO)(NO₂) unfortunately suffered from severe disorder thus limiting the usefulness of the metrical data. We note, however, that although the crystal structures of most of the crystal forms of (TpivPP)Fe(NO)(NO₂) (TpivPP = picket fence porphyrinato dianion) display linear FeNO geometries, that of (T(p-OMe)PP)Fe(NO)(NO₂) displays an Fe–N–O angle of 160°.²³

To further characterize these (por)Fe(NO)(NO₂) compounds, we performed DFT calculations on the parent (porphine)Fe(NO)(NO₂) model compound.^{24,25} The calculations confirmed that the η^1 -NO nitrosyl and η^1 -NO₂ nitro binding modes were present in the ground-state (GS) structure. The presence of a bent FeNO moiety in this formally {FeNO}⁶ compound was evident; the two FeNO angles of 156.4° (GS||) and 159.8° (GS⊥) corresponded to the two GS conformations with mutually parallel (||) and perpendicular (⊥) axial FeNO and FeNO₂ planes, respectively. Importantly, the calculated electron localization function (ELF) revealed a non-cylindrical electron pairing region on the nitrosyl N-atom (Figure 8). This result demonstrated, for the first time, a “ferrous {FeNO}⁷-like” electron pair localization in a formally “ferric” {FeNO}⁶ porphyrin system.

Photolysis of (TPP)Fe(NO)(NO₂) as a KBr pellet (330 < λ < 500 nm; Xe lamp) at low temperature resulted in several IR spectral shifts that could be rationalized by the transformations to generate both nitrosyl and nitrite linkage isomers shown in Figure 9. Irradiation of this compound at 11 K for 10 min produces shifts in the IR spectrum consistent with the transformation of the nitrosyl to an isonitrosyl Fe(η^1 -ON) group (ν_{NO} 1699 cm⁻¹) as well as an Fe(NO₂)-to-Fe(ONO) conversion. The identities of the bands were confirmed by ¹⁵N-labeling. The 1699 cm⁻¹ band was observable only at very low temperatures (e.g., temperatures < 50 K); warming the sample to 200 K results in the disappearance of this band. The new bands due to the FeONO group were, however, retained even at 200 K. We were unable to use the IR data obtained at 11 K to distinguish between a mixture of the singly isomerized species (i.e., MSa + MSb) and the doubly isomerized species (MSc). Thus, we employed DFT calculations to help provide

insight into the existence and stabilities of these single and double linkage isomers shown in Figure 9. In all, ten optimized structures were determined; two for the ground-state (GS|| and

GS $\perp$), three for the nitrosyl-nitrito (MSa $\parallel$, MSa $\perp$, and MSa $\perp$), two for the isonitrosyl-nitro (MSb $\parallel$ and MSb $\perp$), and three for the isonitrosyl-nitrito double-linkage isomers (MSc $\parallel$, MSc $\perp$, and MSc $\perp$). The “L” notation symbolizes those conformations with linear FeNO and FeON moieties; interestingly, these were calculated to be *higher* in energy than their bent counterparts in these formally {FeNO}6 systems. The calculated energies and sketches of representative examples of these linkage isomers are shown in Figure 10. The energies increase in the order GS $\ll$ nitrito (MSa) < isonitrosyl (MSb) < nitrito-isonitrosyl (MSc). The double linkage isomer MSc $\perp$ was determined to be the least stable, with an energy of 1.73 eV (40 kcal/mol) above the ground-state isomer GS $\parallel$.²⁴

The Fe–ON bonds in the isonitrosyl isomers (with calculated Mayer bond orders of 0.5–0.6) are longer than the Fe–NO bonds in the nitrosyl isomers (with calculated Mayer bond orders of 0.9–1.0). We attributed this feature to reduced back-donation into the antibonding π^* -orbital in the Fe(η^1 -ON) isonitrosyls. This analysis was consistent with the calculated smaller Fe apical displacement out of the porphyrin-N₄ plane in the isonitrosyls relative to that determined for the nitrosyls. Indeed, examination of the relevant MOs revealed that the shortening of the Fe–N(por) bonds in the isonitrosyls (when compared with the nitrosyls) was due to the increased π overlap in the Fe–N(por) bonds that accompanied the decrease in backbonding to the axial isonitrosyl group.

The results of time-dependent DFT calculations confirmed that both the nitrosylto-isonitrosyl and nitro-to-nitrito isomerizations could be induced by 300–500 nm light. Indeed, Ford and coworkers had previously shown that flash photolysis of (TPP)Fe(NO)(NO₂) in toluene solution at 298 K resulted in competitive dissociation of the axial NO and NO₂ ligands.²⁶ In our photolysis experiments with (TPP)Fe(NO)(NO₂) as a KBr pellet at low temperatures, however, both NO and NO₂ ligands were retained at the metal center in this medium.

Nitrite Linkage Isomers

The nitrite anion (NO₂[−]; pK_a 3.2 at 20 °C)²⁷ displays several coordination modes in its metal complexes.²⁸ Of relevance to this article are the three binding modes shown in Figure 11. We have already considered the nitro (metal-NO₂) and the nitrito (metal-ONO) forms in the previous section. The nitrite *O,O'*-bidentate binding mode has been determined crystallographically for the nitrite adducts of the copper-containing nitrite reductase (NiR) from *Alcaligenes xylosoxidans* (the His313Gly mutant),²⁹ the soil bacterium *Achromobacter cycloclastes*,⁸ and in synthetic copper complexes.³⁰ However, this *O,O'*-bidentate mode has not been determined for any metalloporphyrin or heme protein to date, hence will not be considered further.

Model Complexes

Our interest in nitrite coordination modes in heme models and heme proteins was to a large degree inspired by the evidence we obtained for low energy differences between the nitro Fe-NO₂ and nitrito Fe-ONO geometries during our photoinduced linkage isomerization experiments discussed above.^{24,25} In particular, the calculated (and rather small) energy difference of 4.3 kcal/mol between the GS $\parallel$ and MSa $\parallel$ isomers of (porphine)Fe(NO)(NO₂)

raised our interest in examining the factors that could determine the coordination geometry of nitrite in its metalloporphyrin complexes. The crystal structures of synthetic iron porphyrin nitrite complexes display, regardless of the iron oxidation state, the nitro *N*-binding mode.^{31–33} The only exception is that for the [(TpivPP)Fe(NO)(NO₂)][–] anion that exhibits a 40:60 disorder of the FeNO₂:FeONO binding modes in the same crystal.³⁴ It is reasonable to assume that the “distal pocket” provided by the –NH groups of the picket fence porphyrin probably assist in the stabilization of the FeONO isomer in this complex anion.

The nitro binding mode prevails in the case of cobalt porphyrin nitrite complexes as well.^{31,35,36} Demonstration of this prevalent *N*-binding mode can be obtained from the results of laser flash photolysis experiments performed on the five-coordinate (TPP)Co(NO₂) (Figure 12A).³⁷ Photolysis of this compound in benzene resulted in the formation of (TPP)Co^{II} (determined spectroscopically) via photodissociation of the NO₂ ligand. The photodissociated NO₂ ligand then recombined with (TPP)Co^{II} to give initially the metastable (TPP)Co(ONO) compound; a nitrito-to-nitro isomerization occurs to regenerate the thermally stable nitro (TPP)Co(NO₂) compound.

In contrast, the nitrite ligand is found to adopt a stable nitrito *O*-binding mode in the (TPP)Mn(ONO) compound.³⁸ Indeed, and in what appears to directly contrast the cobalt case just described, flash photolysis of the (TPP)Mn(ONO) complex in toluene results in the formation of (TPP)Mn^{II} (determined spectroscopically) via dissociation of the NO₂ ligand; recombination produces the intermediate nitro complex (TPP)Mn(NO₂) that then isomerizes to the stable nitrito species (TPP)Mn(ONO) (Figure 12B).³⁹ The stable nitrito *O*-binding mode is also the only one observed to date in the crystal structures of the nitrite adducts of synthetic metalloporphyrins of Ru^{40–43} and Os.⁴⁴

Such *O*-binding of nitrite to Fe porphyrins have been demonstrated spectroscopically by Ford and coworkers during the low-temperature reactions of NO₂ with sublimed layers of the four-coordinate (por)Fe compounds (por = TPP, TTP).⁴⁵ The reaction employing the TPP macrocycle is illustrated in Figure 13. In these reactions, the initially-generated five-coordinate nitrito (por)Fe(ONO) compounds react further with the sixth ligands (L = NO, NH₃) to give the six-coordinate (por)Fe(ONO)(L) intermediates that isomerize upon warming to their more stable nitro isomers.^{46–48} DFT calculations on the model five-coordinate (porphine)Fe(nitrite) compound (B3LYP functional; LACVP* basis set) predicted near-identical energies for the experimentally observed nitrito and the not-yet-observed nitro isomers.⁴⁶

An earlier DFT calculation (B3LYP functional) on the model compound (porphine)Fe(NO₂)(NH₃) revealed a thermodynamic preference for the nitro binding mode in the ferric form by over 10 kcal/mol.⁴⁹ Related DFT calculations (UBP86 functional) on the six-coordinate (porphine)Fe(NO₂)(ImdH) showed a preference, by 4.5 kcal/mol, for the nitro binding mode (6 kcal/mol in the ferrous form).⁵⁰ Similar results were obtained in a recent study, and the results show a preference, by 5–10 kcal/mol (depending on the basis sets used), for the nitro binding modes.⁵¹

Heme Proteins

The heme-nitrite interaction in proteins has a rather long history. In bacterial denitrification, the NiR enzymes convert nitrite to NO.^{52–56} Initial binding of nitrite to the heme iron in the active site is generally regarded as a requirement for the NiR activity of these proteins (eq 1).

Nitrite has also been used for many generations in the curing of meat.^{57–60} In addition to its antimicrobial and antioxidant activity, nitrite restores the pink color of meat due to the formation of the myoglobin (Mb) heme-NO pigment.⁶¹ However, nitrite can also be harmful to mammals, as it can oxidize ferrous hemoglobin (Hb) and increase the in vivo levels of ferric Hb to result in the blood disorder methemoglobinemia.^{62,63}

The relevance of nitrite to mammalian physiology is currently being actively studied, and a biannual international conference on The Role of Nitrite in Physiology, Pathophysiology and Therapeutics has been established.^{64,65} It is now known that several mammalian metalloenzymes will reduce nitrite to NO.^{66,67} The demonstration, by Hendgen-Cotta et al. that nitrite protects against myocardial infarction in Mb^{+/+} mice but not in Mb^{–/–} knockout mice implicates Mb as an in vivo NiR.⁶⁸ The chemistry of the heme-nitrite interactions relevant to mammalian physiology has been recently reviewed.⁴⁵

Despite the generally acknowledged medical importance of the nitrite interactions with the mammalian proteins Mb and Hb, we were surprised to find out that there was no crystal structural data for either the Mb-nitrite or Hb-nitrite adducts. The reported crystal structures for the nitrite adducts of the cytochrome *cd1* NiR from *Paraccoccus pantotrophus*,⁶⁹ the sulfite reductase hemoprotein from *Escherichia coli*,⁷⁰ and the cytochrome *c* NiR from *Wolinella succinogenes*⁴⁹ all revealed the *N*-binding of nitrite to the active site Fe centers (i.e., nitro binding mode) as shown in Figure 14. In all cases, the bound nitrite ligand engaged in more than one hydrogen-bonding interaction with distal pocket residues. Recently, the crystal structure of the nitrite adduct of the cytochrome *c* NiR Y218F mutant was reported; the bound nitrite retained its *N*-binding mode in this mutant that has a conserved Tyr residue mutated to Phe (Figure 14, bottom right).⁷¹

We mentioned earlier that despite the importance of the Mb-nitrite and Hb-nitrite interactions, there were no reported crystal structures of these compounds. We were successful at crystallizing the nitrite adduct of ferric horse heart Mb and determining its crystal structure at 1.20 Å resolution (top of Figure 15).⁷² The nitrite ligand in this formally Mb^{III}(ONO) complex binds to the ferric center through one of its O atoms, namely in the nitrito *O*-binding mode. At the time, this was the first report of *O*-binding of nitrite to any heme protein. The nitrito ligand was stabilized by H-bonding to the distal His64 residue using its O1 atom. Importantly, the same Fe-ONO geometry was obtained regardless of whether the complex was generated by soaking crystals of preformed metMb with nitrite, or by crystallizing pre-formed Mb-nitrite from solution. This suggested that the nitrito FeONO formation, with Fe in the *d*⁵ electronic configuration, was the ground-state geometry.

Various attempts to generate a ferrous Mb-nitrite complex for a crystal structural determination were unsuccessful. We thus proceeded to prepare and determine the structures of the related Mn^{III}-substituted (a d^4 system) and Co^{III}-substituted (a d^6 system) derivatives.

The 1.60 Å resolution crystal structure of the Mn^{III}-substituted Mb-nitrite complex is shown in the middle of Figure 15; the nitrite ligand is also *O*-bound in this derivative.⁷³ This is not too surprising, as the nitrite adduct of the synthetic compound (TPP)Mn(ONO) reveals this stable *O*-binding mode.³⁸ What was surprising to us, however, was the retention of the *O*-binding mode of nitrite in the d^6 Co^{III}-substituted derivative (bottom of Figure 15).⁷³ We noted earlier that the crystal structures of all reported nitrite adducts of Co-porphyrins displayed the *N*-binding mode of nitrite. This suggested to us that the distal His64 residue in Mb played a critical role in directing the nitrite ligand towards this *O*-binding mode in this d^6 Co^{III}-nitrite complex (formally valence isoelectronic with the Fe^{II}-nitrite compound).

We then examined the nitrite binding mode(s) in the nitrite adduct of ferric human Hb. The heme sites of the nitrite adduct of the ferric Hb, as determined from the 1.80 Å crystal structure, are shown in Figure 16. As is evident in the figure, the nitrite ligand also displays the *O*-binding mode to the iron centers in both the α and β subunits.⁷⁴ However, the FeONO conformations were found to differ between these two subunits. The FeONO conformation was *trans* in the α subunit (Fe–O–N–O torsion angle of 174°), but deviated from *trans* towards a distorted *cis*-like conformation in the β subunit (Fe–O–N–O torsion angle of –91°). The limiting *trans* and *cis* conformations are shown in Figure 17.

Our observation of the previously “rare” *O*-binding mode of nitrite in both Mb and Hb led us to further examine the role of the distal His64 (Mb numbering) residue in the active sites of these proteins. We hypothesized that it was this single H-bonding residue that directed the nitrite ligand towards the *O*-binding mode. If this were the case, then removing this H-bonding residue should allow the nitrite ligand to adopt its more “common” *N*-binding mode as observed in almost all ground-state synthetic models lacking distal pockets. For example, the H64V mutant of Mb would not have this distal H-bonding capacity, as sketched in the middle of Figure 18. To test this hypothesis, we prepared the nitrite adduct of the H64V mutant of Mb, crystallized it, and solved its 1.95 Å resolution crystal structure. The heme site of this H64V Mb-nitrite compound is shown at the top of Figure 19.⁷⁵ In this structure solution, the Fe–nitrite bond distance was unrestrained throughout the refinement, and the nitrite ligand was modeled in the *N*-binding mode at 65% occupancy. The lack of a distal His64V residue to stabilize the bound nitrite allows for a water channel to form from the exterior of the protein to contact the bound nitrite ligand. We speculate, based on the available data at this resolution, that the rather long Fe–NO₂ distance of 2.6 Å is probably due to a weak electrostatic interaction of the nitrite ligand with the ferric center within the hydrophobic pocket of this mutant.

Did the lack of a H-bonding residue in the distal pocket of Mb direct the nitrite ligand towards the *N*-binding mode? We further hypothesized that reintroduction of a H-bonding residue into the pocket, as would be extant in the H64V/V67R double mutant shown on the right of Figure 18 (where Arg has replaced the non-H-bonding Val67 residue), would permit the nitrite to again adopt the *O*-binding mode. The heme site of the nitrite adduct of the Mb

H64V/V67R is shown at the bottom of Figure 19.⁷⁵ In this structure, the nitrite ligand was modeled in the active site in the *O*-binding mode (as a distorted *cis*-FeONO conformation) at 65% occupancy, and it Hbonds to a water molecule near the surface of the protein.

Clearly, it is evident that the single H-bonding residue in Mb, namely the His64 residue, is important in determining the binding conformation of the nitrite ligand in Mb (and probably in Hb as well). The effects of these mutations on the NiR activities of these mutants were explored; the rates follow the order wt > H64V/V67R >> H64V, suggesting a significant role of the distal pocket H-bonding residue in the reduction of nitrite to NO.⁷⁵ The classic nitrite *N*-binding mode has been used to help understand nitrite reduction in bacterial denitrifying enzymes (namely a formal double protonation of a terminal O-atom initiates nitrite reduction), it has been calculated that the *O*-binding mode is also viable for nitrite reduction (a formal protonation of the O1 atom would result in the release of NO) for cytochrome *cd*₁⁵⁰ and for Hb.⁵¹ Both these pathways are shown schematically in Figure 20, and in the case of Hb presumes the involvement of the distal His residue as a proton donor.⁵¹

The crystallographic demonstration of both *N*-binding (previous) and *O*-binding (recent) of nitrite to heme proteins now raises new possibilities to study differential nitrite reduction by various heme proteins under different conditions. It should prove interesting to eventually determine which linkage isomers are operative under normal and abnormal physiological conditions.

Metal Hyponitrite Complexes

Hyponitrites can be considered as anionic derivatives of the NO dimer (Figure 21), hence it is appropriate to provide a brief discussion of the NO dimer.

NO dimer formation is enhanced at low temperature in condensed phases of NO,⁷⁶ and by its location in hydrophobic environments such as those encountered in single-walled carbon nanotubes⁷⁷ and aromatic environments.⁷⁸ The neutral NO dimer, (NO)₂, consists of two NO moieties with a weak N–N bond with a binding energy of ~2 kcal/mol.^{78,79} Results of several experimental and computational studies of the neutral dimer (NO)₂ indicate that the ground state *cis*-ONNO geometry is the most stable for this compound.⁸⁰ Fuster and coworkers have reexamined the bonding interactions between the NO moieties in the neutral dimer and mono- and dianionic redox partners using theoretical calculations.⁷⁹ They concluded that although the *cis*-ONNO isomer was favored for the neutral species, the *trans*-ONNO geometry was favored for both the reduced monoanion (NO)₂[–], and dianion (NO)₂^{2–} derivatives (the *cis* and *trans* forms of the dianion are sketched in Figure 22). Further, they calculated that the N–N bonds became shorter in the order (NO)₂ > (NO)₂[–] > (NO)₂^{2–} in both the *cis* and *trans* geometries; these results reaffirmed the earlier and similar report by Snis and Panas.⁸¹ Theoretical treatments^{82–84} and infrared experimental data⁸⁵ for the monoanionic hyponitrite radical have been reported. The increased strength of the ON–NO interaction in the anionic derivatives is consistent with the successful experimental isolation of several dianionic (NO)₂^{2–} salts, termed hyponitrite salts, that contain formal N=N double bonds.

The reaction of alkali metals with NO generates alkali metal hyponitrites. For example, the reaction of Na with NO in liquid ammonia produces a solid that was later identified by IR spectroscopy as *cis*-Na₂N₂O₂.⁸⁶ Andrews and coworkers have prepared several such hyponitrite salts from the reactions of laser-ablated metals with NO at 4–7 K in inert gas matrices.^{87–89} Sodium hyponitrite can also be prepared from the chemical reduction of sodium nitrite.⁹⁰ The reaction of N₂O with Na₂O generates the *cis*-Na₂N₂O₂ compound identified by X-ray structural analysis.^{91–93} Bohle and coworkers have prepared and characterized a series of organic soluble hyponitrite salts of the form [organic]²⁺[N₂O₂]^{2–} and determined their X-ray crystal structures; they also determined the crystal structure of *trans*-Na₂N₂O₂.⁹⁴

Coordination Compounds

A few transition metal hyponitrite compounds have been reported in the literature, and these are generally prepared by (i) the coupling of two NO molecules by metal complexes^{95,96} (ii) coupling of chemisorbed NO metal surfaces,^{97–99} (iii) attack of NO on a metal–NO group,^{100–102} and (iv) from the transfer of the hyponitrite moiety from an organic diazeniumdiolate to a metal.^{103,104} The binding modes of hyponitrites in transition metal complexes that have been structurally characterized by X-ray diffraction are shown in Figure 23. The *cis*-hyponitrite binding mode to a single metal (structure **A**) has been demonstrated for the complexes (PPh₃)₂Pt(N₂O₂)^{104,105} and (dppf)Ni(N₂O₂) (dppf = 1,1'-bid(diphenylphosphanyl)ferrocene).¹⁰³ A *cis*-hyponitrite *N,O*-binding mode (structure **B**) has been established for the bimetallic cation [(NH₃)₅Co(μ-N₂O₂)Co(NH₃)₅]⁴⁺.^{106,107} The structure **C** that contains a *trans*hyponitrite *N,O*-binding mode was determined for the Ru₂(CO)₄(μ-H)(μ-PBu^t₂)(μ-dppm)(μ-N₂O₂) product obtained from the reductive dimerization of NO by its bimetallic Ru precursor Ru₂(CO)₄(μ-H)(μ-PBu^t₂)(μ-dppm) (dppm = Ph₂PCH₂PPh₂).⁹⁵ The complex [(NO)₂Co(μ-NO₂)]₂(μ-N₂O₂), isolated as a minor product from the reaction of Co(CO)₃NO with NO, contains the tetradentate planar hyponitrite ligand sketched as structure **D**.¹⁰⁸

Heme and Heme Models

The reduction of two molecules of NO to N₂O is a formal two-electron process (eq 2) that can be carried out by the bimetallic active sites in

the heme-containing bacterial NO reductases (NORs).¹⁰⁹ In contrast, the fungal NORs contain a monometallic heme-thiolate active site.¹¹⁰ Lehnert and coworkers have provided an excellent theoretical treatment of NO reduction by the single metal center in a fungal NO reductase model.¹¹¹ Our emphasis in this article, however, is on NO reduction by the bimetallic NORs.

In the active sites of the bacterial NORs, the heme (referred to as heme *b*₃) is in close proximity to a non-heme iron center (referred to as Fe_B) and current evidence suggests that both the heme and non-heme iron centers play active roles in the reduction of NO to N₂O. A major drawback in firmly establishing the mechanism(s) for the reduction of NO by NORs

is that it has been difficult to identify intermediates along the NO reduction pathway. Three mechanisms are commonly discussed in the literature (Figure 24),¹⁰⁹ and all three involve the coupling of two NO molecules at a diferrous active site followed by the formation of a putative diferrous hyponitrite intermediate prior to the release of N₂O and generation of the resting oxo-bridged diferrous NOR (with an Fe-Fe separation of ~3.5 Å in *P.d. cNOR*).^{109,112} Moënné-Loccoz has provided a comprehensive treatise on the available experimental spectroscopic data that argue for one or more of these putative mechanisms shown in Figure 24.¹⁰⁹ The *cis-heme b₃* mechanism involves attack of a second NO molecule on a heme {FeNO}⁷ species to generate an asymmetrically-bridged hyponitrite ligand. In the case of the *cis-Fe_B* mechanism, a non-heme dinitrosyl iron complex (DNIC) forms prior to NO coupling and hyponitrite formation. The *trans* mechanism presumes formation of both heme and nonheme {FeNO}⁷ moieties that couple via N–N bond formation.

The NORs are evolutionarily related to the heme copper oxidases (HCOs) that also contain bimetallic active sites.^{113,114} In the case of HCOs, the heme (heme *a₃*) is in close proximity to a Cu site (rather than a non-heme Fe site as found in the NORs). Some prokaryotic HCOs such as the cytochromes *ba₃* and *caa₃* from *Thermus thermophilus*, and the cytochrome *cbb₃* from *Paracoccus stutzeri* display NOR activities.¹¹⁴

Varotsis and coworkers have employed resonance Raman spectroscopy to identify an intermediate during the *Tt ba₃* NOR reaction pathway that they assign to a bridged protonated hyponitrite compound **E** (Figure 25) that forms subsequent to the initial generation of a bis-nitrosyl heme–NO/Cu–NO species.¹¹⁵ The proposed identity of this bridged hyponitrite compound is derived from DFT calculations (B3LYP functional; 6–31G* basis set)¹¹⁶ and from the bands in the resonance Raman spectra at 626 cm^{–1} and 1334 cm^{–1} that they assign to the heme *a₃* Fe–N–OH bending and the N–N stretching vibrations, respectively;¹¹⁵ the lower N–N stretching vibration (*c.f.*, free hyponitrite at 1392 cm^{–1})⁸⁷ suggests less double-character in the proposed structure **E**.

Blomberg and coworkers have performed DFT calculations (B3LYP functional) to probe the likely mechanisms of NOR activity in a bacterial NOR model¹¹⁷ and in a *ba₃*-type HCO.¹¹⁸ Their results appear to favor attack of a second NO molecule on a heme-*b₃* (NOR) or heme-*a₃* (HCO) {FeNO}⁷ species (*i.e.*, the *cis-heme b₃* mechanism in Figure 24; where M = Fe in NOR, and M = Cu in HCO).

Clearly, bioinorganic chemistry has a vital role to play in helping elucidate the coordination and reaction chemistry of heme-hyponitrites. For example, Lu and coworkers have engineered a Cu binding site into the distal pocket of Mb, and have clearly demonstrated that this CuBMb mutant catalyzes the reduction of NO to N₂O.¹¹⁹ Importantly, they have also engineered an Fe(II) binding site into Mb, and this Fe_BMb mutant displays ~10-fold faster NO reduction activity than the CuBMb mutant.¹²⁰ Collman and coworkers have utilized the results from the reaction of a functional diferrous synthetic model of the active site of NOR with NO to propose that a *trans*-bisnitrosyl intermediate forms (*i.e.*, the *trans* mechanism in Figure 24) at the active site of NOR followed by NO coupling to give N₂O and a bis-ferric product.^{121,122} The heme and non-heme nitrosyl intermediates were

characterized spectroscopically at low temperatures. Karlin has provided clear evidence that a synthetic heme/Cu model of HCO will also reductively couple NO to N₂O in the presence of acid, thus substantiating the requirement for both metal and acid for this NOR reaction to occur.¹²³

Our research into hyponitrites stems from our interest in designing tractable compounds that can be used to study the coordination chemistry of hyponitrites in a systematic fashion. Prior to our work in this area, there were no crystallographically characterized heme or heme model hyponitrites. We thus set out to prepare an isolable heme hyponitrite compound that we would subject to crystallization efforts and structure solution. The reaction of the oxo-bridged dimer [(OEP)Fe]₂(μ-O) with hyponitrous acid (eq 3) gave, after workup, the stable hyponitrite-bridged iron porphyrin product in good yields.¹²⁴

A band at 982 cm⁻¹ in the IR spectrum of the product was assigned to the ν_{as} of the N–O group ($\nu_{\text{as}}(^{15}\text{NO})$ 973 cm⁻¹). The X-ray crystal structure of this compound is shown in Figure 26. The compound crystallizes as a tetra-dichloromethane solvate where the CH₂Cl₂ molecules surround the hyponitrite bridge. Figure 26 (top) shows the molecule without the CH₂Cl₂ solvates. As is seen in the Figure, the hyponitrite ligand is bound to each Fe atom via the η^1 -O binding mode, and the hyponitrite ligand is *trans* (i.e., structure **F** in Figure 25). The N–N bond length of 1.250(3) Å is indicative of doublebond character (c.f., 1.256(2) Å in *trans*-Na₂N₂O₂),⁹⁴ and suggests substantial hyponitrite (i.e., dianionic) character to this bridging ligand. DFT calculations were performed for geometry optimization for the porphine analogue [(por)Fe]₂(μ-N₂O₂) using the BLYP functional with TZP basis set and frozen core for all atoms, and for subsequent singlepoint energy calculations using the B3LYP functional and all-electron TZP basis set. The DFT calculations on the porphine analog reveal that the calculated geometry of a ferric high-spin *trans*-N₂O₂ system most closely reproduces the crystal structural data, consistent with the EPR data for the product as a CH₂Cl₂/toluene glass at 77 K.¹²⁴ The frontier spin orbitals from the unrestricted open-shell calculations (shown in Figure 27) reveal that the N atoms of the hyponitrite bridge form a bonding interaction in both highest occupied spin orbitals. The detailed magnetic behavior of this complex remains to be explored, however.

We then explored the possibility of N₂O generation from this complex. Addition of hydrochloric acid to the hyponitrite-bridged complex results in the formation of N₂O and (OEP)FeCl (eq 4).

The N₂O was identified by IR spectroscopy of the headspace; new bands at 2236/2213 and 1298/1266 cm⁻¹ were attributed to ν_{as} and ν_{s} of N₂O, respectively.^{125,126} The use of the ¹⁵N-labeled hyponitrite shifts the ν_{as} bands to 2167/2144 cm⁻¹; the corresponding ν_{s} bands were not observed due to its occurrence outside the detection window.

The successful preparation of this [(OEP)Fe]₂(μ-ONNO) complex is only the beginning of what could be a fruitful area of research into heme hyponitrites. However, we note that the *O,O*-binding of the hyponitrite in this complex might be possible because of the generous Fe-Fe distance of 6.7 Å in this complex (i.e., can accommodate an *O,O*-binding mode). This 6.7 Å distance is longer than the 4.4 Å distance between the Fe and Cu centers in *T. thermophilus* cyt *ba3* that exhibits NOR activity.¹²⁷ Ongoing work in our laboratories are centered on the design and preparation of iron porphyrin hyponitrite complexes exhibiting different binding modes of the hyponitrite ligand (e.g., a possible *N*-binding mode in a ferrous derivative) and exploring the differences in chemical reactivities of the hyponitrite linkage isomers.

Conclusions

To a large extent, the study of linkage isomerization in heme-NO_x compounds is still in its infancy. It is possible that the intense coloration of nitrosyl metalloporphyrins hinders efficient light penetration for obtaining high yields of the photogenerated isonitrosyl (η^1 -ON) and/or side-on (η^2 -NO) linkage isomers starting from their groundstate nitrosyl precursors. Their demonstrated existence, however, in synthetic iron porphyrins suggests that they cannot be readily ignored in discussions about heme-NO interactions. Indeed, a recent calculation on Mb(NO) suggests the possible existence of the metastable Mb(η^1 -ON) linkage isomer in this protein.¹²⁸ Clearly, more work needs to be done to elucidate any contributions such linkage isomers may make towards the overall biological activity of NO. In the case of nitrite binding, the existence of its *N*-binding and *O*-binding to heme proteins provides an entry into studies that may differentiate between the nitrite reductase activities of the proteins as a function of nitrite binding mode. Only one synthetic metalloporphyrin hyponitrite compound has been reported to date.

We fully expect that synthetic bioinorganic chemistry of heme-NO_x compounds will continue to play an important role in delineating the rather diverse roles that NO plays in mammalian physiology.

Acknowledgments

We are grateful to the National Institutes of Health (GM064476) and the Oklahoma Center for the Advancement of Science and Technology (HR09-081) for funding for this work.

References

1. Cheng, L.; Richter-Addo, GB. The Porphyrin Handbook. In: Guillard, R.; Smith, K.; Kadish, KM., editors. Biochemistry and Binding: Activation of Small Molecules. Vol. 4. Academic Press; New York: 2000. p. 219-291.
2. Richter-Addo, GB.; Legzdins, P. Metal Nitrosyls. Oxford University Press; New York: 1992.
3. Carducci MD, Pressprich MR, Coppens P. J Am Chem Soc. 1997; 119:2669–2678.
4. Fomitchev DV, Coppens P. Inorg Chem. 1996; 35:7021–7026. [PubMed: 11666882]
5. Fomitchev DV, Coppens P. Comments Inorg Chem. 1999; 21:131–148.
6. Fomitchev DV, Furlani TR, Coppens P. Inorg Chem. 1998; 37:1519–1526.
7. Tocheva EI, Rosell FI, Mauk AG, Murphy MEP. Science. 2004; 304:867–870. [PubMed: 15131305]

8. Antonyuk SV, Strange RW, Sawers G, Eady RR, Hasnain SS. *Proc Natl Acad Sci USA*. 2005; 102:12041–12046. [PubMed: 16093314]
9. Merkle AC, Lehnert N. *Inorg Chem*. 2009; 48:11504–11506. [PubMed: 19938869]
10. Feltham RD, Enemark JH. *Top Stereochem*. 1981; 12:155–215.
11. Enemark JH, Feltham RD. *Coord Chem Rev*. 1974; 13:339–406.
12. Fomitchev DV, Coppens P, Li T, Bagley KA, Chen L, Richter-Addo GB. *Chem Commun*. 1999:2013–2014.
13. Ghosh A. *Acc Chem Res*. 2005; 38:943–954. [PubMed: 16359166]
14. Wondimagegn T, Ghosh A. *J Am Chem Soc*. 2001; 123:5680–5683. [PubMed: 11403599]
15. Cheng L, Novozhilova I, Kim C, Kovalevsky A, Bagley KA, Coppens P, Richter-Addo GB. *J Am Chem Soc*. 2000; 122:7142–7143.
16. Ellison MK, Scheidt WR. *J Am Chem Soc*. 1997; 119:7404–7405.
17. Scheidt WR, Duval HF, Neal TJ, Ellison MK. *J Am Chem Soc*. 2000; 122:4651–4659. and references therein.
18. Ghosh A, Wondimagegn T. *J Am Chem Soc*. 2000; 122:8101–8102.
19. Coppens P, Novozhilova I, Kovalevsky A. *Chem Rev*. 2002; 102:861–883. [PubMed: 11942781]
20. Praneeth VKK, Nather C, Peters G, Lehnert N. *Inorg Chem*. 2006; 45:2795–2811. [PubMed: 16562937]
21. Yoshimura T. *Inorg Chim Acta*. 1984; 83:17–21.
22. Settin MF, Fanning JC. *Inorg Chem*. 1988; 27:1431–1435.
23. Ellison MK, Schulz CE, Scheidt WR. *Inorg Chem*. 1999; 38:100–108.
24. Novozhilova IV, Coppens P, Lee J, Richter-Addo GB, Bagley KA. *J Am Chem Soc*. 2006; 128:2093–2104. [PubMed: 16464112]
25. Lee J, Kovalevsky AY, Novozhilova IV, Bagley KA, Coppens P, Richter-Addo GB. *J Am Chem Soc*. 2004; 126:7180–7181. [PubMed: 15186147]
26. Lim MD, Lorkovic IM, Wedeking K, Zanella AW, Works CF, Massick SM, Ford PC. *J Am Chem Soc*. 2002; 124:9737–9743. [PubMed: 12175231]
27. Braidia W, Ong SK. *Water, Air, and Soil Pollut*. 2000; 118:13–26.
28. Hitchman MA, Rowbottom GL. *Coord Chem Rev*. 1982; 42:55–132.
29. Barrett ML, Harris RL, Antonyuk S, Hough MA, Ellis MJ, Sawers G, Eady RR, Hasnain SS. *Biochemistry*. 2004; 43:16311–16319. [PubMed: 15610025]
30. Lehnert N, Cornelissen U, Neese F, Ono T, Noguchi Y, Okamoto K-i, Fujisawa K. *Inorg Chem*. 2007; 46:3916–3933. [PubMed: 17447754]
31. Wyllie GRA, Scheidt WR. *Chem Rev*. 2002; 102:1067–1089. [PubMed: 11942787]
32. Nasri H, Ellison MK, Shang M, Schultz CE, Scheidt WR. *Inorg Chem*. 2004; 43:2932–2942. [PubMed: 15106981]
33. Cheng L, Powell DR, Khan MA, Richter-Addo GB. *Chem Commun*. 2000; 2301–2302
34. Nasri H, Ellison MK, Chen S, Huynh BH, Scheidt WRJ. *Am Chem Soc*. 1997; 119:6274–6283.
35. Adachi H, Suzuki H, Miyazaki Y, Iimura Y, Hoshino M. *Inorg Chem*. 2002; 41:2518–2524. [PubMed: 11978120]
36. Goodwin J, Kurtikyan T, Standard J, Walsh R, Zheng B, Parmley D, Howard J, Green S, Mardyukov A, Przybla DE. *Inorg Chem*. 2005; 44:2215–2223. [PubMed: 15792456]
37. Seki H, Okada K, Iimura Y, Hoshino M. *J Phys Chem A*. 1997; 101:8174–8178.
38. Suslick KS, Watson RA. *Inorg Chem*. 1991; 30:912–919.
39. Hoshino M, Nagashima Y, Seki H, Leo MD, Ford PC. *Inorg Chem*. 1998; 37:2464–2469.
40. Kadish KM, Adamian VA, Caemelbecke EV, Tan Z, Tagliatesta P, Bianco P, Boschi T, Yi GB, Khan MA, Richter-Addo GB. *Inorg Chem*. 1996; 35:1343–1348. [PubMed: 11666330]
41. Miranda KM, Bu X, Lorkovic I, Ford PC. *Inorg Chem*. 1997; 36:4838–4848. [PubMed: 11670164]
42. Bohle DS, Hung CH, Smith BD. *Inorg Chem*. 1998; 37:5798–5806.
43. Lim MH, Lippard SJ. *Inorg Chem*. 2004; 43:6366–6370. [PubMed: 15446885]

44. Leal FA, Lorkovic IM, Ford PC, Lee J, Chen L, Torres L, Khan MA, Richter-Addo GB. *Can J Chem*. 2003; 81:872–881.
45. Heinecke J, Ford PC. *Coord Chem Rev*. 2010; 254:235–247.
46. Kurtikyan TS, Hovhannisyan AA, Hakobyan ME, Patterson JC, Iretskii A, Ford PC. *J Am Chem Soc*. 2007; 129:3576–3585. [PubMed: 17338521]
47. Kurtikyan TS, Ford PC. *Angew Chem Int Ed*. 2006; 45:492–496.
48. Kurtikyan TS, Hovhannisyan AA, Gulyan GM, Ford PC. *Inorg Chem*. 2007; 46:7024–7031. [PubMed: 17636900]
49. Einsle O, Messerschmidt A, Huber R, Kroneck PMH, Neese F. *J Am Chem Soc*. 2002; 124:11737–11745. [PubMed: 12296741]
50. Silaghi-Dumitrescu R. *Inorg Chem*. 2004; 43:3715–3718. [PubMed: 15180427]
51. Perissinotti LL, Marti MA, Doctorovich F, Luque FJ, Estrin DA. *Biochemistry*. 2008; 47:9793–9802. [PubMed: 18717599]
52. Averill BA. *Chem Rev*. 1996; 96:2951–2964. [PubMed: 11848847]
53. Eady, RR.; Hasnain, SS. *Comprehensive Coordination Chemistry II*. In: Que, L., Jr; Tolman, WB., editors. *Biocoordination chemistry*. Vol. 8. Elsevier; San Diego, CA: 2004. p. 759-786.
54. Hollocher, TC. *Nitric Oxide Principles and Applications*. Lancaster, J., editor. Academic Press; San Diego: 1996. p. 289-344.
55. Hollocher, TC.; Hibbs, JB, Jr. *Methods in Nitric Oxide Research*. Feelish, M.; Stamler, JS., editors. John Wiley and Sons; Chichester: 1996. p. 119-146.
56. Tavares P, Pereira AS, Moura JGG. *J Inorg Biochem*. 2006; 100:2087–2100. [PubMed: 17070915]
57. Skibsted, LH. *The Chemistry of Muscle-Based Foods*. Johnson, DE.; Knight, MK.; Ledward, DA., editors. Royal Society of Chemistry; Cambridge: 1992. p. 266-286.
58. Livingston DJ, Brown WD. *Food Technol*. 1981; (May Issue):244–252. and references therein.
59. Killday KB, Tempesta MS, Bailey ME, Metral CJ. *J Agric Food Chem*. 1988; 36:909–914.
60. Bruun-Jensen L, Skibsted LH. *Meat Science*. 1996; 44:145–149. [PubMed: 22060824]
61. Møller JKS, Skibsted LH. *Chem Rev*. 2002; 102:1167–1178. [PubMed: 11942791]
62. Percy MJ, McFerran NV, Lappin TRJ. *Blood Reviews*. 2005; 19:61–68. [PubMed: 15603910]
63. Chui JS, Poon WT, Chan KC, Chan AY, Buckley TA. *Anaesthesia*. 2005; 60:496–500. [PubMed: 15819771]
64. Gladwin MT, Schechter AN, Kim-Shapiro DB, Patel RP, Hogg N, Shiva S, Cannon RO III, Kelm M, Wink DA, Espey MG, Oldfield EH, Pluta RM, Freeman BA, Lancaster JR Jr, Feelisch M, Lundberg JO. *Nat Chem Biol*. 2005; 1:308–314. and references therein [Erratum: 2006, 2(2), 110.]. [PubMed: 16408064]
65. Lundberg JO, Gladwin MT, Ahluwalia A, Benjamin N, Bryan NS, Butler A, Cabrales P, Fago A, Feelisch M, Ford PC, Freeman BA, Frenneaux M, Friedman J, Kelm M, Kevil CG, Kim-Shapiro DB, Kozlov AV, Lancaster JR, Lefer DJ, McColl K, McCurry K, Patel RP, Petersson J, Rassaf T, Reutov VP, Richter-Addo GB, Schechter A, Shiva S, Tsuchiya K, van Faassen EE, Webb AJ, Zuckerbraun BS, Zweier JL, Weitzberg E. *Nat Chem Biol*. 2009; 5:865–869. [PubMed: 19915529]
66. van Faassen EE, Babrami S, Feelisch M, Hogg N, Kelm M, Kim-Shapiro DB, Kozlov AV, Li HT, Lundberg JO, Mason R, Nohl H, Rassaf T, Samouilov A, Slama-Schwok A, Shiva S, Vanin AF, Weitzberg E, Zweier J, Gladwin MT. *Med Res Rev*. 2009; 29:683–741. [PubMed: 19219851]
67. Feelisch M, Fernandez BO, Bryan NS, Garcia-Saura MF, Bauer S, Whitlock DR, Ford PC, Janero DR, Rodriguez J, Ashrafian H. *J Biol Chem*. 2008; 283:33927–33934. [PubMed: 18835812]
68. Hendgen-Cotta UB, Merx MW, Shiva S, Schmitz J, Becher S, Klare JP, Steinhoff HJ, Goedecke A, Schrader J, Gladwin MT, Kelm M, Rassaf T. *Proc Natl Acad Sci USA*. 2008; 105:10256–10261. [Erratum: p 12636]. [PubMed: 18632562]
69. Williams PA, Fulop V, Garman EF, Saunders NFW, Ferguson SJ, Hajdu J. *Nature*. 1997; 389:406–412. [PubMed: 9311786]
70. Crane BR, Siegel LM, Getzoff ED. *Biochemistry*. 1997; 36:12120–12137. [PubMed: 9315849]
71. Lukat P, Rudolf M, Stach P, Messerschmidt A, Kroneck PMH, Simon J, Einsle O. *Biochemistry*. 2008; 47:2080–2086. [PubMed: 18201106]

72. Copeland DM, Soares A, West AH, Richter-Addo GB. *J Inorg Biochem.* 2006; 100:1413–1425. [PubMed: 16777231]
73. Zahran ZN, Chooback L, Copeland DM, West AH, Richter-Addo GB. *J Inorg Biochem.* 2008; 102:216–233. [PubMed: 17905436]
74. Yi J, Safo MK, Richter-Addo GB. *Biochemistry.* 2008; 47:8247–8249. [PubMed: 18630930]
75. Yi J, Heinecke J, Tan H, Ford PC, Richter-Addo GB. *J Am Chem Soc.* 2009; 131:18119–18128. [PubMed: 19924902]
76. Mckellar ARW, Watson JKG, Howard BJ. *Mol Phys.* 1995; 86:273–286.
77. Byl O, Kondratyuk P, Yates J, JT. *J Phys Chem B.* 2003; 107:4277–4279.
78. Zhao YL, Bartberger MD, Goto K, Shimada K, Kawashima T, Houk KN. *J Am Chem Soc.* 2005; 127:7964–7965. [PubMed: 15926791]
79. Fuster F, Dezarnaud-Dandine C, Chevreau H, Sevin A. *Phys Chem Chem Phys.* 2004; 6:3228–3234.
80. Taguchi N, Mochizuki Y, Ishikawa T, Tanaka K. *Chem Phys Lett.* 2008; 451:31–36.
81. Snis A, Panas I. *Chem Phys.* 1997; 221:1–10.
82. Dutton AS, Fukuto JM, Houk KN. *Inorg Chem.* 2005; 44:4024–4028. [Erratum: pp 7687–7688]. [PubMed: 15907131]
83. Poskrebyshev GA, Shafirovich V, Lymar SV. *J Am Chem Soc.* 2004; 126:891–899. [PubMed: 14733565]
84. Poskrebyshev GA, Shafirovich V, Lymar SV. *J Phys Chem A.* 2008; 112:8295–8302. [PubMed: 18707066]
85. Andrews L, Zhou MF, Willson SP, Kushto GP, Snis A, Panas I. *J Chem Phys.* 1998; 109:177–185.
86. Goubeau J, Laitenberger K. *Z Anorg Allg Chem.* 1963; 320:78–85.
87. Andrews L, Liang B. *J Am Chem Soc.* 2001; 123:1997–2002. [PubMed: 11456821]
88. Andrews L, Wang X, Zhou M, Liang B. *J Phys Chem A.* 2002; 106:92–95.
89. Andrews L, Citra A. *Chem Rev.* 2002; 102:885–911. [PubMed: 11942782]
90. McGraw GE, Bernitt DL, Hisatsune IC. *Spectrochim Acta.* 1967; 23A:25–34.
91. Feldmann C, Jansen M. *Angew Chem, Int Ed Engl.* 1996; 35:1728–1730.
92. Feldmann C, Jansen M. *Z Anorg Allg Chem.* 1997; 623:1803–1809.
93. Feldmann C, Jansen M. *Z Kristallogr.* 2000; 215:343–345.
94. Arulsamy N, Bohle DS, Imonigie JA, Sagan ES. *Inorg Chem.* 1999; 38:2716–2725.
95. Bottcher HC, Graf M, Mereiter K, Kirchner K. *Organometallics.* 2004; 23:1269–1273.
96. Cenini S, Ugo R, La Monica G, Robinson SD. *Inorg Chim Acta.* 1972; 6:182–184.
97. Lorenzelli V, Busca G, Sheppard N, Al-Mashta F. *J Mol Struct.* 1982; 80:181–186.
98. Ramprasad R, Hass KC, Schneider WF, Adams JB. *J Phys Chem B.* 1997; 101:6903–6913.
99. Azambre B, Zenbourny L, Koch A, Weber JV. *J Phys Chem C.* 2009; 113:13287–13299.
100. Franz KJ, Lippard SJ. *J Am Chem Soc.* 1999; 121:10504–10512.
101. Gwost D, Caulton KG. *Inorg Chem.* 1974; 13:414–417.
102. Schneider JL, Carrier SM, Ruggiero CE, Young VG, Tolman WB. *J Am Chem Soc.* 1998; 120:11408–11418.
103. Arulsamy N, Bohle DS, Imonigie JA, Levine S. *Angew Chem Int Ed.* 2002; 41:2371–2373.
104. Arulsamy N, Bohle DS, Imonigie JA, Moore RC. *Polyhedron.* 2007; 26:4737–4745.
105. Bhaduri S, Johnson BFG, Pickard A, Raithby PR, Sheldrick GM, Zuccaro CI. *J Chem Soc, Chem Commun.* 1977:354–355.
106. Hoskins BF, Whillans FD, Dale DH, Hodgkin DC. *J Chem Soc, Chem Commun.* 1969:69–70.
107. Villalba MEC, Navaza A, Guida JA, Varetto EL, Aymonino PJ. *Inorg Chim Acta.* 2006; 359:707–712.
108. Bau R, Sabherwal IH, Burg AB. *J Am Chem Soc.* 1971; 93:4926–4928.
109. Moënné-Loccoz P. *Nat Prod Rep.* 2007; 24:610–620. [PubMed: 17534533]
110. Daiber A, Shoun H, Ullrich V. *J Inorg Biochem.* 2005; 99:185–193. [PubMed: 15598501]

111. Lehnert N, Praneeth VKK, Paulat F. *J Comput Chem.* 2006; 27:1338–1351. [PubMed: 16788909]
112. Moënné-Loccoz P, Richter OMH, Huang HW, Wasser IM, Ghiladi RA, Karlin KD, de Vries S. *J Am Chem Soc.* 2000; 122:9344–9345.
113. Pinakoulaki E, Varotsis C. *J Inorg Biochem.* 2008; 102:1277–1287. [PubMed: 18334269]
114. Zumft WG. *J Inorg Biochem.* 2005; 99:194–215. [PubMed: 15598502]
115. Varotsis C, Ohta T, Kitagawa T, Soulimane T, Pinakoulaki E. *Angew Chem Int Ed.* 2007; 46:2210–2214.
116. Ohta T, Kitagawa T, Varotsis C. *Inorg Chem.* 2006; 45:3187–3190. [PubMed: 16602774]
117. Blomberg LM, Blomberg MRA, Siegbahn PEM. *Biochim Biophys Acta.* 2006; 1757:240–252. [PubMed: 16774734]
118. Blomberg LM, Blomberg MRA, Siegbahn PEM. *Biochim Biophys Acta.* 2006; 1757:31–46. [PubMed: 16375849]
119. Zhao X, Yeung N, Russell BS, Garner DK, Lu Y. *J Am Chem Soc.* 2006; 128:6766–6767. [PubMed: 16719438]
120. Yeung N, Lu Y. *Chem Biodiversity.* 2008; 5:1437–1454.
121. Collman JP, Dey A, Yang Y, Decreau RA, Ohta T, Solomon EI. *J Am Chem Soc.* 2008; 130:16498–16499. [PubMed: 19049449]
122. Collman JP, Yang Y, Dey A, Decreau RA, Ghosh S, Ohta T, Solomon EI. *Proc Natl Acad Sci USA.* 2008; 105:15660–15665. [PubMed: 18838684]
123. Wang J, Schopfer MP, Sarjeant AAN, Karlin KD. *J Am Chem Soc.* 2009; 131:450–451. [PubMed: 19099478]
124. Xu N, Campbell ALO, Powell DR, Khandogin J, Richter-Addo GB. *J Am Chem Soc.* 2009; 131:2460–2461. [PubMed: 19191487]
125. Captain DK, Amiridis MD. *J Catal.* 2000; 194:222–232.
126. Nightingale RE, Downie AR, Rotenberg DL, Crawford B, Ogg RA. *J Phys Chem.* 1954; 58:1047–1050.
127. Soulimane T, Buse G, Bourenkov GP, Bartunik HD, Huber R, Than ME. *Embo J.* 2000; 19:1766–1776. [PubMed: 10775261]
128. Nutt DR, Karplus M, Meuwly M. *J Phys Chem B.* 2005; 109:21118–21125. [PubMed: 16853735]

Figure 1.

Representative approaches of the NO molecule towards the Fe center in heme proteins.

Figure 2.
Metal-NO binding modes.

Figure 3.

Difference spectra (spectrum after 15 min irradiation minus spectrum prior to irradiation) for the ¹⁴N (bottom) and ¹⁵N (top) labeled (OEP)Ru(NO)(O-*i*-C₅H₁₁) compound.¹²

Figure 4.

Light-induced generation of metastable nitrosyl linkage isomers during irradiation of (OEP)Ru(NO)(O-*i*-C₅H₁₁) as a KBr pellet.

Figure 5.

Optimized geometry of the metastable side-on nitrosyl linkage isomer of (OEP)Ru(NO)Cl.¹³

Figure 6.

Light-induced generation of the metastable isonitrosyl linkage isomer of (TTP)Fe(NO) as a KBr pellet.

Figure 7.

Calculated geometries of the ground-state (porphine)Fe(NO) (top) and its metastable isonitrosyl derivative (bottom) viewed from three different directions (reproduced in part from reference ¹⁵). Valence shells of the H, C, N, and O atoms were described by a double- ζ STO basis set extended with a polarization function, whereas the 3s, 3p, and 3d shells on Fe were described by a triple- ζ STO basis set (ADF program package).

Figure 8.

Electron localization function (ELF) of the ground-state (porphine)Fe(NO)(NO₂), with a plotted isosurface value of 0.8 (Reproduced with permission from reference ²⁴. Copyright 2006 The American Chemical Society.)

Figure 9.

Light-induced transformations upon irradiation of (TPP)Fe(NO)(NO₂) as a KBr pellet at low temperatures.

Figure 10.

Calculated energies and representative structures for the linkage isomers of (porphine)Fe(NO)(NO₂).// = axial ligand planes are coplanar; ⊥ = axial ligand planes are mutually perpendicular; MSa_L and MSC_L are the isomers displaying linear FeNO and FeON groups, respectively. (Reproduced from reference ²⁵. Copyright 2004 The American Chemical Society.)

Figure 11.
Nitrite binding modes to monometallic centers.

Figure 12.

Proposed mechanisms for the linkage isomerization reactions after photolysis of the ground-state nitro (TPP)Co(NO₂) (**A**) and nitrito (TPP)Mn(ONO) (**B**) compounds.

Figure 13.

Reaction of nitrogen dioxide with sublimed layers of (TPP)Fe to give initially (TPP)Fe(ONO) followed by exogenous ligand-induced isomerization to the nitro isomer.

Figure 14.

The heme active sites of the *N*-bound nitrite adducts of cytochrome *cd₁* NiR from *Paracoccus pantotrophus* (top left; 1.8 Å resolution; PDB access code 1AAQ), the sulfite reductase hemoprotein from *Escherichia coli* (top right; 2.1 Å resolution; PDB access code 3GEO), cytochrome *c* NiR from *Wolinella succinogenes* (bottom left; 1.6 Å resolution) and its Y218F mutant (bottom right; 1.75 Å resolution; PDB access code 3BNH).

Figure 15.

The $F_o - F_c$ omit electron density maps (contoured at 3σ) and final models of the heme environments of the *O*-bound nitrite adducts of (A) wildtype horse heart ferric Mb (1.20 Å resolution; PDB access code 2FRF),⁷² (B) Mn^{III}-substituted Mb (1.60 Å resolution; PDB access code 2O5O),⁷³ and (C) Co^{III}-substituted Mb (1.60 Å resolution; PDB access code 2O5S).⁷³

Figure 16.

The $F_o - F_c$ omit electron density maps (contoured at 3σ) and final models of the heme environments of the *O*-bound nitrite adduct of ferric human Hb (1.80 Å resolution; PDB access code 3D7O).⁷⁴

Figure 17.

Sketches of the *trans*- and *cis*-FeONO conformations.

Figure 18.

Sketches of the active sites of wild-type Mb (left), the H64V mutant (middle), and the H64V/V67R double-mutant (right).

Figure 19.

The $F_o - F_c$ omit electron density maps (contoured at 3σ) and final models of the heme environments of (A) the *N*-bound nitrite adduct of the ferric Mb H64V mutant (1.95 Å resolution; PDB access code 3HEP), and (B) the *O*-bound nitrite adduct of the ferric Mb H64V/V67R double mutant (2.0 Å resolution; PDB access code 3HEO).⁷⁵

Figure 20.

Probable protonation pathways involving the bound nitrite ligands to generate FeNO (left; I) and free NO (right, II).

Figure 21.
Redox congeners of the NO dimer.

Figure 22.
The *cis* and *trans* forms of the hyponitrite dianion.

Figure 23.

Structurally characterized metal hyponitrite binding modes in inorganic coordination compounds.

Figure 24.

Intermediates in the three putative mechanisms for NO reduction by NORs and HCOs.

Figure 25.

A proposed intermediate (**E**) and a structurally characterized (**F**) bimetallic heme-hyponitrite moiety.

Figure 26.

Molecular structure of $[(\text{OEP})\text{Fe}]_2(\mu\text{-ONNO})$. *Top*: Hydrogen atoms and the CH_2Cl_2 solvates have been omitted for clarity. *Bottom*: With CH_2Cl_2 solvates, but without non-solvate H atoms. Selected bond lengths (Å) and angles (°): $\text{Fe}-\text{O} = 1.889(2)$, $\text{O}-\text{N} = 1.375(2)$, $\text{N}-\text{N} = 1.250(3)$, $\text{Fe}-\text{N}(\text{por}) = 2.049(2)$ – $2.064(2)$, $\angle\text{FeON} = 118.56(12)$, $\angle\text{NNO} = 108.5(2)$. (Reproduced from reference ¹²⁴. Copyright 2009 The American Chemical Society.)

Figure 27.

Frontier spin orbitals for high-spin $[(\text{porphine})\text{Fe}]_2(\mu\text{-ONNO})$. HOSO and LUSO denote the highest occupied and the lowest unoccupied spin orbitals, respectively (reproduced from reference 124).

Table 1

IR Stretching Frequencies (cm^{-1}) and Shifts of Absorption Bands of the Ground State and Metastable States of $(\text{OEP})\text{Ru}(\text{NO})(\text{O-}i\text{-C}_5\text{H}_{11})$ and $(\text{OEP})\text{Ru}(\text{NO})(\text{SCH}_2\text{CF}_3)$.¹²

	$\nu(\text{RuN-O})$	$\nu(\text{RuO-N})$	$\nu(\text{Ru}(\eta^2\text{-NO}))$
$(\text{OEP})\text{Ru}(\text{NO})(\text{O-}i\text{-C}_5\text{H}_{11})$			
^{14}N	1791	1645 (−146)	1497 (−294)
^{15}N	1755	1609 (−146)	1480 (−275)
$[\nu(^{14}\text{N}) - \nu(^{15}\text{N})]$	36	36	17
$(\text{OEP})\text{Ru}(\text{NO})(\text{SCH}_2\text{CF}_3)$			
^{14}N	1788	1660 (−128)	1546 (−242)
^{15}N	1753	1633 (−120)	1527 (−226)
$[\nu(^{14}\text{N}) - \nu(^{15}\text{N})]$	35	27	19

Table 2

Selected Calculated Geometrical Parameters (in Å and deg) for the Ground State and Isonitrosyl Isomers of the Unsubstituted (porphine)Fe(NO) Compound.¹⁵

	(P')Fe(NO)	(P')Fe(ON)
Fe–NO	1.666	-
Fe–ON	-	1.797
N–O	1.169	1.163
Fe–N–O	142.5	-
Fe–O–N	-	141.0
Fe–NO/–ON tilt	7.8	10.8
	1.971	1.949
Fe–N(por)		
	1.946	1.938
Fe (por–N ₄)	0.224	0.171