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Forum Article

Reactions of NO and Nitrite with Heme Models and Proteins

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Presented is an overview of the fundamental chemical properties of nitric oxide and nitrite ion in relation to the reactions with ferri- and ferroheme models and proteins potentially relevant to the roles of these species in mammalian biology.

Introduction

It has been more than 2 decades since the initial reports that nitric oxide (NO, aka nitrogen monoxide) is an endogenous bioregulator of cardiovascular systems. These were followed quickly by additional discoveries that NO has important roles in mammalian neurochemistry and immunology. Since then, there have been well over 10^4 publications discussing the mammalian biology and pathobiology of NO and other reactive nitrogen species. More recently, the nitrite ion has received renewed attention, and much of the mammalian biology of both species involves heme proteins. The goal of this article is to outline the chemical properties of NO and NO_2^- involving hemes relevant to the physiological properties of these species.

The singularly most important chemical feature of NO is that *it is a stable free radical*. As a consequence, NO readily reacts with other free radicals,[1] for example, with superoxide ion (O_2^-; eq 1), at near diffusion-limited rates ($k_d \sim 10^{10}$ M^{-1} s^{-1} in an aqueous solution).[1b] However, unlike many free radicals, NO does not readily dimerize. Although the $(NO)_2$ dimer is known, its N−N bond is weak, so $(NO)_2$ is found only at high NO pressures or at very low temperature.[2] Moreover, NO is relatively unreactive toward substrates requiring two-electron processes to give stable products. Thus, NO autoxidation in aqueous media (eq 2) is fast only at high NO concentrations because it occurs by third-order kinetics and the reaction rate depends on $[NO]^2$.[3]

$$O_2^- + NO \longrightarrow ONOO^- \quad (1)$$

$$4NO + O_2 + 2H_2O \longrightarrow 4H^+ + 4NO_2^- \quad (2)$$

The principal chemical targets of NO under bioregulatory conditions are redox-active metal centers, primarily those in heme proteins,[4] although NO also reacts with copper models and proteins[5] and with nonheme iron.[6] Heme centers are also involved in the in vivo NO generation from arginine by the nitric oxide synthase (NOS) enzymes.[7] (Note: a list of acronyms and abbreviations is given at the end of the article.)

From a bioregulatory perspective, the best characterized NO target is the ferroheme enzyme soluble guanylyl cyclase (sGC). Indeed, it was the effort to characterize the endothelium-derived relaxation factor that activates sGC that uncovered NO as that factor,[8] a discovery that led to the 1998 Nobel Prize in Medicine awarded to Furchgott, Ignarro, and Murad.[8c−e] Formation of a NO complex with the sGC heme is thought to labilize the trans axial (proximal) histidine ligand, leading to a change in the protein conformation and activation of the enzyme.[9] The resulting formation of cyclic guanylyl monophosphate from guanylyl triphosphate (GTP) leads to the relaxation of vascular smooth muscle tissue and lowers blood pressure. Only nanomolar concentrations of NO are needed to activate sGC;[10] thus, the reaction of NO

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(1) (a) Neta, P.; Huie, R. E. *J. Phys. Chem. Ref. Data* **1988**, *17*, 1027–1284. (b) Goldstein, S.; Czapski, G. *Free Radical Biol. Med.* **1995**, *19*, 505–510.

(2) (a) Wade, E. A.; Cline, J. I.; Lorenz, K. T.; Hayden, C.; Chandler, D. W. *J. Chem. Phys.* **2002**, *116*, 4755–4757. (b) Zhao, Y.-L.; Bartberger, M.; Houk, K. N.; Goto, K. *J. Am. Chem. Soc.* **2005**, *127*, 7964–7965 and references cited therein.

(3) (a) Ford, P. C.; Wink, D. A.; Stanbury, D. M. *FEBS Lett.* **1993**, *326*, 1–3. (b) Wink, D. A.; Darbyshire, J. F.; Nims, R. W.; Saavedra, J. E.; Ford, P. C. *Chem. Res. Toxicol.* **1993**, *6*, 23.

(4) (a) *Nitric Oxide: Biology and Pathobiology*; Ignarro, L. J., Ed.; Academic Press: San Diego, 2000. (b) Hoshino, M.; Laverman, L.; Ford, P. C. *Coord. Chem. Rev.* **1999**, *187*, 75. (c) Cheng, L.; Richter-Addo, G. B. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard,R., Eds.; Academic Press: New York, 2000; Vol. *4*, Chapter 33.

(5) (a) Tran, D.; Shelton, B. W.; White, Al. H.; Laverman, L. E.; Ford, P. C. *Inorg. Chem.* **1998**, *37*, 2505. (b) Shiva, S.; Wang, X.; Ringwood, L. A.; Xu, X.; Yuditskaya, S.; Annavajjhala, V.; Miyajima, H.; Hogg, N.; Harris, Z. L.; Gladwin, M. T. *Nat. Chem. Biol.* **2006**, *2*, 486–493. (c) Fujisawa, K.; Tateda, A.; Miyashita, Y.; Okamoto, K.; Paulat, F.; Praneeth, V. K. K.; Merkle, A.; Lehnert, N. *J. Am. Chem. Soc.* **2008**, *130*, 1205–1213. (d) Ruggiero, C. E.; Carrier, S. M.; Tolman, W. B. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 895–897.

(6) Butler, A. R.; Megson, I. L. *Chem. Rev.* **2002**, *102*, 1155–1165.

(7) Griffith, O. W.; Stuehr, D. J. *Annu. Rev. Physiol.* **1995**, *57*, 707–736.

(8) (a) Ignarro, L. J.; Buga, G. M.; Wood, K. S.; Byrns, R. E.; Chaudhuri, G. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 9265–9269. (b) Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. *Nature* **1987**, *327*, 524–526. (c) Murad, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 1856–1868. (d) Furchgott, R. F. *Angew. Chem., Int. Ed.* **1999**, *38*, 1870–1880. (e) Ignarro, L. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1882–1892.

(9) Traylor, T. G.; Sharma, V. S. *Biochemistry* **1992**, *31*, 2847.

(10) (a) Bellamy, T. C.; Griffiths, C.; Garthwaite, J. *J. Biol. Chem.* **2002**, *277*, 31801. (b) Bellamy, T. C.; Garthwaite, J. *J. Biol. Chem.* **2001**, *276*, 4287.

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with sGC must be exceedingly fast for the enzyme to compete effectively with other processes that deplete NO in cellular tissue. Other metalloproteins are also potential targets; for example, NO inhibits various enzymes including cytochrome *c* oxidase (thus having an impact on mitochondrial respiration),[11] it is a substrate for mammalian peroxidases,[12] and it is released from the salivary ferriheme proteins of certain blood-sucking insects.[13]

The brief discussion regarding sGC introduces two salient issues relevant to the mechanistic inorganic chemistry of NO and heme systems. The first is concerned with the rates, mechanism, and thermodynamics regarding the formation of NO---heme iron complexes. The second is concerned with the effect of NO coordination both on the properties of the heme model or protein and on the reactivity of the coordinated NO itself. Similar considerations need to be taken into account with regard to the interactions of other nitrogen oxide derivatives with heme centers. This is especially important given the extensive interest in the biomedical field in the therapeutic, signaling, and/or deleterious effects of NO_x species such as nitroxyl (HNO), nitrite ion (NO_2^-), nitrogen dioxide, and peroxynitrite ($OONO^-$) as well as more complex species such as *S*-nitrosothiols (RSNO) and *N*-nitrosoamines (RR′NNO).

The efficacy of NO in biological signaling depends on the location, timing, and concentration of NO production. Enzymatic formation occurs at specific locations within an organism followed by relatively nondirectional diffusion to target sites. The activation of signals results from the reaction with NO or with some product of NO oxidation or reduction. Additional complications to consider are the varied solubilities of these species in different media because cells and physiological structures are heterogeneous. For example, NO is nearly 1 order of magnitude more soluble in hydrophobic environments such as cell membranes than in aqueous media (1.9 mM atm^{-1} in 25 °C water)[14] and partitions selectively into lipid membranes.[15] Partitioning into the gas phase is even higher. For an aqueous medium at ambient temperature in equilibrium with a gas phase, the NO concentration in the gas will be ~20 times higher than that in solution.[14] Adding further to the complexity is that such partitioning is not instantaneous. Thus, the NO concentration at a specific site is a complex function of the rates of its formation, consumption, diffusion, and partitioning.

Recent discoveries that inorganic nitrite may protect mammalian organs during ischemia/reperfusion injury,[16] that it induces vasodilation,[17] and that it may have other therapeutic applications[18] have drawn considerable attention. Although the mechanisms of these functions have not been conclusively established, it is generally thought that the presence of NO_2^- under hypoxic conditions leads to NO formation[19] and that such NO production inhibits the formation of damaging reactive oxygen species by inhibiting mitochondrial respiration.

Feelisch and co-workers have shown quantitatively that mammalian fluids and tissue include surprisingly high nitrite concentrations, at 50−300 nM and 0.5−25 μM, respectively,[20] so it is relevant to envision potential physiological functions. Although there are several possible sources for such as nitrite, this topic has not been quantitatively addressed. Autoxidation of endogenous NO (eq 2) is one possibility, but the third-order reaction rate ($-d[NO]/dt = 4k_{aq}[NO]^2[O_2]$, $4k_{aq} = \sim 8 \times 10^6\ M^{-2}\ s^{-1}$),[3] will be quite slow at bioregulatory concentrations given the low steady-state [NO] under nonpathological conditions. Furthermore, a major NO sink in the cardiovascular system involves rapid oxidation by oxyhemoglobin [$Hb(O_2)$] to give nitrate[21] (eq 3; $k_2 = 9 \times 10^7\ M^{-1}\ s^{-1}$ in a buffered solution).[22] On the other hand, nitrite is the product of NO oxidation in the plasma by the copper protein ceruloplasmin (eq 4; $Cu^{II}P$ is ceruloplasmin),[23] although the importance of this pathway has yet to be evaluated. Another possibility would be NO oxidation by the ferric forms of hemoglobin (metHb) or myoglobin (metMb) or other ferriheme proteins (e.g., eq 5), but again there are no quantitative measures of the extent to which such reactions lead to NO_2^- formation in the organism. Last, another potential source of various NO_x is ionic nitrate, which is present in physiological fluids and tissues at much higher concentrations than either NO or NO_2^-.[24] Certain mammalian enzymes such as xanthine oxidoreductase have been shown to reduce NO_3^-,[25] but it is unclear what roles these have in human nitrogen cycles. On the other hand, nitrate reductases that convert NO_3^- to NO_2^- are present in oral bacteria, and in certain intestinal bacteria, and thereby constitute potential physiological sources of ionic nitrite.[26]

$$NO + Hb(O_2) \longrightarrow metHb + NO_3^- \quad (3)$$

$$Cu^{II}P + NO + H_2O \longrightarrow Cu^{I}P + NO_2^- + 2H^+ \quad (4)$$

$$metHb + xsNO \longrightarrow Hb(NO) + NO_2^- \quad (5)$$

An important yet sometimes underappreciated feature of nitrite biochemistry is the effect of the pH. Nitrite is the conjugate base of nitrous acid ($pK_a = 3.16$ at 25 °C and 3.11

(11) Brown, G. C. *FEBS Lett*. **1995**, *369*, 136–139.
(12) Thomas, D. D.; Miranda, K. M.; Colton, C. A.; Citrin, D.; Espey, M. G.; Wink, D. A. *Antioxid. Redox Signaling* **2003**, *5*, 307–317.
(13) Walker, F. A.; Montfort, W. R. *Adv. Inorg. Chem*. **2001**, *51*, 295–358.
(14) Young, C. L., Ed. *IUPAC Solubility Series: Oxides of Nitrogen*; Pergamon Press: Oxford, U.K., 1983
(15) Liu, X.; Miller, M. J. S.; Joshi, M. S.; Thomas, D. D.; Lancaster, J. R., Jr. *Proc. Natl. Acad. Sci. U.S.A*. **1998**, *95*, 2175–2179.
(16) Shiva, S.; Sack, M. N.; Greer, J. J.; Duranski, M.; Ringwood, L. A.; Burwell, L.; Wang, X.; MacArthur, P. H.; Shoja, A.; Raghavachari, N.; Calvert, J. W.; Brookes, P. S.; Lefer, D. J.; Gladwin, M. T. *J. Exp. Med*. **2007**, *204*, 2089–2102.
(17) Cosby, K.; Partovi, K. S.; Crawford, J. H.; Patel, R. P.; Reiter, C. D.; Martyr, S.; Yang, B. K.; Waclawiw, M. A.; Zalos, G.; Xu, X.; Huang, K. T.; Shields, H.; Kim-Shapiro, D. B.; Schechter, A. N.; Cannon, R. O.; Gladwin, M. T. *Nat. Med*. **2003**, *9*, 1498.
(18) Lundberg, J. O.; Weitzberg, E.; Gladwin, M. T. *Nat. Rev. Drug Discovery* **2008**, *7*, 156–167.
(19) Feelisch, M.; Fernandez, B. O.; Bryan, N. S.; Garcia-Saura, M. F.; Bauer, S.; Whitlock, D. R.; Ford, P. C.; Janero, D. R.; Rodriguez, J.; Ashrafian, H. *J. Biol. Chem*. **2008**, *283*, 33927.
(20) (a) Feelisch, M.; Rassaf, T.; Mnaimneh, S.; Singh, N.; Bryan, N. S.; Jourd'Heuil, D.; Kelm, M. *FASEB J*. **2002**, *16*, 1775. (b) Rodriguez, J.; Maloney, R. E.; Rassaf, T.; Bryan, N. S.; Feelisch, M. *Proc. Natl. Acad. Sci. U.S.A*. **2003**, *100*, 336.
(21) Lancaster, J. R., Jr. *Proc. Natl. Acad. Sci. U.S.A*. **1994**, *91*, 8137.
(22) Doyle, M. P.; Hoekstra, J. W. *J. Inorg. Biochem*. **1981**, *14*, 351.
(23) Shiva, S.; Wang, X.; Ringwood, L. A.; Xu, X.; Yuditskaya, S.; Annavajjhala, V.; Miyajima, H.; Hogg, N.; Harris, Z. L.; Gladwin, M. T. *Nat. Chem. Biol*. **2006**, *2*, 486.
(24) Rassaf, T.; Feelisch, M.; Kelm, M. *Free Radical Biol. Med*. **2004**, *36*, 413.
(25) Jansson, E. A.; Huang, L.; Malkey, R.; Govoni, M.; Nihlen, C.; Olsson, A.; Stensdotter, M.; Petersson, J.; Holm, L.; Weitzberg, E.; Lundberg, J. O. *Nat. Chem. Biol*. **2008**, *4*, 411.
(26) Knobeloch, L.; Salna, B.; Hogan, A.; Postle, J.; Anderson, H. *Environ. Health Perspect*. **2000**, *108*, 675.

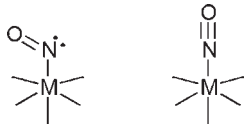


Figure 1. Illustration of the limiting cases of NO binding to a metal center as the nitroxyl anion (NO^-) with a M−N−O bond angle of ~120° (left) or as the nitrosyl cation (NO^+) with a M−N−O bond angle of ~180° (right).

at 37 °C).[27] The reduction to NO (eq 6) requires 2 equiv of acid; hence, HNO_2/NO_2^- is a strong oxidant under acidic conditions ($E° = 0.99$ V vs NHE), but the reduction potential drops to 0.37 V at pH 7 and to −0.46 V in 1 M base.[28]

$$HNO_2 + H^+ + e^- \longrightarrow NO + H_2O \qquad (6)$$

At pH 7.4, nitrite is ~99.99% NO_2^-, but there is more HNO_2 at lower pH. This is significant in the context of nitrous acid dehydration (eq 7),[29] for which the equilibrium constant was reported to be 3×10^{-3} M^{-1}. The N_2O_3 product is a strong nitrosating agent[29] and can further disproportionate (eq 8; $K = 2 \times 10^{-5}$ M).[30] This combination (eqs 7 and 8) has been proposed as a source of endogenous NO via nitrate reduction to nitrite in saliva, followed by ingestion of the resulting nitrite and then protonation/dehydration/disproportionation in acidic gastric fluids.[31] Similar reactions may also be relevant during ischemic events, where metabolic activity under reduced blood flow leads to tissues becoming more acidic.[19,32]

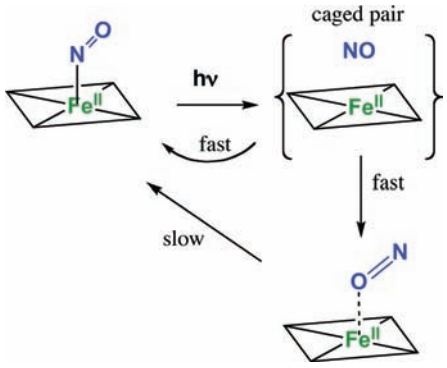
$$2HNO_2 \rightleftharpoons H_2O + N_2O_3 \qquad (7)$$

$$N_2O_3 \rightleftharpoons NO + NO_2 \qquad (8)$$

Bonding of NO and NO_2^- to Metal Centers

NO bonding to metal centers is the subject of other articles in this special Forum issue of *Inorganic Chemistry*, so the comments here are relatively qualitative. Typically, NO binds to the metal via the nitrogen atom, and in such complexes, its character can range (formally) from that of a nitrosyl cation (NO^+), where considerable charge transfer to the metal has occurred, to that of a nitroxyl anion (NO^-), where the charge transfer is in the opposite direction.[33] In the former case, the M−N−O angle is typically ~180°, while in the latter, a "bent" bond angle of ~120° might be anticipated (Figure 1). In this context, the addition of NO to the *meso*-tetraphenylporphyrinato (TPP) complexes Mn^{II}(TPP), Fe^{II}(TPP), and Co^{II}(TPP) gives the NO adducts Mn^{II}(TPP)(NO), Fe^{II}(TPP)(NO), and Co^{II}(TPP)(NO), which display the respective M−N−O bond angles 176.2°, 149.2°, and 135°.[34] The first is consistent with the nitrosyl cation formulation Mn^{I}(TPP)(NO^+), the third with that of a nitroxyl anion complex Co^{III}(TPP)(NO^-), and the Fe^{II}(TPP)(NO) adduct is intermediate in character. Oxidation of Fe^{II}(TPP)(NO) gives Fe^{III}(TPP)(H_2O)(NO), with a 174.4° Fe−N−O angle and a shift of ν_{NO} (in CH_2Cl_2) from 1670 to 1937 cm^{-1};[34] both changes are consistent with a Fe^{II}(TPP)(NO^+) formulation. Qualitatively, one might envision NO^+ complexes as being susceptible to nucleophilic attack and NO^- complexes to electrophilic attack.[35] However, one needs to be cautious in relying on such oversimplified designations because these sometimes give incorrect predictions of qualitative chemical properties.[36]

Scheme 1. Likely Scenario for the Photochemical Formation of the Isonitrosyl Complex Fe(TPP)(*O*N) in a Solid or Low-Temperature Matrix[a]



[a] The rectangle represents a porphyrinato ligand such as TPP^{2-}.

Other NO bonding modes, such as the isonitrosyl form M−O−N, are known; these were generated photochemically and proved to be metastable at best.[37] A likely scenario for the isonitrosyl formation from a complex such as Fe(TPP)(NO) is shown in Scheme 1.

A description of metal−NO interactions was offered some time ago by Enemark and Feltham,[38] who proposed the $\{MNO\}^n$ formulation, where n is the sum of metal d electrons and nitrosyl π^* electrons, and simple Walsh-type molecular orbital diagrams were used to predict the bond angle. When the other ligands provide a strong C_{4v} perturbation, as is the case with metalloporphyrins, the M−N−O angle is predicted to be linear for $n \leq 6$ but bent for $n > 6$. For a six-coordinate complex, the model also predicts that going from $n = 6$ to 7 leads to M−N−O bending and weakens the metal−ligand bond trans to the nitrosyl. This trans effect of NO on the bonding and lability of the proximal ligand in tetragonal $\{MNO\}^7$ systems has been documented in several experimental and computational studies.[39]

This trans-labilizing effect led Traylor and Sharma[9] to propose the mechanism by which sGC is activated by NO coordination at the heme center to give a $\{FeNO\}^7$ complex, leading to weakened bonding of the proximal histidine and thereby activating the enzyme. Burstyn and co-workers tested this premise by investigating the activity of non-native

(27) da Silva, G.; Kennedy, E. M.; Dlugogorski, B. Z. *J. Phys. Chem.* **2006**, *110*, 11371.
(28) Bard, A. J.; Parsons, R.; Jordan, J. *Standard Potentials in Aqueous Solutions*; Marcel Dekker: New York, 1985.
(29) Williams, D. L. H. *Nitrosation Reactions and the Chemistry of Nitric Oxide*; Elsevier: Amsterdam, The Netherlands, 2004.
(30) Treinin, A.; Hayon, E. *J. Am. Chem. Soc.* **1970**, *92*, 5821.
(31) Lundberg, J. O.; Weitzberg, E.; Cole, J. A.; Benjamin, N. *Nat. Rev. Microbiol.* **2004**, *2*, 593.
(32) Samouilov, A.; Kuppusamy, P.; Zweier, J. L. *Arch. Biochem. Biophys.* **1998**, *357*, 1.
(33) (a) Wayland, B. B.; Minkiewixz, J. V.; Abd-Elmageed, M. E. *J. Am. Chem. Soc.* **1974**, *96*, 2795–2801. (b) Eisenberg, R.; Meyer, C. D. *Acc. Chem. Res.* **1975**, *8*, 26. (c) Richter-Addo, G. B.; Legzdins, P. *Metal Nitrosyls*; Oxford University Press: New York, 1992. (d) Greenwood, N. N.; Earnshaw, A. *Chemistry of the Elements*; Pergamon Press: Oxford, U.K., 1993; Chapter 11.
(34) Wyllie, G. R. A.; Scheidt, W. R. *Chem. Rev.* **2002**, *102*, 1067–1090.
(35) Ford, P. C.; Lorkovic, I. M. *Chem. Rev.* **2002**, *102*, 993–1017.
(36) Bordini, J.; Novaes, D. O.; Borissevitch, I. E.; Owens, B. T.; Ford, P. C.; Tfouni, E. *Inorg. Chim. Acta* **2008**, *361*, 2252–2258.
(37) Novozhilova, I. V.; Coppens, P.; Lee, J.; Richter-Addo, G. B.; Bagley, K. A. *J. Am. Chem. Soc.* **2006**, *128*, 2093–2104.
(38) (a) Enemark, J. H.; Feltham, R. D. *J. Am. Chem. Soc.* **1974**, *96*, 5002. (b) Feltham, R. D.; Enemark, J. H. *Top. Stereochem.* **1981**, *12*, 155.

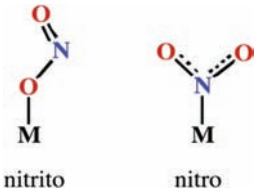


Figure 2. Linkage isomers known for nitrite ion heme complexes.

sGC with Mn^{II}(PPIX) or Co^{II}(PPIX) substituted for Fe^{II}-(PPIX) of the native enzyme (PPIX = protoporphyrin IX dianion).[39e,40] The addition of NO did not activate sGC(Mn) above basal levels, presumably because the proximal histidine for this $\{MnNO\}^6$ complex was not labilized, while NO activation of sGC(Co) gave even greater activity than that with sGC(Fe), consistent with the trans effect on metal center lability having a major role in the NO activation of sGC.

Several linkage isomers represent nitrite ion coordination to metal centers (Figure 2). For heme models and proteins, the most common is the *N*-nitrito ("nitro") structure.[41] However, there have been several recently published structures of heme proteins with *O*-nitrito ("nitrito") coordination. The energy difference between these two structures is only a few kilojoules per mole,[41] so the nature of the proximal ligand trans to the nitrite ion may well affect the relative stabilities of these linkage isomers. Kurtikyan and co-workers have shown that the five-coordinate species Fe(TPP)-(ONO) is the nitrito isomer, but once a proximal ligand such as NH_3 is added, the nitro isomer Fe(TPP)$(NH_3)(NO_2)$ is the more stable six-coordinate complex.[42] Similarly, in heme proteins, hydrogen bonding from amino acids in the distal pocket may determine whether the nitrito or nitro forms are favored. Richter-Addo and co-workers have determined crystal structures of nitrite ion complexes for the ferric forms of myoglobin and hemoglobin (metMb and metHb, respectively) and found both to be *O*-nitrito species.[43] However, the H64 V mutant of metMb, which has the distal histidine replaced by a valine, binds NO_2^- as a nitro complex.[44] This change, which may be triggered by differences in the hydrogen-bonding capability of the relevant amino acid residues or by shifts in the polarity of the binding pocket, illustrates the subtlety of the energetic differences between the isomers.

Metal complexes having bidentate nitrite coordination as well as structures with NO_2^- bridging two metal centers are also known,[33d] and these have been shown to play a role in the catalytic cycle of copper-based nitrite reductases (NiRs).[45] However, although bidentate nitrate coordination has been demonstrated for the ferriheme complex Fe^{III}(TPP)-(NO_3),[46] to our knowledge, such coordination has not been established for a nitrite heme complex.

Table 1. Representative k_{on} and k_{off} (298 K) Values for Fe^{II} and Fe^{III} Heme Models and Proteins in Near-Neutral Aqueous Solutions unless Noted

ferric models and proteins[a]	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	ref
Fe^{III}(TPPS)[b]	4.5×10^5	500	47
Fe^{III}(TMPS)[c]	9.6×10^5	51	48
Fe^{III} (TMPS)(OH)[d]	7.4×10^3	1.5	49
metMb[e]	1.9×10^5	13.6	50
metMb[f]	4.8×10^4	43	51
Cyt^{IIIg}	7.2×10^2	4.4×10^{-2}	50
Cat[h]	3.0×10^7	1.7×10^2	50
eNOS[i]	8.2×10^5	70	52
nNOS[j]	2.1×10^7	40	53
NPn[k]	$1.5-5.5 \times 10^6$	0.006−12.7	54
P450 CYP125[l]	17.1×10^6	11.2	55
$P450_{cam}$ CYP101[m]	0.32×10^6 (34.5×10^6)	0.35 (1.93)	56

ferrous models and proteins	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	ref
Fe^{II}(TPPS)[b]	1.5×10^9	6.4×10^{-4}	47
Hb_4^{Tn}	2.6×10^7	3.0×10^{-3}	57
Hb_4^{Rn}	2.6×10^7	1.5×10^{-4}	57
sGC[o]	1.4×10^8	$(6-8) \times 10^{-4}$	58
sGC[p]		5.0×10^{-2}	58
Mb[q]	1.7×10^7	1.2×10^{-4}	57
Cyt^{IIr}	8.3	2.9×10^{-5}	50
eNOS[s]	1.1×10^6	70	52
nNOS[t]	1.1×10^7	~0	53
P450 BM3[u]	4.7×10^6	13.8	59
P. aeruginosa cd_1 NiR[v]	3.9×10^8	~27.5, 3.8	60

[a] Cyt^{II} = ferrocytochrome *c*, Cyt^{III} = ferricytochrome *c*, eNOS = endothelial nitric oxide synthase, Hb = hemoglobin, Mb = myoglobin, metMb = ferrimyoglobin, nNOS = neuronal nitric oxide synthase, NPn = nitrophorin, sGC = soluble guanylyl cyclase, TMPS = *meso*-tetrakis(sulfonatomesityl)porphinato, TPPS = tetrakis(4-sulfonatophenyl)porphyrinato. [b] 298 K, pH 3. [c] 282 K, pH 3. [d] 283 K, pH 11. [e] 298 K, sperm whale skeletal metMb. [f] 298 K, horse heart metMb. [g] 293 K. [h] 293 K. [i] 283 K, 1 mM arginine. [j] pH 7.8, 293 K, heme domain. [k] Range of 298 K rate constants for NPn1, NPn2, NPn3, and NPn4, pH 5 and 8; k_{off} displays two phases. [l] 10 °C. [m] 25 °C, pH 7.4; the values in parentheses are rate constants for a camphor-bound protein. [n] 293 K; two phases are observed for NO binding. [o] pH 7.4, 293 K, 3 mM Mg^{2+}, 0.5 mM GTP. [p] Phosphate buffer, pH 7, 293 K. [q] H_2O, pH 6.5. [r] 283 K, 1 mM arginine. [s] pH 7.8, 293 K, heme domain. [t] pH 7, 283 K. [u] 30 °C, pH 7. [v] 20 °C, pH 7.

Substitution Reactions Leading to the Formation of Heme Nitrosyls

Table 1[47−60] lists some representative rate constants, k_{on} and k_{off}, for the reactions of NO with ferri- and ferroheme

(39) (a) Choi, I.-K.; Ryan, M. D. *Inorg. Chim. Acta* **1988**, *153*, 25–30. (b) Bohle, D. S.; Hung, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 9584–9585. (c) Patterson, J. C.; Lorkovic, I. M.; Ford, P. C. *Inorg. Chem.* **2003**, *42*, 4902–4908. (d) Praneeth, V. K. K.; Näther, C.; Peters, G.; Lehnert, N. *Inorg. Chem.* **2006**, *45*, 2795–2811. (e) Dierks, E. A.; Hu, S. Z.; Vogel, K. M.; Yu, A. E.; Spiro, T. G.; Burstyn, J. N. *J. Am. Chem. Soc.* **1997**, *119*, 7316–7323.

(40) Heinecke, J.; Ford, P. C. *Coord. Chem. Rev.* **2010**, *254*, 235–247.

(41) Silaghi-Dumitrescu, R. *Inorg. Chem.* **2004**, *43*, 3715.

(42) (a) Kurtikyan, T. S.; Ford, P. C. *Angew. Chem., Int. Ed.* **2006**, *45*, 492. (b) Kurtikyan, T. S.; Hovhannisyan, A. A.; Hakobyan, M. E.; Patterson, J. C.; Iretskii, A.; Ford, P. C. *J. Am. Chem. Soc.* **2007**, *129*, 3576. (c) Kurtikyan, T. S.; Ford, P. C. *Coord. Chem. Rev.* **2008**, *252*, 1486. (d) Kurtikyan, T. S.; Hovhannisyan, A. A.; Iretskii, A.; Ford, P. C. *Aust. J. Chem.* **2009**, *62*, 1226–1230.

(43) (a) Yi, J.; Safo, M. K.; Richter-Addo, G. B. *Biochemistry* **2008**, *47*, 8247–8249. (b) Copeland, D. M.; Soares, A. S.; West, A. H.; Richter-Addo, G. B. *J. Inorg. Biochem.* **2006**, *100*, 1413–1425.

(44) Yi, J.; Heinecke, J.; Tan, H.; Ford, P. C.; Richter-Addo, G. B. *J. Am. Chem. Soc.* **2009**, *131*, 18119–18128.

(45) (a) Boulanger, M. J.; Murphy, M. E. P. *Protein Sci.* **2003**, *12*, 248–256. (b) Lehnert, N.; Cornelissen, U.; Neese, F.; Ono, T.; Noguchi, Y.; Okamoto, K.-I.; Fujisawa, K. *Inorg. Chem.* **2007**, *46*, 3916–3933. (c) Ghosh, S.; Dey, A.; Sun, Y.; Scholes, C. P.; Solomon, E. I. *J. Am. Chem. Soc.* **2009**, *131*, 277–288.

(46) Phillippi, M. A.; Baenziger, N.; Goff, H. M. *Inorg. Chem.* **1981**, *20*, 3904–3911.

(47) (a) Laverman, L. E.; Hoshino, M.; Ford, P. C. *J. Am. Chem. Soc.* **1997**, *119*, 12663–12665. (b) Laverman, L. E.; Ford, P. C. *J. Am. Chem. Soc.* **2001**, *123*, 11614–11622.

(48) Franke, A.; Stochel, G.; Suzuki, N.; Higuchi, T.; Okuzono, K.; van Eldik, R. *J. Am. Chem. Soc.* **2005**, *127*, 5360–5375.

(49) Wolak, M.; van Eldik, R. *J. Am. Chem. Soc.* **2005**, *127*, 13312–13315.

(50) Hoshino, M.; Ozawa, K.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc.* **1993**, *115*, 9568–9575.

(51) Laverman, L. E.; Wanat, A.; Oszajca, J.; Stochel, G.; Ford, P. C.; van Eldik, R. *J. Am. Chem. Soc.* **2001**, *123*, 285–293.

models and proteins (eq 9) determined by flash photolysis or stopped-flow kinetics. For the water-soluble heme model complexes Fe^{II}(TPPS) and Fe^{III}(TPPS) [TPPS = tetrakis(4-sulfonatophenyl)porphyrinato], as well as for the oxygen-carrying globin proteins Mb and Hb, the ferrous systems show significantly larger k_{on} and smaller k_{off} values, thus much larger association constants K than the ferric analogues. While this trend would appear to extend to other heme models and proteins, it becomes less obvious; the on and off rates are as much controlled by the nature of the distal and proximal ligands on the hemes and by the protein structure as by the oxidation state of the iron center. Examples are the ferri- and ferrocytochrome *c* proteins (Cyt^{III} and Cyt^{II}), both of which show quite small k_{on} and k_{off} values.[50] A logical argument for the low reactivities both of Cyt^{II} and of Cyt^{III} toward NO is the coordination at both metal center axial sites by protein amino acids, an imidazole nitrogen at one and a methionine sulfur at the other. Thus, formation of the nitrosyl complex for either Cyt^{II} or Cyt^{III} not only involves ligand displacement but also significant protein conformational changes.

$$M(Por) + NO \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} M(Por)(NO) \qquad (9)$$

One might pose a simple question regarding the reaction between NO and various metal centers to form a nitrosyl complex (eq 10): Does the free-radical nature of NO lead to different mechanisms for ligand substitution reactions than those seen for other small ligands? The answer is at best ambiguous. In many cases, the reactivity of NO does appear to be similar to that seen for other small Lewis bases such as CO,[47] but this may be the result of other parameters, such as ligand dissociation, controlling the dynamics of the overall reaction. However, certain systems do clearly show marked mechanistic differences between the metal bond-forming reactions of NO and those of other Lewis base ligands. For example, the back-reactions of geminate pairs {L_nM, AB} formed by flash photolysis of a L_nM-AB complex (or from an analogous encounter pair formed by the diffusion of L_nM and AB together) display markedly different reactivity patterns for NO and CO.[61−63]

$$ML_nX + NO \rightleftharpoons ML_n(NO) + X \qquad (10)$$

Until recently, there had been few systematic studies of the reaction mechanism(s) of metal−NO bond formation. In an early study, Taube and co-workers[64] examined the kinetics of nitrosylation of the Ru^{III} complex $Ru(NH_3)_6^{3+}$ in an acidic aqueous solution (eq 11) and found the rate constant (k_{NO} = 0.2 M^{-1} s^{-1} at 298 K) to be much larger than that for NH_3 substitution by other ligands. They concluded that the reaction very likely proceeds by an associative mechanism, whereby the unpaired electron of the d^5 Ru^{III} center engages the odd electron of the NO to give a seven-coordinate transient $Ru(NH_3)_6(NO)^{3+}$. Later activation parameter measurements[65] proved consistent with this proposal given that $\Delta H^{\ddagger}$ is small (41 kJ mol^{-1}), while $\Delta S^{\ddagger}$ is large and negative (−114 J K^{-1} mol^{-1}), as is the activation volume $\Delta V^{\ddagger}$ (−14 cm^3 mol^{-1}).[65b]

$$Ru(NH_3)_6^{3+} + NO + H^+ \longrightarrow Ru(NH_3)_5(NO)^{3+} + NH_4^+ \qquad (11)$$

Should a similar mechanism be expected for the ferriheme models and proteins that also have the d^5 electronic configuration (albeit, mostly in the high-spin state rather than low-spin state characteristic of Ru^{III} complexes)? In this context, we examined the mechanism of this ligand substitution for Fe^{III}(TPPS)$(H_2O)_2$ using flash photolysis techniques.[47] (Note: we will often use Fe^{III}(TPPS) as a shorthand designation for Fe^{III}(TPPS)$(H_2O)_2$.) NO photodissociation from nitrosyl metalloporphyrins is commonly reversible, so pulsed laser techniques are well suited for investigation of the kinetics of eq 9. In such studies, the NO is labilized from the M(Por)(NO) precursor during the nanosecond pulse, and the subsequent relaxation of the system back to equilibrium is monitored spectroscopically (Figure 3). Under excess NO, the transient spectra decay exponentially to give the rate constants k_{obs}, and the relationship described by eq 12 should hold true. A plot of k_{obs} vs [NO] would then be linear with a slope of k_{on} and an intercept of k_{off}. For the examples shown in Figure 4, k_{off} is sufficiently large that one can determine an accurate value of the intercept; thus, the slope/intercept ratio (k_{on}/k_{off}) gives a reasonable estimate of the equilibrium constant (K) for nitrosyl complex formation. By using this technique, K's determined for nitrosyl complexes of metmyoglobin, ferricytochrome *c*, and catalase (Cat)[50] in aqueous media were in good agreement with values determined by spectroscopic titration. For cases where the "off" reaction is too slow to give accurate intercept values, it is sometimes possible to measure k_{off} by using an efficient trapping agent like $Ru(EDTA)^-$ to sequester any labilized NO.[47b,51]

$$k_{obs} = k_{on}[NO] + k_{off}, \quad K = k_{on}/k_{off} \qquad (12)$$

Laverman et al.[47] determined the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ (from the temperature dependence, 298−318 K, e.g., Figure 4) and $\Delta V^{\ddagger}$ (from the hydrostatic pressure

(52) Abu-Soud, H. M.; Ichimori, K.; Presta, A.; Stuehr, D. J. *J. Biol. Chem.* **2000**, *275*, 17349–57.
(53) Scheele, J. S.; Bruner, E.; Kharitonov, V. G.; Martasek, P.; Roman, L. J.; Masters, B. S.; Sharma, V. S.; Magde, D. *J. Biol. Chem.* **1999**, *274*, 13105–13110.
(54) Andersen, J. F.; Ding, X. D.; Balfour, C.; Shokhireva, T. K.; Champagne, D. E.; Walker, F. A.; Montfort, W. R. *Biochemistry* **2000**, *39*, 10118.
(55) Ouellet, H.; Lang, J.; Couture, M.; Ortiz de Montellano, P. R. *Biochemistry* **2009**, *48*, 863–872.
(56) Franke, A.; Stochel, G.; Jung, C.; Van Eldik, R. *J. Am. Chem. Soc.* **2004**, *126*, 4181–4191.
(57) (a) Olson, J. S.; Phillips, G. N. *J. Biol. Chem.* **1996**, *271*, 17593. (b) Ikeda-Saito, M.; Dou, Y.; Yonetani, T.; Olson, J. S.; Li, T.; Regan, R.; Gibson, Q. H. *J. Biol. Chem.* **1993**, *268*, 6855. (c) Olson, J. S.; Phillips, G. N. *J. Biol. Chem.* **1996**, *271*, 17593.
(58) (a) Sharma, V. S.; Magde, D. *Methods−A Companion to Methods in Enzymology* **1999**, *19*, 494. (b) Kharitonov, V. G.; Russwurm, M.; Madge, D.; Sharma, V. S.; Koesling, D. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 284.
(59) Quaroni, L. G.; Seward, H. E.; McLean, K. J.; Girvan, H. M.; Ost, T. W. B.; Noble, M. A.; Kelly, S. M.; Price, N. C.; Cheesman, M. R.; Smith, W. E.; Munro, A. W. *Biochemistry* **2004**, *43*, 16416–16431.
(60) Rinaldo, S.; Arcovito, A.; Brunori, M.; Cutruzzolà, F. *J. Biol. Chem.* **2007**, *282*, 14761–14767.
(61) Walda, K. N.; Liu, X. Y.; Sharma, V. S.; Magde, D. *Biochemistry* **1994**, *33*, 2198–2209.
(62) Traylor, T. G.; Luo, J.; Simon, J. A.; Ford, P. C. *J. Am. Chem. Soc.* **1992**, *114*, 4340–4345.
(63) Strickland, N.; Harvey, J. N. *J. Phys. Chem. B* **2007**, *111*, 841–852.
(64) Armor, J. N.; Scheidegger, H. A.; Taube, H. *J. Am. Chem. Soc.* **1968**, *90*, 5928.
(65) (a) Armor, J. N.; Pell, S. D. *J. Am. Chem. Soc.* **1973**, *95*, 7625. (b) Czap, A.; van Eldik, R. *Dalton Trans.* **2003**, 665–671.

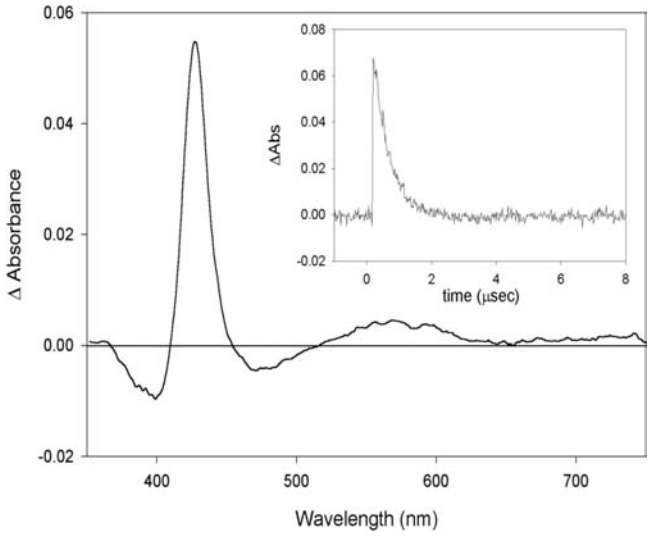


Figure 3. Transient difference spectrum of Fe^{II}(TMPS)(NO) in an aqueous solution under a NO atmosphere ([NO] = 1.8 mM) recorded 50 ns after 355 nm flash photolysis. The generation of excess Fe^{II}(TMPS) by NO photodissociation from Fe^{II}(TMPS)(NO) is indicated by the strong Soret band absorbance at 426 nm [TMPS = *meso*-tetrakis-(sulfonatomesityl)porphinato]. Inset: Temporal profile of the relaxation back to equilibrium recorded at 426 nm [$k_{obs} = (k_{on}[NO] + k_{off}) = 2.0 \times 10^6\ s^{-1}$ for these conditions (296 K)]. Adapted from ref 47b.

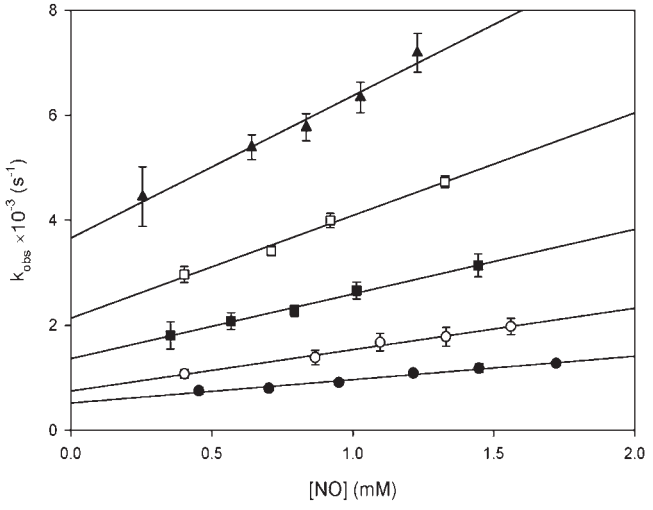


Figure 4. Plots of k_{obs} vs [NO] for the reaction of NO with Fe^{III}(TPPS) as a function of the temperature in an aqueous solution (filled circles, 25 °C; open circles, 30 °C; filled squares, 35 °C; open squares, 40 °C; triangles, 45 °C; from ref 47b). The slopes of such plots are the rate constants k_{on}, while the intercepts are the k_{off} values at different temperatures.

dependence, 0.1−250 MPa) for k_{on} and k_{off} values measured for aqueous solution NO reactions with Fe^{III}(TPPS) (Table 2). Notably, although the "on" reaction for Fe^{III}(TPPS) is much faster than that for $Ru(NH_3)_6^{3+}$, $\Delta H^{\ddagger}_{on}$ is larger for the ferriheme (69 vs 41 kJ mol^{-1}). The key difference is that $\Delta S^{\ddagger}_{on}$ for the ferriheme complexes is large and positive (+ 95 J mol^{-1} K^{-1})[47] while that for the Ru^{III} complex is large and negative (−114 J K^{-1} mol^{-1}).[47] Similarly, there is a marked difference in the activation volumes ($\Delta V^{\ddagger}$ = +9 and −14 cm^3 mol^{-1}, respectively).[47,65b] This pattern of the activation parameters for the k_{on} values for $Fe^{III}(TPPS)(H_2O)_2$ is a clear indication of a dissociative mechanism controlled by the lability of the axial water molecules (eqs 13 and 14) and is in direct contrast to the pattern for the NO reaction of $Ru(NH_3)_6^{3+}$, for which

Table 2. Kinetics Data for NO "On" and "Off" Reactions for Selected Heme Centers (Data from Refs 47 and 51

"on" reactions	k_{on} (M^{-1} s^{-1})	$\Delta H^{\ddagger}_{on}$ (kJ mol^{-1})	$\Delta S^{\ddagger}_{on}$ (J mol^{-1} K^{-1})	$\Delta V^{\ddagger}_{on}$ (cm^3 mol^{-1})
Fe^{III}(TPPS) + NO	4.5×10^5	69 ± 3	95 ± 10	9 ± 1
metMb + NO	4.8×10^4	63 ± 2	55 ± 8	20 ± 6
Fe^{II}(TPPS) + NO	1.5×10^9	24 ± 3	12 ± 10	5 ± 1
"off" reactions	k_{off} (s^{-1})	$\Delta H^{\ddagger}_{off}$ (kJ mol^{-1})	$\Delta S^{\ddagger}_{off}$ (J mol^{-1} K^{-1})	$\Delta V^{\ddagger}_{off}$ (cm^3 mol^{-1})
Fe^{III}(TPPS)(NO)	0.5×10^3	76 ± 6	60 ± 11	18 ± 2
metMb(NO)	42	68 ± 4	14 ± 13	18 ± 3

an associative mechanism was assigned.

$$Fe^{III}(TPPS)(H_2O)_2 \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} Fe^{III}(TPPS)(H_2O) + H_2O \quad (13)$$

$$Fe^{III}(TPPS)(H_2O) + NO \underset{k_{-ii}}{\overset{k_{ii}}{\rightleftharpoons}} Fe^{III}(TPPS)(H_2O)NO \quad (14)$$

The mechanism defined by eqs 13 and 14 also suggests that water exchange for $Fe^{III}(TPPS)(H_2O)_2$ should be much faster than the reaction with NO, which experimentally was present only at millimolar or lower concentrations. This indeed is the case; Hunt et al.[66] have reported that water exchange between the solvent and $Fe^{III}(TPPS)(H_2O)_2$ occurs with a first-order rate constant ($k_{ex} = 1.4 \times 10^7\ s^{-1}$ in 298 K water) far exceeding the k_{obs} values determined at any [NO]. Also consistent with a dissociative mechanism are the activation parameters measured for exchange, $\Delta H^{\ddagger}_{ex}$ (57 kJ mol^{-1}) and $\Delta S^{\ddagger}_{ex}$ (+84 J K^{-1} mol^{-1}), which parallel the respective k_{on} activation parameters. The exchange reaction was subsequently reexamined by Schneppensieper et al.[67] using variable-temperature/pressure NMR techniques, and those workers reported the values $\Delta H^{\ddagger}_{ex}$ = 67 kJ mol^{-1}, $\Delta S^{\ddagger}_{ex}$ = 99 J mol^{-1} K^{-1}, and $\Delta V^{\ddagger}_{ex}$ = 7.9 cm^3 mol^{-1}, which are in even better agreement with those found by Laverman et al. for the k_{on} pathway with NO.[47] Thus, the factors that determine the solvent-exchange kinetics for $Fe^{III}(TPPS)(H_2O)_2$ dominate the NO reaction with the same species, and it is clearly evident that the activation parameters of both processes are largely defined by a dissociative mechanism, with the limiting step being eq 13.

Analogously, the activation parameters determined for the "off" reaction from Fe^{III}(TPPS)(NO) are consistent with NO dissociation being the rate-limiting process, as would be expected according to the principle of microscopic reversibility. The dissociative intermediate(s) formed in the "off" step should be the same as those generated along the "on" pathway, with iron nitrosyl bond breaking (k_{-ii}) being the dominant step. The observation that $\Delta V^{\ddagger}_{off}$ is substantially larger than $\Delta V^{\ddagger}_{on}$ may reflect solvation changes due to charge transfer from NO to Fe^{III} upon coordination to give a linearly bonded diamagnetic $\{FeNO\}^6$ complex that can be formally represented as $Fe^{II}(NO^+)$. Thus, NO dissociation is accompanied by spin change and solvent reorganization as the charge relocalizes on the metal.

Table 2 also summarizes studies in this laboratory in collaboration with van Eldik, Stochel, and co-workers[51] to

(66) Ostrich, I. J.; Gordon, L.; Dodgen, H. W.; Hunt, J. P. *Inorg. Chem.* **1980**, *19*, 61.
(67) Schneppensieper, T.; Zahl, A.; van Eldik, R. *Angew. Chem., Int. Ed.* **2001**, *40*, 1678.

determine the activation parameters for the k_{on} and k_{off} rate constants for the NO reaction with metMb (eq 15). Comparison of these activation parameters with those determined for reactions of Fe^{III}(TPPS) demonstrates strong similarities. Again, the k_{on} step appears to be defined largely by the lability of the metal center of metMb(H_2O). However, it is clear that diffusion through protein channels, distal residues, and the coordinated proximal histidine must influence the NO binding kinetics. These issues may indeed be reflected in the lower $\Delta S^{\ddagger}$ values for both the "on" and "off" reactions of metMb.

$$\text{metMb} + \text{NO} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{metMb(NO)} \qquad (15)$$

In a related study, Cao et al. reported observing the five-coordinate intermediate generated by flash photolysis of metMb(NO).[68] They further showed that k_{on} is several orders of magnitude larger for the metMb mutant H64G, where glycine is substituted for the distal histidine, than for native hh metMb. This disparity was interpreted in terms of the coordinated water in the native protein being stabilized through hydrogen bonding to His-64.

For the water-soluble ferrous complex Fe^{II}(TPPS), the NO "on" rate is about 3 orders of magnitude faster than that for the ferric analogue (Table 1). Correspondingly, the activation parameters $\Delta H^{\ddagger}_{on}$, $\Delta S^{\ddagger}_{on}$, and $\Delta V^{\ddagger}_{on}$ are much smaller than those for the analogous reaction with Fe^{III}(TPPS) (Table 2) and are consistent with rates largely defined by diffusional factors.[47b] The data in Table 1 illustrate the common observation that high-spin Fe^{II}(Por) complexes tend to be considerably more reactive with NO than the Fe^{III}(Por) analogues, not only for model compounds but also for myoglobin and hemoglobin. Because the ferroheme center may be five-coordinate in such cases, formation of the Fe^{II}−NO bond does not require the initial displacement of another ligand, and nitrosyl complex formation is thus not limited by the rate of ligand labilization.

Such a very large k_{on} value (and, correspondingly, a high binding constant K) would appear to be essential for the activation of sGC as a key step in blood pressure control. It is well established that sGC is regulated at nanomolar NO concentrations, and these concentrations could only be effective if the rate constant for sGC(NO) formation were very large, as has been demonstrated (Table 1).

Although this ferrous versus ferric trend appears to have validity for numerous heme proteins, it is important to reemphasize that the pattern of reactivity is strongly dependent on the other ligands bound to the metal center as well as on the protein structure.[54] The Cyt^{II} and Cyt^{III} cases were discussed above. More subtle examples are seen for the k_{on} values of the ferric hemes. These tend to be higher for the more strongly donating proximal ligands such as thiolates as compared to the histidine of the globins. An example of an extreme perturbation was the replacement of the dianionic porphyrinato macrocycle Por^{2-} with the trianionic corrolato ligand C^{3-}. Limited data indicate that NO reactions with the Fe^{III}(C) complexes display high k_{on} and K values comparable to those of analogous ferrous Fe^{II}(Por) species.[69]

Furthermore, the corollary of the observation that NO coordination to a ferrous heme leads to labilization of the proximal ligand in some cases[9] would be that a strongly bonding proximal ligand should correspondingly weaken the Fe^{II}−NO interaction. This may well explain the higher value of k_{off} found for the bacterial ferrous heme protein P450 BM3, which has a thiolato distal axial ligand.[59] The view that the nitrosyl bonding will be dependent on the nature of the proximal ligand is substantiated by IR spectral studies.[70,71]

The key point is that while typically the "off" rates of the ferrous nitrosyl proteins tend to be slower than those in the ferric analogues, the k_{off} values are also sensitive to the nature of the specific ligand in the proximal site and of the protein structure. Thus, there may be quite some variation in the nitrosyl lability.

The small k_{off} values typically found for ferroheme proteins are relevant to the question of how sGC, once activated by the formation of an NO complex, might then deactivate. Kharitonov et al.[58b] have used stopped-flow kinetics techniques to determine the first-order loss of NO from sGC−NO. They measured a rate constant of $\sim 7 \times 10^{-4}$ s^{-1} in a 293 K, pH 7.4, buffered solution, much slower than would be necessary for reversible deactivation of the enzyme. However, in the presence of excess substrate GTP (5 mM) plus the Mn^{2+} cofactor (3 mM), the rate was about 70-fold faster ($k_{off} \sim 5 \times 10^{-2}$ s^{-1} at 293 K). An in vivo study suggests that the actual rate of sGC deactivation may be significantly faster (3.7 s^{-1} at 310 K),[10b] an observation that illustrates the complexity of comparing in vitro kinetics of purified proteins to analogous reactions in vivo.

Reactions of Coordinated NO

As noted above, M−NO coordination often involves charge transfer between the metal and the NO ligand, and this would be expected to affect reactivity. Thus, one can easily speculate that a NO linearly coordinated to an oxidizing metal center may be susceptible to nucleophilic attack, whereas the bent nitroxyl complexes would be more inclined to react with electrophiles such as H^+. This qualitative picture has indeed been realized for each limiting case. An example of the former is the long-known, reversible reaction of a hydroxide ion with the substitution-inert iron(III) nitrosyl nitroprusside to give a nitro species that is formally an Fe^{II} complex (eq 16). This can be considered an intramolecular redox reaction mediated by facile nucleophilic attack at a coordinated nitrosyl, although this description might be challenged on the basis that $Fe^{II}(CN)_5(NO^+)^{2-}$ is a valid resonance structure of nitroprusside, so the transformation does not really involve an electron transfer. Less debatable are numerous reactions of metal centers, including heme systems, where NO serves as a one-electron reductant of a transition-metal center while itself being oxidized to a labile nitrite or another N^{III} species (see below). Related reactions of other metal complexes are summarized elsewhere.[35,72,73]

$$Fe^{III}(CN)_5(NO)^{2-} + 2OH^- \rightleftharpoons Fe^{II}(CN)_5(NO_2)^{4-} + H_2O \qquad (16)$$

(68) Cao, W.; Christian, J. F.; Champion, P. M.; Rosca, F.; Sage, J. T. *Biochemistry* **2001**, *40*, 5728.
(69) Joseph, C. A.; Ford, P. C. *J. Am. Chem. Soc.* **2005**, *127*, 6737–6743.
(70) Paulat, F.; Berto, T. C.; DeBeer George, S.; Goodrich, L. E.; Praneeth, V. K. K.; Sulok, C. D.; Lehnert, N. *Inorg. Chem.* **2008**, *47*, 11449–11451.
(71) (a) Swinehart, J. H. *Coord. Chem. Rev.* **1967**, *2*, 385. (b) Olabe, J. A.; Gentil, L. A.; Rigotti, G.; Navaza, A. *Inorg. Chem.* **1984**, *23*, 4297.
(72) (a) Bottomley, F. *React. Coord. Ligands* **1989**, *2*, 115. (b) McCleverty, J. A. *Chem. Rev.* **1979**, *79*, 53.
(73) Ford, P. C.; Fernandez, B. O.; Lim, M. D. *Chem. Rev.* **2005**, *105*, 2439–2455.

Nitrosyl protonation was reported by Roper et al.,[74] who showed that the addition of HCl to the osmium complex $Os(PPh_3)_2Cl(NO)$ gave the first characterized example of an N-coordinated HNO ("nitroxyl") complex $OsCl_2(PPh_3)_2(HNO)$[75] (eq 17). An alternative path to nitroxyl complexes is the reduction of the M−NO unit, followed by protonation, and such coupled reduction/protonation schemes have been argued to have relevance to enzymatic nitrogen oxide reductases. Farmer and co-workers[76] have reported using graphite electrodes modified by the deposition of surfactant films of Mb(NO) on the surface. Electrochemical reduction ($E_{1/2}$ = −0.63 V vs NHE) to $Mb(NO^-)_{surface}$ was accompanied by protonation to provide a $Mb(HNO)_{surface}$ complex. A solution-phase version of Mb(HNO), characterized by a ^{1}H NMR singlet at 14.8 ppm, was prepared in an aqueous solution by reducing Mb(NO) with Cr^{2+}.[77] Nitroxyl complexes and reactions of HNO with biologically relevant targets are subjects of another article in this Forum issue.

$$Os(PPh_3)_2Cl(NO) + HCl \rightleftharpoons OsCl_2(PPh_3)_2(H\underline{N}O) \quad (17)$$

Reductive Nitrosylation. Ferric porphyrinato complexes and ferriheme proteins have long been known to undergo "reductive nitrosylation" (sometimes called "autoreduction") in the presence of excess NO. For example, the iron(III) porphyrin complex $Fe^{III}(TPP)(Cl)$ reacts with NO in toluene containing a small amount of methanol to give $Fe^{II}(TPP)(NO)$, consistent with the reductive nitrosylation sequence shown in eqs 18−20.[78] The key step in this sequence is the transfer of NO^+ from the activated $Fe^{III}NO$ complex to a nucleophile such as methanol to give *O*-nitrosomethoxide, that is, methyl nitrite.

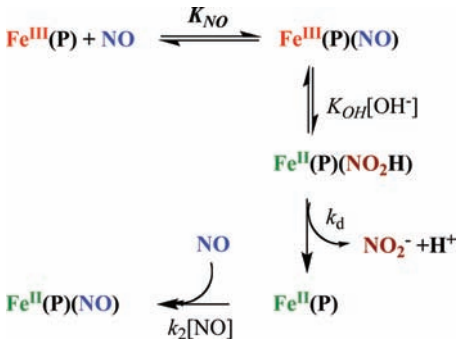
$$Fe^{III}(TPP)Cl + NO \rightleftharpoons Fe^{III}(TPP)(Cl)(NO) \quad (18)$$

$$Fe^{III}(TPP)(Cl)(NO) + CH_3OH \longrightarrow Fe^{II}(TPP) + CH_3ONO + HCl \quad (19)$$

$$Fe^{II}(TPP) + NO \longrightarrow Fe^{II}(TPP)(NO) \quad (20)$$

When ferriheme proteins such as methemoglobin are exposed to excess NO in near-neutral aqueous solutions, the products are often the ferroheme nitrosyl adducts (e.g., eq 5).[79,80] In this context, Hoshino et al. followed up flash photolysis studies[50] of the ferriheme proteins Cyt^{III}, metMb, and metHb (eq 9) with a quantitative investigation of the reductive nitrosylations of the same proteins at various pH's.[81,82] On the basis of the rate dependencies on the pH and [NO], the mechanism shown in Scheme 2 [where $Fe^{III}(P)$ is the ferriheme protein] was proposed.

Scheme 2. Proposed Mechanism for the Base-Catalyzed Reductive Nitrosylation of Ferriheme Proteins $Fe^{III}(P)$ (Ref 81)



For Mb and Hb, the rate constant for the reaction of $Fe^{II}(P)$ with NO is large, and the product is $Fe^{II}(P)(NO)$, so reduction of the ferric protein is the rate-limiting step, leading to the rate law depicted in eq 21.[81] An exception is the cytochrome *c* system. Because Cyt^{II} reacts quite slowly with NO (Table 1), it was possible to observe the formation of Cyt^{II} directly, but the same rate law would apply. For metMb and Cyt^{III}, the observed rates were functions of [NO] and [OH^-], as predicted by eq 21, namely, $k_{obs} = k_{OH}K_{NO}[NO][OH^-]/(1 + K_{NO}[NO])$ at low pH (where $k_{OH} = k_dK_{OH}$) and $k_{obs} = k_{OH}[OH^-]$ at high [NO]. The proposed N-coordinated nitrous acid complexes were not observed, so either the formation of this intermediate is rate-limiting or K_{OH} is very small. The k_{OH} values had the respective values 0.32×10^3, 1.5×10^3, and 3.2×10^3 M^{-1} s^{-1} for metMb, Cyt^{III}, and metHb in 298 K aqueous media.

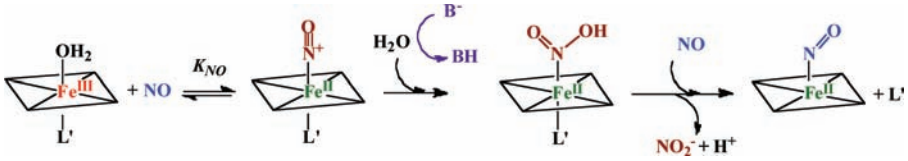
$$d[Fe^{II}(P)]_{tot}/dt = k_d\frac{K_{NO}[NO]}{1 + K_{NO}[NO]} \times \frac{K_{OH}[OH^-]}{1 + K_{OH}[OH]}[Fe^{III}(P)] \quad (21)$$

Unlike the other two proteins, metHb undergoes reductive nitrosylation at low pH values (< 6), implying that metHb(NO) reacts not only with OH^- but also with H_2O.[50,81] Under conditions of relatively high [NO], where metHb is fully in the nitrosyl form, k_{H_2O} was determined to be 1.1×10^{-3} s^{-1} in a 298 K aqueous solution.

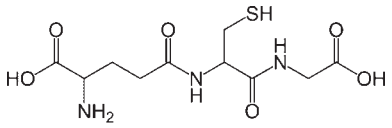
Nucleophiles other than OH^- or H_2O may also participate. For example, the NO reduction of metMb in the presence of the biological antioxidant glutathione (GSH) has been reported to give Mb(NO) as one product and *S*-nitrosoglutathione (GSNO) as the other (eq 22). The second-order rate constant for the reaction of GSH with metMb(NO) was recorded as 47 M^{-1} s^{-1}, which is somewhat surprising given that k_{OH} for the smaller hydroxide ion is but 1 order of magnitude higher. In this context, one might keep in mind that metMb(NO) has a much higher reduction potential than does wt metMb itself, owing to the greater stability of ferrous Mb(NO). Because recent studies have demonstrated that cysteine slowly reduces metMb at physiological pH even in the absence of NO,[44] it is not unreasonable to expect that the reaction of metMb(NO) plus GSH might be faster, even without

(74) Grundy, K. R.; Reed, C. A.; Roper, W. R. *Chem. Commun.* **1971**, 1501.
(75) (a) Wilson, R. B.; Ibers, J. A. *Inorg. Chem.* **1979**, *18*, 336. (b) Marhenke, J.; Joseph, C. A.; Corliss, M. Z.; Dunn, T. J.; Ford, P. C. *Polyhedron* **2007**, *26*, 4638–4644.
(76) Bayachou, M.; Lin, R.; Cho, W.; Farmer, P. J. *J. Am. Chem. Soc.* **1998**, *102*, 9888.
(77) Lin, R.; Farmer, P. J. *J. Am. Chem. Soc.* **2000**, *122*, 2393.
(78) (a) Wayland, B. B.; Olson, L. W. *J. Chem. Soc., Chem. Commun.* **1973**, 897. (b) Wayland, B. B.; Olson, L. W. *J. Am. Chem. Soc.* **1974**, *96*, 6037.
(79) Ehrenberg, A.; Szczepkowski, T. W. *Acta Chem. Scand.* **1960**, *14*, 1.
(80) Chien, J. C. W. *J. Am. Chem. Soc.* **1969**, *91*, 2166.
(81) Hoshino, M.; Maeda, M.; Konishi, R.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc.* **1996**, *118*, 5702–5707.
(82) Reichenbach, G.; Sabatini, S.; Palombari, R.; Palmerini, C. A. *Nitric Oxide* **2001**, *5*, 395.

Scheme 3



invoking nucleophilic attack at the coordinated NO. This system needs more detailed investigation.



GSH

$$\text{metMb} + 2\text{NO} + \text{GSH} \longrightarrow \text{Mb(NO)} + \text{GSNO} + \text{H}^+ \quad (22)$$

Hoshino et al.[81] found it difficult to quantify the nitrite formed by the NO reduction of ferriheme proteins, with their measured values being ~30−40% short of that predicted by Scheme 2. This was attributed to analytical difficulties. However, Luchsinger et al.[83] have suggested that, in the case of metHb, the low NO_2^- analysis may result from the formation of another reductive nitrosylation product, hemoglobin modified at the cysteine-93 of the β-chain by S-nitrosation, often called "SNO-Hb",

The observation that metHb was reduced by NO via a hydroxide-independent pathway led Fernandez et al.[84,85] to examine more closely the kinetics for the water-soluble ferriheme model Fe^{III}(TPPS) in moderately acidic, buffered aqueous solutions (pH 4−6). The question was whether reductive nitrosylation would display general base catalysis. Under such conditions, there were progressive spectral changes consistent with the reduction of the ferric species to the ferrous analogue, giving, for example, k_{obs} of 2.36×10^{-4} s^{-1} for the NO reduction of Fe^{III}(TPPS) in a pH 5 acetate-buffered solution (20 mM) under 1.0 atm of NO. The effects of pH and of NO and buffer concentrations were probed in detail, and the reduction rates gave curved k_{obs} vs [NO] plots that approached saturation at $P_{NO} \sim 1$ atm, consistent with the general mechanism described by Scheme 2. These plots could be modeled accurately using the K_{NO} value of 1.3×10^3 M^{-1}, which was consistent with earlier measurements.[50]

The kinetics of this reaction were pH-independent in moderately acidic solutions (pH 4−5); thus, specific base catalysis was not responsible. However, the k_{obs} values were dependent on the nature and concentration of the buffer.[85] For example, a plot of k_{obs} vs [buffer] (NaOAc/HOAc at 0.1 M ionic strength) was linear, indicating the relationship $k_{red} = k_0 + k_{buffer}$[buffer], where k_0 is the buffer-independent term and k_{buffer} is the catalytic term due to the buffer. For $P_{NO} = 1$ atm, the term $K_{NO}/(1 + K_{NO}[NO])$ has the value 0.7, and from this, the values $k_0 = 2.8 \times 10^{-4}$ s^{-1} and $k_{acetate} = 2.4 \times 10^{-3}$ M^{-1} s^{-1} were calculated. At the same pH and P_{NO}, the buffer DESPEN exhibited a 5-fold greater slope. Similar catalysis was noted for a phosphate buffer at pH 6. Consistent with these data would be a mechanism for NO reduction of Fe^{III}(TPPS), as illustrated by Scheme 3.

Reactions Involving Nitrite

In the course of probing the buffer catalysis of the reductive nitrosylation of Fe^{III}(TPPS), Fernandez et al.[84−86] made the remarkable discovery that ferriheme reduction by NO is also dramatically catalyzed by traces of a nitrite ion, one of the products. Notably, NO_2^- is also a ubiquitous impurity in aqueous NO solutions because it is the product of NO autoxidation.[3] The k_{obs} values proved to be a linear function of [NO_2^-] [eq 23, where f(NO) = (K_{NO}[NO]/(1 + K_{NO}[NO])] and accounts for the preequilibrium with NO. From these data for Fe^{III}(TPPS), the catalytic rate constant $k_{nitrite}$ was calculated as 3.1 M^{-1} s^{-1}, 3 orders of magnitude larger than the effect of the acetate buffer. Reduction of Fe^{III}(TPPS) was not observed unless NO was added.

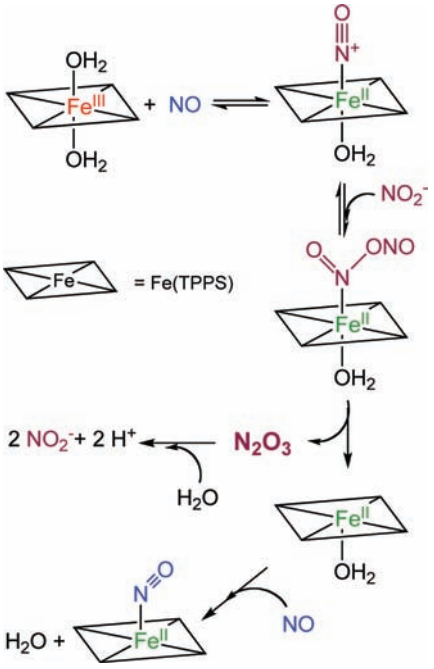
$$k_{obs} = (k_{red} + k_{nitrite}[NO_2^-])f(NO) \quad (23)$$

Nitrite catalysis of reductive nitrosylation was also demonstrated for the ferriheme proteins metMb and metHb.[85] Consistent with the earlier studies,[81] reactions in a pH 7 solution without added nitrite showed that metHb and metMb react rapidly with NO to generate an equilibrium mixture of Fe^{III}(P) and Fe^{III}(P)(NO) that undergoes slow reductive nitrosylation. Adding $NaNO_2$ dramatically accelerated the rates, consistent with a catalytic role of nitrite, and values of $k_{nitrite}$ were measured as 0.14 and 1.1×10^{-2} M^{-1} s^{-1} for metHb and metMb, respectively.[85,86]

Two limiting mechanisms were proposed to explain this catalysis, one involving nucleophilic attack of NO_2^- on an Fe^{III}-coordinated NO and the other involving the outer-sphere reduction of the Fe^{III}(NO) complex to give Fe^{II}(NO) and the NO_2 radical, which would be rapidly trapped by NO to give N_2O_3.[85] While one cannot fully exclude the outer-sphere model, several arguments favor the nucleophilic attack pathway (Scheme 4). Because other nucleophiles such as azide ion also markedly enhance NO reduction of Fe^{III}-(TPPS), it is likely that the catalytic character of nitrite is due to the relatively high nucleophilicity of NO_2^-.[87] The question has not been fully resolved, although recent mechanistic studies by Jee et al.[88] of several related model ferriheme

(83) Luchsinger, B. P.; Rich, E. N.; Gow, A. J.; Williams, E. M.; Stamler, J. S.; Singel, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 461.
(84) Fernandez, B. O.; Lorkovic, I. M.; Ford, P. C. *Inorg. Chem.* **2003**, *42*, 2–4.
(85) Fernandez, B. O.; Lorkovic, I. M.; Ford, P. C. *Inorg. Chem.* **2004**, *43*, 5393.
(86) Fernandez, B. O.; Ford, P. C. *J. Am. Chem. Soc.* **2003**, *125*, 10510.
(87) Fernandez, B. O. Ph.D. Dissertation, University of California, Santa Barbara, Santa Barbara, CA, **2004**.
(88) (a) Jee, J.-E.; van Eldik, R. *Inorg. Chem.* **2006**, *45*, 6523–6534. (b) Jee, J.-E.; Eigler, S.; Jux, N.; Zahl, A.; van Eldik, R. *Inorg. Chem.* **2007**, *46*, 3336–3352.

Scheme 4. Adapted from Ref 85

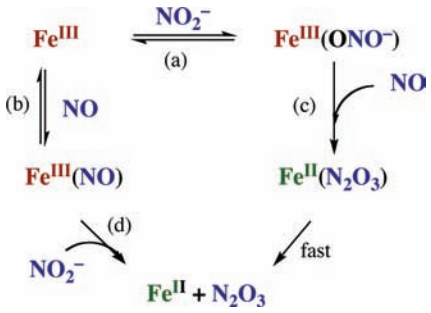


complexes are supportive of the mechanism described by Scheme 4.

A common feature of the two proposed schemes for nitrite catalysis is the formation of a N_2O_3 reactive intermediate. In aqueous solution, N_2O_3 would be expected to hydrolyze readily to nitrous acid. If, however, it were formed in the hydrophobic pocket of a protein, there would be some interesting potential consequences because N_2O_3 is a nitrosating agent and could conceivably react with protein amines or thiols to generate *N*-nitrosoamines or *S*-nitrosothiols. For example, we have speculated[85] that nitrite catalysis of metHb reduction by NO could lead to the generation of SNO hemoglobin. As will be seen below, the nitrite plus NO plus metHb to generate N_2O_3 scenario has received considerable attention,[89] and a recent publication from Navati and Friedman[90] reports the observation of a reactive species believed to be a N_2O_3/Hb conjugate formed as an intermediate along the reaction coordinate of these three components in a sol−gel matrix.

In the context of the above discussion of nitrite catalysis of the autoreduction of ferriheme models and proteins, it should be noted that an alternative mechanism has been proposed by Kim-Shapiro and co-workers.[91] In work carried out at very low NO concentrations ($< 10^{-4}$ M) but with added nitrite, they found the rate of Fe^{III} reduction to the ferrous state higher than that predicted by the model described by Scheme 4. They attributed this discovery to the reaction of free NO with Fe^{III}-coordinated nitrite in the manner illustrated by the right side of the cycle (steps a and c) of Scheme 5 (Fe^{III} = metHb). These authors report that ferriheme reduction via Scheme 4 still functions (steps b and d of Scheme 5) but is slower. They argue that the unexpectedly faster rate at low [NO] is the result of competitive inhibition of the NO_2^- binding at higher [NO] owing to metHb's affinity for NO ($K_b = 1.3 \times 10^4$ M^{-1} at 298 K).[81]

Scheme 5. Expanded Model for the Reduction of metHb by NO and Nitrite



There is no reason that the reaction should not proceed around either side of Scheme 5. Furthermore, density functional theory (DFT) studies[91,92] argue that a NO_2^- ion O-coordinated to a ferriheme iron as a nitrito complex would have some radical character that might enhance its reactivity toward NO, supporting the likelihood that both pathways are functioning. Although, because both are dependent on the concentrations of NO and NO_2^-, it is not clear how steps a and c would lead to enhanced Fe^{III} reduction rates at low [NO].[40] One problem in making the comparison is that the conditions of the two studies were quite different. In the Fernandez et al. studies, the ferriheme complexes were the limiting reactants,[85] while in the latter study, NO was limiting.[91]

N_2O_3 formed by either pathway would be expected to hydrolyze to nitrous acid or to react with nucleophiles such as the thiols cysteine or GSH to form *S*-nitrosothiols. Basu and co-workers proposed that N_2O_3 itself or a *S*-nitrosothiol derivative such as CysSNO formed by N_2O_3 nitrosation provides a mechanism for NO equivalents to escape from the red blood cells (RBCs) into the plasma. Regardless, while some NO generation has been noted upon reaction of nitrite salts with hypoxic RBCs, the quantities are quite small,[19] undoubtedly the result of NO trapping by deoxyHb or by $Hb(O_2)$ (see below). It is not clear whether there is an enhanced release of *S*-nitrosothiols from RBCs.

NiR Activity. The reduction of nitrite by ferrous hemes is in effect the reverse of the "autoreduction" described above. Excess NO drives the autoreduction, owing to the strong NO association constants commonly seen for ferroheme models and proteins. Although mechanisms of bacterial and fungal NiR enzymes have long been of interest,[93] new attention has turned to the activity of mammalian proteins after reports that nitrite induces vasodilation in humans.[17] In explanation, it was proposed that NiR activity of ferroheme proteins such as Hb and Mb generates NO. Hb has long been known to reduce nitrite to NO (e.g., eq 24),[94] but the NO produced is rapidly trapped by the remaining deoxygenated Hb (eq 25; $K = 9 \times 10^9$ M^{-1} T state, 1×10^{11} M^{-1} R state).[95]

(89) Gladwin, M. T.; Grubina, R.; Doyle, M. P. *Acc. Chem. Res.* **2009**, *42*, 157–167.
(90) Navati, M. S.; Friedman, J. M. *J. Am. Chem. Soc.* **2009**, *131*, 12273–12279.
(91) Basu, S.; Grubina, R.; Huang, J.; Conradie, J.; Huang, Z.; Jeffers, A.; Jiang, A.; He, X.; Azarov, L; Seibert, R.; Mehta, A.; Patel, R.; King, S. B.; Hogg, N.; Ghosh, A.; Gladwin, M. T.; Kim-Shapiro, D. B. *Nat. Chem. Biol.* **2007**, *3*, 785.
(92) Ireteskii, A.; Ford P. C. Reported at the 2nd International Symposium on Nitrite, Bethesda, MD, **2007**.
(93) Averill, B. A. *Chem. Rev.* **1996**, *96*, 2951.
(94) Doyle, M. P.; Hoekstra, J. W. *J. Inorg. Biochem.* **1981**, *14*, 351.
(95) Moore, E. G.; Gibson, Q. H. *J. Biol. Chem.* **1976**, *251*, 2788.

The reaction kinetics showed behavior consistent with allosteric effects, with the rates being slower with the T state tetramer, as evidenced by an initial lag phase. Second-order rate constants $k_{nitrite}$ for the T and R states were determined as 0.12 and 6 M^{-1} s^{-1}, respectively, at 25 °C and pH 7.4.[96]

$$Hb + NO_2^- + 2H^+ \xrightarrow{k_{nitrite}} metHb + NO + H_2O \quad (24)$$

$$Hb + NO \rightleftharpoons Hb(NO) \quad (25)$$

As discussed above, the rapid trapping of any NO produced in the RBCs by deoxyHb and $Hb(O_2)$ makes it problematic that significant amounts of NO would be released into the plasma. For this reason, there has been increasing attention among cardiovascular researchers on the creative combination of NO production via Hb NiR activity and N_2O_3 production via the reaction of NO with nitrite mediated by metHb, leading overall to the conversion of NO_2^- to N_2O_3 (eq 26, i.e., a nitrite dehydrase reaction) catalyzed by hemoglobin. However, from this reviewer's perspective, it seems that nitrite-derived cardiovascular-relevant NO is more likely to be produced by tissue myoglobin than in the RBCs.

$$2NO_2^- + 2H^+ \overset{Hb}{\rightleftharpoons} {}_{N2}O_3 + H_2O \quad (26)$$

$k_{nitrite}$ (6 M^{-1} s^{-1}) for Mb is the same as that for R state Hb presumably because of the similar redox potentials.[97] The reaction with Mb may play a critical biological role because nitrite levels are much higher in tissue than in plasma,[20] and under hypoxia the production of NO through this pathway may adjust muscle energetics to the limited oxygen supply.[98] This idea is reinforced by the observation that certain protective effects of nitrite are no longer evident for myoglobin knockout mice.[98b]

$$Mb + NO_2^- + 2H^+ \xrightarrow{k_{nitrite}} metMb + NO + H_2O \quad (27)$$

DFT calculations examining nitrite binding to the ferrous heme d_1 in bacterial NiRs have concluded that the N-bound nitro isomer is favored by ~19 kJ mol^{-1} over the O-bound nitrito isomer.[99] However, this difference is small, and it is notable that the crystal structures of the nitrite complexes of both metMb and metHb show these to be the nitrito isomers.[43] Indeed, only the metMb H64 V mutant not having the hydrogen-bonding distal histidine demonstrated N-coordinated nitro bonding.[44] Whether such considerations carry over to the ferroheme complexes remain uncertain, but it is notable that the nitrite reduction by wt hh Mb is 16 times faster than that by the Mb H64 V mutant.[44] In this context, we should note that a recent computational study of hypothetical NiR mechanisms by Mb favors nitro coordination, followed by stepwise proton transfers from the distal histidine to the nitrite.[100] Thus, the absence of the distal histidine in H64 V could also interrupt the proton transfers, leading to lower reactivity.

Mammalian NiR activity has also been shown to occur in blood-free tissue and, to this activity, has been attributed nitrite's protective effects during incidents of ischemia/reperfusion. For example, homogenates prepared from blood-free rat livers are quite active in reducing added nitrite to NO under hypoxia.[19] Kinetics measurements show first-order dependence on [nitrite] and, to a lesser extent, on the tissue protein concentration, but NO production is largely quenched under normoxic conditions. Inhibition studies indicate that free thiols and heme proteins are both important to this process. The dominant reductase activity was in the microsomal fraction, where the cytochrome P450 proteins are located,[19] so one can speculate that these also have NiR activity in their reduced forms. NiR activity has also been reported for the reduced *Mycobacterium tuberculosis* P450 proteins Cyp130 and CYP51.[55]

Oxygen-Atom-Transfer (OAT) Reactions of Coordinated Nitrite. The Fe^{III}-mediated OAT from coordinated nitrite to a suitable substrate S (eq 28) is another potential source of physiological NO. OAT reactions involving coordinated nitrite have been reported for several heme systems[101−105] but have avoided much discussion in the mammalian physiology community. This situation may change given the recent demonstration that such heme-mediated OAT can be effected in aqueous media.[104]

$$Fe^{III}(Por)(NO_2^-) + S \longrightarrow Fe^{II}(Por)(NO) + SO \quad (28)$$

Although OAT from coordinated nitrite had been described previously, the reports by Castro et al.[101a,b] that stoichiometric oxygen transfers to various substrates can be accomplished in acetic acid/*N*-methylpyrrolidone solutions of $[K(18\text{-C-}6)]NO_2$ (18-C-6 is a crown ether) and Fe(OEP)Cl (OEP = octaethylporphyrinato dianion) are a good starting point for this discussion. A ferric nitro complex $Fe^{III}(OEP)(NO_2^-)$ was postulated as a key reactive intermediate in analogy to OAT reactions of similar Co^{III} complexes.[106] Concerted oxygen transfer from the nitro group would give the very stable ferrous nitrosyl $Fe^{II}(OEP)(NO)$. Subsequently, DFT computations were used[103] to probe the hypothetical OAT from Fe^{III}(porphine)(NO_2) and from Fe^{III}(porphine)(L)(NO_2) (L = pyridine) to the substrate dimethyl sulfide (DMS).

(96) Huang, K. T.; Keszler, A.; Patel, N.; Patel, R. P.; Gladwin, M. T.; Kim-Shapiro, D. B.; Hogg, N. *J. Biol. Chem.* **2005**, *280*, 31126.
(97) Shiva, S.; Huang, Z.; Grubina, R.; Sun, J.; Ringwood, S. A.; MacArthur, P. H.; Xu, X.; Murphy, E.; Darley-Usmar, V. M.; Gladwin, M. T. *Circ. Res.* **2007**, *100*, 654–661.
(98) (a) Rassaf, T.; Floegel, U.; Drexhage, C.; Hendgen-Cotta, U.; Kelm, M.; Schrader, J. *Circ. Res.* **2007**, *100*, 1749–1754. (b) Hendgen-Cotta, U. B.; Merx, M. W.; Shiva, S.; Schmitz, J.; Becher, S.; Klare, J. P.; Steinhoff, H. J.; Goedecke, A.; Schrader, J.; Gladwin, M. T.; Kelm, M.; Rassaf, T. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 10256.
(99) Silaghi-Dumitrescu, R. *Inorg. Chem.* **2004**, *43*, 3715.
(100) Perissinotti, L. L.; Marti, M. A.; Doctorovich, F.; Luque, F. J.; Estrin, D. A. *Biochemistry* **2008**, *47*, 9793.
(101) (a) Castro, C. E.; O'Shea, S. K. *J. Org. Chem.* **1995**, *60*, 1922. (b) Castro, C. E. *J. Org. Chem.* **1996**, *61*, 6388. (c) O'Shea, S. K.; Wall, T.; Lin, D. *Transition Met. Chem.* **2007**, *32*, 514.
(102) Cheng, L.; Powell, D. R.; Khan, M. A.; Richter-Addo, G. B. *Chem. Commun.* **2000**, 2301.
(103) Conradie, J.; Ghosh, A. *Inorg. Chem.* **2006**, *45*, 4902.
(104) Khin, C.; Heinecke, J.; Ford, P. C. *J. Am. Chem. Soc.* **2008**, *130*, 13830.
(105) Kurtikyan, T. S.; Hovhannisyan, A. A.; Iretskii, A. V.; Ford, P. C. *Inorg. Chem.* **2009**, *48*, 11236–11241.
(106) Tovrog, B. S.; Mares, F. M.; Diamond, S. E. *J. Am. Chem. Soc.* **1980**, *102*, 6616.

Concerted OAT from either nitro complex was shown to be a viable process, although the former reaction was calculated to have a lower activation energy by ~30 kJ mol^{-1}. One can easily rationalize this higher E_a on the basis that the binding of the proximal pyridine to the ferric nitro complex is likely to be significantly stronger than that to the ferrous nitrosyl analogue. This would result in a larger reorganization energy for the six-coordinate species, and thus a higher E_a. Notably, the six-coordinate form is the likely ground state of ferriheme nitro complexes in a coordinating solvent.

The analogous OAT pathways for nitrito complexes Fe^{III}(porphine)(L)(ONO) were not computed.[103] However, DFT calculations[105] on related systems have since concluded that the isonitrosyl product Fe^{II}(porphine)-(ON) of OAT from a Fe^{III}(ONO) species would be ~67 kJ mol^{-1} more energetic that the nitrosyl product; thus, a pathway involving concerted OAT to give this intermediate (eq 29) would present a higher energy barrier than OAT from the $Fe^{III}(NO_2)$ analogue.

$$Fe^{III}(Por)(ONO^-) + S \longrightarrow Fe^{II}(Por)(ON) + SO \longrightarrow Fe^{II}(Por)(NO) + SO \quad (29)$$

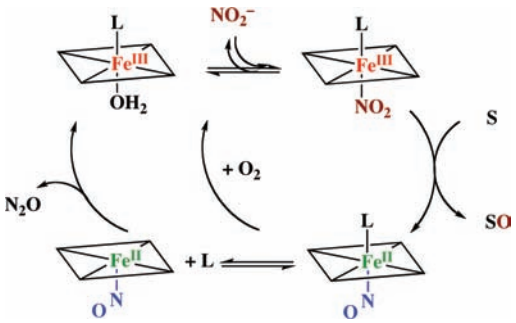
Near-neutral, aqueous solutions of $NaNO_2$ and Fe^{III}-(TPPS)$(H_2O)_2$ have been shown to oxidize trisulfonated triphenylphosphine (TPPTS), DMS and the biological thiols cysteine and GSH (Scheme 6).[104] The products are the respective monoxides TPPTSO and DMSO and the disulfides CysSSCys and GSSG. Although the observed disulfides might suggest a mechanism proceeding via a one-electron oxidation of the thiols to radicals, sulfenic acids [RS(O)H], the expected OAT products, are known to react with excess RSH to form disulfides.[107] Thus, disulfide products would be consistent with either pathway. The latter reaction would be particularly significant given recent attention focused on cysteine to Cys sulfenic acid protein modifications in redox-mediated signal transduction.[108] Our ongoing studies have indeed identified CysS(O)H as an OAT product from Fe^{III}(TPPS)-(NO_2^-) to CysSH.

Notably, when the solution containing the ferrous nitrosyl product Fe^{II}(TPPS)(NO) was exposed to small quantities of air, the initial ferric complexes were quickly regenerated. However, in the absence of O_2, the ferrous complexes persisted as long as excess substrate was present. More surprising was the observation[104] that, once the substrate was expended, the reduced system slowly, yet spontaneously, regenerated the ferric complexes. Gas-phase analysis indicated the formation of nitrous oxide (N_2O) consistent with the stoichiometry of eq 30 for S = TPPTS; thus, Fe(TPPS) serves as a catalyst for the overall process.

$$2S + 2NO_2^- + 2H^+ \xrightarrow{Fe(TPPS)} 2SO + N_2O + H_2O \quad (30)$$

Initial rate studies show that the transformation of Fe^{III}-(TPPS) to Fe^{II}(TPPS)(NO) is approximately first-order in

Scheme 6. OAT from Nitrite-Coordinated Fe^{III}(TPPS) to Various Substrates in Aqueous solution



substrate, in nitrite, and in the ferric complex, consistent with the OAT step following preequilibrium formation of $Fe^{III}(TPPS)(NO_2^-)$ ($K = 3\ M^{-1}$).[85] On the basis of the pH dependence, we have speculated[104] that spontaneous Fe^{III}-(TPPS) regeneration involves the unprecedented protonation of the ferrous nitrosyl, followed by dissociation to give Fe^{III}(TPPS) plus HNO, which dimerizes to N_2O. However, this mechanism needs to be more critically interrogated.

Kurtikyan and co-workers[105] have also observed OAT reactions in a system in which they generated porous layers of the nitrito complex Fe^{III}(TPP)(ONO) from the reaction of solid Fe^{II}(TPP) and NO_2 in a vacuum cryostat.[42a] After removing the residual NO_2, they introduced gaseous DMS to the system and immediately saw the formation of the hexacoordinate nitro species Fe^{III}(TPP)(DMS)(NO_2). This product is consistent with earlier studies showing that coordination of a Lewis base in the trans axial site tends to favor nitrito to nitro linkage isomerization. The six-coordinate complex is moderately stable at ambient temperature, if excess DMS is present, but slowly transforms to Fe^{II}(TPP)(NO) concomitant with the formation of DMSO, as probed by mass spectrometry. When this experiment was conducted using Fe(Por)(η^1-^{18}ON^{18}O), the mass spectrum of DMS^{18}O was registered, confirming the nitrite ligand as the origin of the oxygen atom transferred.[105] It is not clear whether OAT to DMS to give DMSO occurs from the six-coordinate nitro complex Fe^{III}(TPP)(DMS)(NO_2) or from the five-coordinate analogue Fe^{III}(TPP)(NO_2) formed by DMS dissociation from the former, but the DFT computations appear to rule out OAT directly from the nitrito complexes Fe^{III}(TPP)-(ONO) or Fe^{III}(TPP)(DMS)(ONO) (e.g., eq 29) owing to the instability of the isonitrosyl complex.

Overview and Summary

As this Forum article has coalesced, the author has grown increasingly aware of the difficulty in conveying the complexity of the chemistry and chemical biology presented by the roles of NO and its derivatives in mammalian biology. We have limited the discussion to the interactions of NO and nitrite ion with heme models and the very important heme proteins, but it should be obvious that the issues extend far beyond those limits. There is a very diverse, yet relatively unexplored, chemical biology of other NO-derived products such as *S*-nitrosothiols and their roles in key regulatory mechanisms.[109] Another topic that continues to draw

(107) Claiborne, C. A.; Yeh, J. I.; Mallett, T. C.; Crane, E. J.; Charrier, V.; Personage, D. *Biochemistry* **1999**, 28,15407.
(108) Leonard, S. E.; Reddie, K. G.; Carroll, K. S. *ACS Chem. Biol.* **2009**, *4*, 783–799.
(109) Foster, M. W.; Hess, D. T.; Stamler, J. S. *Trends Mol. Med.* **2009**, *15*, 391–404.

enormous attention is the question of whether NO generation is responsible for enhancing or ameliorating oxidative stress. The answer very likely is that either may be the case depending upon the timing and concentration.[110] NO chemical biology is an exceedingly rich field and warrants greater attention from chemists well grounded in mechanistic concepts and thermodynamics but holding a healthy respect for the complexity introduced by the sophisticated structures of proteins, cells, and organisms.

To illustrate this point, we use the example of the ferroheme protein soluble guanylyl cyclase. As we have summarized above, sGC is effectively activated at nanomolar concentrations of NO, and in order for this to be the case, the k_{on} rate constant as well as the binding constant K must be very large ($>10^8$ M^{-1} s^{-1} and $10^{10}-10^{12}$ M^{-1}, respectively; see Table 1). These values are similar to those measured for globins, so what is clearly a distinguishing feature of sGC is that it does not bind O_2, thus providing the basis for selective NO signaling in aerobic cells? The reason is that Hb and Mb have a distal histidine that stabilizes the superoxide form of their dioxygen complexes $Fe^{III}(O_2^-)$ through hydrogen bonding; sGC does not. This rational was nicely confirmed by Boon et al.,[111] who constructed a mutant sGC with an appropriate hydrogen-bonding residue in the distal pocket, thereby transforming this protein into an oxygen sensor.[111]

In a similar context, the mode of bonding between a nitrite ion and metMb is markedly affected by the presence or absence of the distal His-64.[44]

We have surveyed mechanistic studies of the reactions of NO and nitrite with heme models and proteins in the context of the numerous roles such chemistry may play in the biological functions of NO. The volume of relatively recent information regarding the chemical biology and pathobiology even of this more limited topic is too large to survey comprehensively in the present format. However, certain patterns are obvious. The first is that NO reacts readily with heme proteins having open coordination sites, either five-coordinate as are the ferroheme proteins Mb, Hb, and sGC or those with a labile ligand (usually water) such as the ferriheme proteins metMb and metHb. These are all high-spin complexes (d^6 or d^5, respectably), but the nitrosyl complexes formed are low-spin. Six-coordinate heme proteins where both axial ligands are amino acid residues of the protein, e.g., Cyt^{III} or Cyt^{II}, that require intrinsically more protein conformational change are much less reactive. Overall, the rates of formation of nitrosyl complexes are dependent on the labilities of the ligands being replaced.

Generally, if the coordination sites are open, the reaction rates with NO in solution will be significantly faster for the ferroheme systems and the resulting nitrosyl complexes will be more stable (larger k_{on}'s and K's, respectively). However, closely examining even a representative number of systems, such as those listed in Table 1, quickly alerts one to the sensitivity of the k_{off} rate constants to the nature of the ligand coordinated at the proximal axial site. For example, it appears that the nitrosyl complexes of ferrous P450 proteins with proximal thiolate ligands are more labile than the nitrosyl complexes of the ferrous globins with proximal histidines. This greater lability is consistent with the DFT results that suggest that a proximal thiolate weakens $Fe^{II}-NO$ bonding relative to a proximal imidazole.[112] So, it is quite clear that any such generality must be modified by the specifics of the ligand field as well as of the protein structure itself.

Once formed, the nitrosyl complex can serve to activate the coordinated NO toward nucleophilic or electrophilic attack, depending on the nature of the metal and its oxidation state and the ligand field. Of particular interest biologically is the reaction of the nitrosyl complex with nucleophiles because this may well be a mechanism for *S*-nitrosothiol formation as well as for the reductive labilization of metals in insoluble matrices like ferritin.

Tissue and plasma NO_2^- are largely a consequence of NO production by various isoforms of NOS but may also be derived from nitrate reductase action, especially by oral and/or intestinal bacteria or possibly endogenously. Although once thought to be physiologically deleterious by contributing to amine nitrosation, a growing body of evidence suggests that endogenous nitrite may play a role in vasodilation under hypoxic conditions and in organ protection during incidents of ischemia/reperfusion. The nitrite concentrations in plasma and tissues are considerably larger than those of NO, although the latter is much more reactive.

Discussions of nitrite's beneficial roles have largely focused on it being a reservoir that is relatively easily converted to NO or possibly to *S*-nitrosothiols, which also may have key functions in signaling. Metal-mediated reactions of nitrite might also be a source of HNO,[104] another species attracting attention for endogenous and therapeutic possibilities in physiology.[113] Ferroheme proteins such as Hb and Mb can act as NiRs to generate NO. Moreover, under certain conditions, similar ferriheme proteins and models are NO oxidases to form nitrite. Recent studies have mapped out the general landscape of such reactions, but further investigation is needed to provide the detailed mechanistic understanding necessary for elucidating the key roles in physiology.

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(110) (a) Wink, D. A.; Miranda, K. M.; Espey, M. G.; Pluta, R. M.; Hewett, S. J.; Colton, C.; Vitek, M.; Feelisch, M.; Grisham, M. B. *Antioxid. Redox Signaling* **2001**, *3*, 203–213. (b) Thomas, D. D.; Ridnour, L. A.; Isenberg, J. S.; Flores-Santana, W.; Switzer, C. H.; Donzelli, S.; Hussain, P.; Vecoli, C.; Paolocci, N.; Ambs, S.; Colton, C. A.; Harris, C. C.; Roberts, D. D.; Wink, D. A. *Free Radical Biol. Med.* **2008**, *45*, 18–31. (c) Omsland, A.; Miranda, K. M.; Friedman, R. L.; Boitano, S. *FEMS Microbiol. Lett.* **2008**, *284*, 92–101.

(111) Boon, E. M.; Huang, S. H.; Marletta, M. A. *Nat. Chem. Biol.* **2005**, *1*, 53–59.

(112) Praneeth, V. K. K.; Haupt, E.; Lehnert, N. *J. Inorg. Biochem.* **2005**, *99*, 940–948.

(113) (a) Miranda, K. M. *Coord. Chem. Rev.* **2005**, *249*, 433–455. (b) Paolocci, N.; Jackson, M. I.; Lopez, B. E.; Miranda, K. M.; Tocchetti, C. G.; Wink, D. A.; Hobbs, A. J.; Fukuto, J. M. *Pharmacol. Ther.* **2007**, *113*, 442–458. (c) Jackson, M. I.; Han, T. H.; Serbulea, L.; Dutton, A.; Ford, E.; Miranda, K. M.; Houk, K. N.; Wink, D. A.; Fukuto, J. M. *Free Radical Biol. Med.* **2009**, *47*, 1130–1139.

List of Acronyms and Abbreviations

cGMP	cyclic guanylyl monophosphate
CysSH	cysteine
Cyt	cytochrome *c*
DFT	density functional theory
DMS	dimethyl sulfide
eNOS	endothelial nitric oxide synthase
GSH	glutathione
GSNO	*S*-nitrosoglutathione
GTP	guanylyl triphosphate
Hb	hemoglobin
I/R	ischemia/reperfusion
Mb	myoglobin
metHb	methemoglobin
metMb	metmyoglobin
NiR	nitrite reductase
NOS	nitric oxide synthase
OAT	oxygen atom transfer
OEP^{2-}	octaethylporphyrinato
Por^{2-}	porphyrinato dianion
$PPIX^{2-}$	protoporphyrin IX dianion
RBC(s)	red blood cell(s)
sGC	soluble guanylyl cyclase
TPP^{2-}	*meso*-tetraphenylporphyrinato
TPPS	*meso*-tetrakis(4-sulfonatophenyl)porphyrinato anion
TPPTS	trisulfonated triphenylphosphine