Mechanisms of Reductive Nitrosylation in Iron and Copper Models Relevant to Biological Systems

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1. Introduction

The interaction between metal centers and nitric oxide (nitrogen monoxide) has long been of interest to chemists because of the remarkable flexibility of NO as either a net electron donor or electron acceptor in metal−NO binding interactions.[1] However, this topic's appeal broadened considerably upon the discoveries in the late 1980s that endogenously formed NO has key roles in mammalian biology and that some of this activity could be attributed to the formation of nitrosyl complexes of metallo-proteins. These roles include functions in vascular regulation, neurotransmission, and immuno-cytotoxicity.[2,3] Furthermore, numerous disease states have been shown to be characterized by the over- or underproduction of nitric oxide.[4] As a consequence, there has been an enormous outpouring of publications focused on the relevant biochemistry and the biomedical implications of both endogenous and exogenous NO and its derivatives such as neutral and anionic N_yO_x species, nitroso-thiols and -amines, and metal nitrosyls. To understand these biomedical effects, it is essential to elucidate the fundamental reaction mechanisms and dynamics of these seemingly simple molecular systems under relevant conditions.

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One such reaction involving the interaction between NO and metal centers has been termed "reductive nitrosylation". This refers to the reaction between a metal center and NO leading to one-electron reduction of the metal oxidation state and concomitant nitros(yl)ation of a nucleophile, illustrated in very general terms by eq 1.[5]

$$L_nM^{m+} + NO + X^- \rightleftharpoons L_nM^{(m-1)+} + X{-}NO \quad (1)$$

In aqueous media, the nucleophile X^- is likely to be water or hydroxide, so the common nitrogen-bearing product is nitrite ion as illustrated in eq 2.

$$L_nM^{m+} + NO + H_2O \rightleftharpoons L_nM^{(m-1)+} + NO_2^- + 2H^+ \quad (2)$$

Both of these equations are simplistic in the context that the metal ion coordination sphere is represented as unchanged, but a likely scenario is that the coordination sphere of the reduced product is different. For example, reaction of the five-coordinate cupric complex $Cu(dmp)_2(H_2O)^{2+}$ with NO in methanol (dmp = 2,9-dimethyl-1,10-phenanthroline) leads to formation of the four-coordinate cuprous species $Cu(dmp)_2^+$ plus methyl nitrite and (presumably) H_2O released in solution (eq 3).[6]

$$Cu(dmp)_2(H_2O)^{2+} + NO + CH_3OH \rightarrow Cu(dmp)_2^+ + CH_3ONO + H_2O + H^+ \quad (3)$$

In some cases (but not eq 3), the reduced metal center reacts further with a second mole of NO to form a stable metal−nitrosyl product.

In earlier studies, many predating the discoveries that NO has bioregulatory functions, it was noted that certain metallo-proteins, for example, methemoglobin (metHb), undergo reduction in the presence of added nitric oxide,[7] and this process has been termed "autoreduction". Such autoreduction is undoubtedly the same process as the reductive nitrosylation that is the topic of this review; however, the other products, probably nitrite in most cases, were not characterized. In a similar context, Wayland and Olsen[8] in 1973 reported the use of NO and methanol to prepare $Fe^{II}(TPP)(NO)$ from toluene solutions of Fe(TPP)Cl (TPP = tetraphenylporphyrin) and proposed the series of steps shown in Scheme 1 to explain this reaction. In support of this proposal, they demonstrated that the $Fe^{III}(TPP)(Cl)(NO)$ intermedi-

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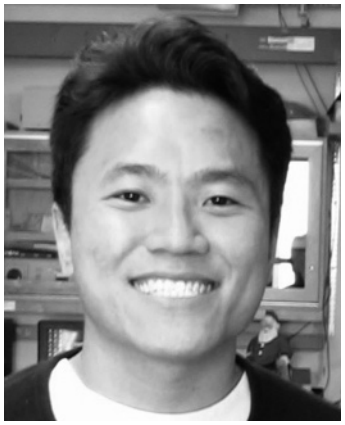
Peter C. Ford was born in California in 1941. His undergraduate work at Caltech was followed by graduate study at Yale with Professor Kenneth Wiberg (Ph.D. 1966) and a year as a NSF Postdoctoral Fellow at Stanford with Professor Henry Taube. In 1967, he joined the faculty of the University of California, Santa Barbara, where he has held the rank of Professor since 1977 and has served as Department Chair (1994–6). He has supervised the research of 50 students who completed their Ph.D. theses as well as a number of B.S., M.S., and postdoctoral students. He has been honored as a Dreyfus Foundation-Teacher Scholar, as a Senior Fulbright Fellow, with an Alexander von Humboldt-Stiftung US Senior Scientist Research Prize, with the 1992 Richard C. Tolman Medal of the American Chemical Society, and with election as a Fellow of the AAAS. He is currently President of the Inter-American Photochemical Society (04–06). His current research interests include the photochemistry/physics of coordination and organometallic compounds, applications of modern kinetics techniques and time-resolved spectroscopy for the study of homogeneous catalysis mechanisms, and the bioinorganic chemistry of the nitrogen oxides.

Bernadette O. Fernandez was born in Manila, Philippines, in 1975, but has spent much of her schooling in Southern California. She graduated with a B.S. degree in Chemistry from Harvey Mudd College in 1997, working with metal carbonyls under the direction of Professor Mits Kubota. After an internship with Janet Blanks at the Doheny Vision Research Center, she entered graduate school in the Department of Chemistry and Biochemistry at the University of California, Santa Barbara, where she earned her Ph.D. degree in 2004 in Inorganic Chemistry working with Professor Peter C. Ford. Her doctoral degree focused on the reactions of nitrogen oxides and heme complexes. She recently began postdoctoral research with Professor Martin Feelisch at the Boston University School of Medicine. Her current research involves understanding the interaction of NO and related metabolites with biological targets.

ate is reversibly formed in toluene solution in the absence of a hydroxylic reactant. In the years since, a number of other workers[9] have reported the NO reductions of various ferriheme model complexes, including those of various substituted tetraarylporhyrins, protoporphyrin, and several water-soluble porphyrins.



Mark D. Lim was born in Pennsylvania in 1976. He received his B.S. degree in Biochemistry/Chemistry from the University of California, San Diego (1999), where he worked with Professor William C. Trogler on the development of a Cu(I) photoluminescent sensor. He earned his Ph.D. degree in Chemistry at the University of California, Santa Barbara (2004), under the direction of Professor Peter C. Ford. His doctoral research included the use of rapid mixing and laser flash photolysis techniques to help elucidate the mechanisms of various nitrogen oxides with transition metal centered model complexes. He began his postdoctoral work in the laboratory of Professor Charles S. Craik at the University of California, San Francisco, in 2005, and his current research interests include the development and use of photoluminescent tags to understand the activity and mechanisms of proteolytic enzymes.

Scheme 1

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

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

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

Early insight into reductive nitrosylation mechanisms was also offered by Gwost and Caulton,[10] who were among the first to utilize NO in the presence of base and alcohol as a reducing agent to prepare organometallic nitrosyl complexes from transition metal salts (e.g., eq 4, L = a phosphine or an amine). These workers also proposed the formation of nitrosyl complexes followed by reaction with alcohol or alkoxide and identified the alkyl nitrite products formed. Similar procedures have seen applications in the syntheses of other organometallic nitrosyls.[1,11]

$$CoCl_2 + 3NO + 2L + NEt_3 + MeOH \rightarrow Co(NO)_2(L)_2Cl + MeONO + Et_3NH^+ + Cl^- \quad (4)$$

With the greatly increased interest in NO reactions under biologically relevant conditions,[12] there has been renewed attention to reductive nitrosylation and its mechanisms involving the reduction of Fe(III) and Cu(II) centers. It is notable that not only the reduction of the metal center but also the formation of the nitrosated product X−NO may have significance in mammalian physiology, because such species may have roles in NO transport[13] and in redox sensing and signaling.[14] For example, reductive nitrosylation has been invoked[13c] as a possible mechanism for the nitrosation of the β-cys-93 of hemoglobin to form *S*-nitrosohemoglobin (SNO-Hb),[13a,b] the subject of a controversial proposal as a nitric oxide carrier in the cardiovascular system.[13d] Furthermore, it is worth noting that reductive nitrosylation under conditions where nitrite is the product is the reverse of nitrite

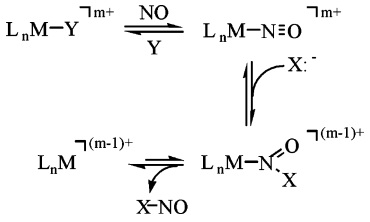
reduction, a reaction that has considerable importance to the biological nitrogen cycle and is catalyzed in certain anaerobic bacteria by nitrite reductases (NIR), which are copper and heme proteins.[14] In this context, the goal of the present review is to examine the details of reductive nitrosylation mechanisms that have now been described for iron and copper models and proteins and to compare such studies with other compounds that have been quantitatively investigated.

2. Prospective Mechanisms To Consider

Before embarking on a discussion of detailed investigations of reductive nitrosylation mechanisms in specific systems, it would be valuable to reflect on the general mechanisms that one might expect for an overall process such as that depicted in eq 1.

One likely mechanism is depicted in Scheme 2. This involves coordination of NO to give a metal

Scheme 2



nitrosyl complex followed by reaction with the nucleophile ($X:^-$), then dissociation to give the nucleophile nitrosyl complex and a reduced metal center. This would follow suggestions in the early studies by Caulton[9] and by Wayland[8] that the formation of a metal−nitrosyl complex with an activated nitrosyl is a logical first step.

In general, the rate of the reaction leading to formation of the nitrosyl complex $L_nM(NO)^{m+}$ is largely determined by the lability of the leaving group Y and would be expected to be facile for the many Fe(III) and most Cu(II) complexes that are substitution labile. For example, the reaction of NO with ferriheme model $Fe^{III}(TPPS)(H_2O)_2$ (TPPS = tetra-(4-sulfonato-phenyl)porphinato) to form $Fe^{III}(TPPS)(NO)$ (eq 5) is relatively rapid ($k_{NO} = 4.5 \times 10^5$ M^{-1} s^{-1}).[16] The activation parameter investigations clearly point to a limiting dissociative mechanism for this high-spin $3d^5$ complex where the lability of the axial waters[16b,c] is the rate-controlling feature of the kinetics.[16] For comparison, the reaction is even faster for the ferro-heme model $Fe^{II}(TPPS)$ ($k_{NO} = 1.5 \times 10^9$ M^{-1} s^{-1}),[16a,d] which is probably five-coordinate, and therefore does not involve displacement of a ligand.

$$Fe^{III}(TPPS)(H_2O)_2 + NO \rightleftharpoons Fe^{III}(TPPS)(H_2O)(NO) + H_2O \quad (5)$$

In contrast, the reaction of hexaammineruthenium(III) ion with NO (eq 6) in acidic aqueous solution is much slower ($k_{NO} = 0.2$ M^{-1} s^{-1}).[17] For this low-spin $4d^5$ complex, the coordinated ammines are effectively inert to dissociation, so the substitution pathway follows an associative mechanism as reflected by the negative activation entropy and volumes for k_{NO}.[18,19] Nonetheless, even for Ru(III) complexes, the leaving group has a kinetically significant effect as illustrated by the 200-fold faster reaction of $Ru(NH_3)_5(H_2O)^{3+}$.[19]

$$Ru(NH_3)_6^{3+} + H^+ + NO \rightarrow Ru(NH_3)_5(NO)^{3+} + NH_4^+ \quad (6)$$

Once the metal nitrosyl complex $L_nM(NO)^{m+}$ is formed, how might one expect the reactivity to be affected? This of course depends on the nature of the metal−ligand bonding. NO coordination is typically at the nitrogen atom, although meta-stable O-bound (isonitrosyl) and η^2-complexes have been observed.[20] A generalized description of the metal−NO interaction was offered some years ago by Feltham and Enemark,[21a] who proposed the $\{MNO\}^n$ formulation, where n is the sum of the metal d-electrons and the nitrosyl π^* electrons. This further emphasizes that the electronic character of the MNO bonding is highly delocalized. Walsh-type diagrams were used to predict the bond angles of this unit. When a strong C_{4v} ligand field is present, the M−N−O angle is predicted to be linear for $n \leq 6$, but bent for $n > 6$. Thus, an Fe^{III} porphyrin will bind to NO linearly ($n = 6$), while the Fe^{II} analogue will bind ($n = 7$) with an angle of about 150° as has been demonstrated by crystallography.[21b]

A qualitative way to envision such bonding is illustrated by the limiting cases illustrated by Figure 1. In forming a metal−NO bond, there may be

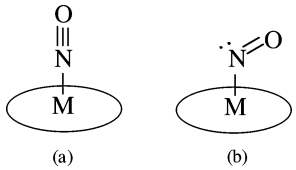


Figure 1. Illustration of limiting cases of NO binding to a metalloporphyrin center as (a) the nitrosyl cation (NO^+) with a M−N−O bond angle of ~180° or as (b) the nitroxyl anion (NO^-) with a M−N−O bond angle of ~120°.

considerable charge transfer between the metal and the NO.[22] If the metal is an oxidant, net charge transfer is to the metal, and we can visualize the coordinated NO as having nitrosonium character (NO^+). Such charge transfer from the π^*_{NO} orbital to the metal is qualitatively consistent with the relatively high ν_{NO} stretching frequencies (~1750−1950 cm^{-1}) usually observed for these species and reflects the triple bond character of the N−O bond. Such a species is isoelectronic to CO, so a linear M−NO bond (~180°) would be anticipated. At the other limit, net charge transfer from a low valent metal would give (formally) a coordinated nitroxyl anion (NO^-) for which a M−N−O bond angle of ~120° might be anticipated,[23] and the ν_{NO} values are lower (~1600−1750 cm^{-1}). From such a picture, one might anticipate that a nitrosonium-type ligand be susceptible to reaction with nucleophiles, while the nitroxyl-type ligand would be susceptible to reaction with electrophiles.

Both "predictions" have proved valid. For example, it is well known that the substitution-inert nitroprusside ion (NP), a ferric nitrosyl complex, reacts (reversibly) in alkaline aqueous solution to give the corresponding nitro ligand via OH^- attack at the coordinated NO (eq 7).[24] Similar reactions have been observed with the homologous ruthenium and osmium complexes[25] as well as with a variety of other ruthenium(III) NO complexes.[26] Alternatively, it has been reported that the one-electron reduction of nitrosyl myoglobin (Mb(NO), a ferroheme nitrosyl complex) gives a new species that is readily protonated to give the corresponding Mb(HNO) complex.[27]

$$Fe(CN)_5(NO)^{2-} + 2OH^- \rightleftharpoons Fe(CN)_5(NO_2)^{4-} + H_2O \quad (7)$$

A clear analogy can be drawn between the reaction of nitrosonium complexes with nucleophiles to similar reactions with certain metal carbonyl complexes (L_xM-CO).[28] The latter species have been shown to react with OH^- to form C-coordinated hydroxycarbonyl complexes $[L_xM-CO_2H]^-$ in equilibrium with the respective conjugate base anions $[L_xM-CO_2]^{2-}$. The dissociation of CO_2 from these leaves strongly reducing species, the metal hydride $[L_xMH]^-$ from the former and the dianion L_xM^{2-} from the latter, that are likely intermediates in the homogeneous catalysis of the water gas shift reaction ($CO + H_2O \rightleftharpoons H_2 + CO_2$).[29] However, an obvious difference is that conversion of CO to CO_2 is a two-electron oxidation, while NO to nitrite is a one-electron change and that nitrite is susceptible to further oxidation to N(IV) and N(V) species.

The third step in Scheme 2 is the dissociation of the X−NO species from the metal center to give a net reaction resulting in the formation of the nitrosylated nucleophile (that is, XNO) and the reduced metal center. This step is of course dependent on the lability of the XNO ligand coordinated to that metal center. For example, with the $Fe(CN)_5(NO_2)^{4-}$ formed in eq 7, the nitrite ligand is not readily released to the solution. If the system is labile, the reduced metal center may react with excess NO to form the nitrosylated complex $L_nM(NO)$ as the final product. For example, the reaction of met-myoglobin (metMb) with excess NO in slightly basic solution leads to formation of nitrite plus Mb(NO) (eq 8).[30]

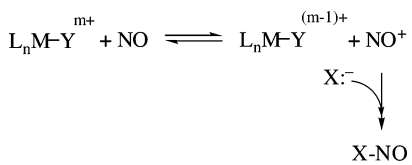
$$metMb + 2NO + OH^- \rightarrow Mb(NO) + NO_2^- + H^+ \quad (8)$$

An alternative mode of reductive nitrosylation is illustrated in Scheme 3. This would be a redox process where the initial activation of the NO toward reaction with a nucleophile is not coordination to the metal center, but involves an outer-sphere electron transfer from the nitric oxide to the oxidizing metal center. Given the positive reduction potential of NO^+ (est. as ~1.2−1.45 V in strongly acidic solution),[31,32] this first step will be thermodynamically uphill for all L_nMY except for very strong oxidants. However, the fast trapping of NO^+ by solution nucleophiles could make this a viable pathway assuming the actual electron-transfer step is sufficiently fast to account for the observed reductive nitrosylation. The rates of the first step (k_{12}) can be estimated by using the Marcus "cross relation" (eq 9, where f_{12} is ~1),[33] but this requires knowing the rate constants for the NO/NO^+ (k_{11}) and $L_nMY^{m+}/L_nMY^{(m-1)+}$ (k_{22}) self-exchange reactions as well as the equilibrium constant K_{12} (which can be calculated from the overall reaction potential) under the relevant conditions. The value of k_{11} has been estimated as $2 \times 10^5\ M^{-1}\ s^{-1}$ in aqueous solution.[34]

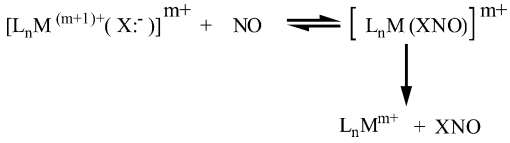
$$k_{12} = (k_{11}k_{22}K_{12}f_{12})^{1/2} \quad (9)$$

Scheme 4 describes a third scenario for reductive nitrosylation that has a parallel in the "inner-sphere" electron-transfer mechanism for metal complexes. In this case, the NO actually attacks a coordinated ligand X^- with concomitant electron transfer to the metal center. The net effect is the transfer of the X radical from the metal to the NO to give XNO plus the metal center that has been reduced by one electron.

Scheme 3



Scheme 4



Other scenarios that are variations upon the themes described in Schemes 2−4 will be discussed when appropriate in the subsequent sections.

Certain thermodynamic considerations must also be taken into account when discussing reductive nitrosylation pathways. This can be illustrated by examining the reaction in aqueous media where a common product is the nitrite ion. The reduction potential of nitrite is strongly dependent on the reaction conditions, especially pH, because two protons are involved in the half-cell for the reduction of NO_2^- to NO (eq 10).

$$NO_2^- + 2H^+ + e^- \rightleftharpoons NO + H_2O \quad (10)$$

As a consequence, NO_2^- with a half-cell potential of 1.20 V under standard conditions[32] (1 M H^+) is a powerful oxidant. On the other hand, this potential drops to 0.37 V at pH 7 and NO reduction of various substrates becomes much more favorable. For example, NO reduction of Fe^{III}(TPPS)(NO) is unfavorable in 1 M H^+ solution ($\Delta E° = -0.62$ V for eq 11) but is favorable at higher pH ($\Delta E = 0.21$ V) in pH 7 solution.[35]

$$Fe^{III}(TPPS)(NO) + H_2O + NO \rightleftharpoons Fe^{II}(TPPS)(NO) + NO_2^- + 2H^+ \quad (11)$$

In this context, the equilibrium constant for eq 11 was determined for Fe^{III}(TPPS) in 298 K, pH 4.5 NaOAc/HOAc buffer (50 mM) with [NO] = 1.9 mM by recording spectral changes as the nitrite concentration was varied from 0 to 40 mM. From these spectral changes and Lineweaver−Burke-type analysis, an equilibrium constant was determined as $(3.35 \pm 0.89) \times 10^{-2}$ M^2.[35] From this value, the reduction potential for Fe^{III}(TPPS)(NO) was calculated as (0.582 ± 0.007) V,[35] a value that compares well with that (0.586 V) determined by Meyer and co-workers using cyclic voltammetry.[36]

3. Mechanistic Studies of Ferriheme Models and Proteins

Although it has long been known that ferriheme proteins such as methemoglobin and ferricytochrome *c* (Cyt^{III}) undergo autoreduction when exposed to nitric oxide,[7] the mechanisms of these reactions were only more recently investigated.

Sharma et al.[37] and Addison et al.[38] have carried out kinetics studies of the NO reductions of various ferriheme proteins including myoglobin,[37,38] human hemoglobin,[38] *Glycera dibranchiata* hemoglobins (Hb_m and Hb_h),[38] and opossum hemoglobin.[37] Electronic spectra, ESR, and circular dichroism studies were used to characterize formation of ferriheme nitrosyl complexes Fe^{III}(P)(NO) (P = porphyrinato ligand of heme protein) and the autoreduction products Fe^{II}(P)(NO) in pH 7 aqueous media. The latter study found that the autoreduction increased with increasing [NO] but leveled off at high [NO].[38] As a consequence, these workers proposed that the Fe^{III}(P)(NO) species must react with a second NO in the rate-determining step and suggested that an Fe(III) dinitrosyl complex $Fe^{III}(P)(NO)_2$, where the proximal histidine has been replaced by NO, may be an intermediate. The nature of the nitrogen-containing product was not clear, although the loss of the species NO^+ (which of course would be rapidly hydrolyzed to nitrite) was suggested.

The basis of the proposal[38] that a second mole of NO is involved in the rate-limiting step of the ferriheme protein autoreductions was drawn from the conclusion that K_{NO} for formation of the ferriheme nitrosyl Fe^{III}(P)(NO) (eq 12) is very large so that this first equilibrium was saturated at $P_{NO} < 0.02$ atm. This assumption, however, is challenged by measurements[30,37] that demonstrated smaller K_{NO} values than assumed, for example, $K_{NO} = 1.3 \times 10^4$ M^{-1} for metMb. Given the low solubility of NO in aqueous media (1.8 mmol L^{-1} atm^{-1}), saturation of eq 12 would have required a much higher P_{NO} than suggested. Nonetheless, despite this unfortunately high estimate, the k_{obs} values reported by these workers[38] increase at $P_{NO} > 1$ atm, in a manner suggesting the possible participation of a second NO at these elevated pressures.

$$Fe^{III}(P) + NO \rightleftharpoons Fe^{III}(P)(NO) \quad (12)$$

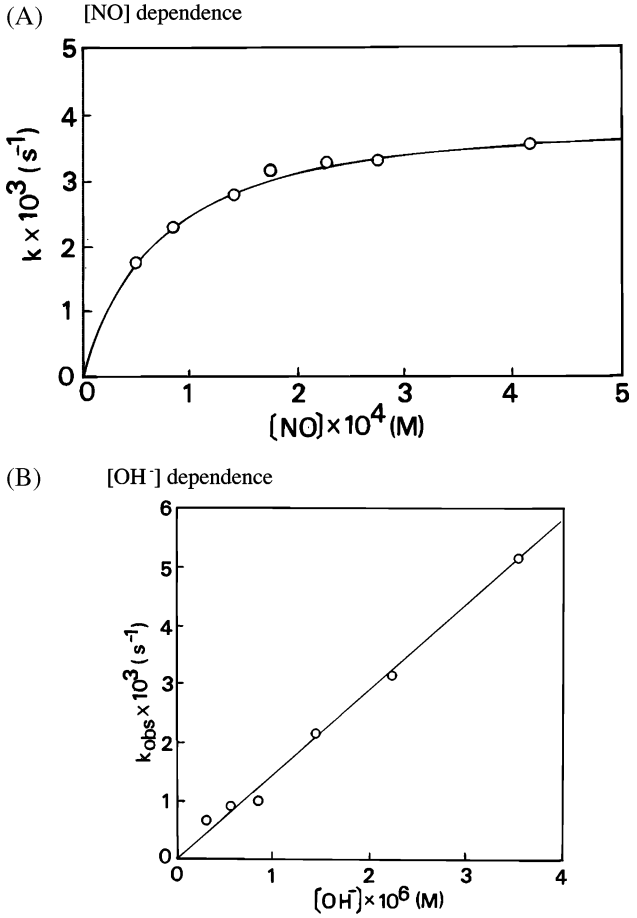
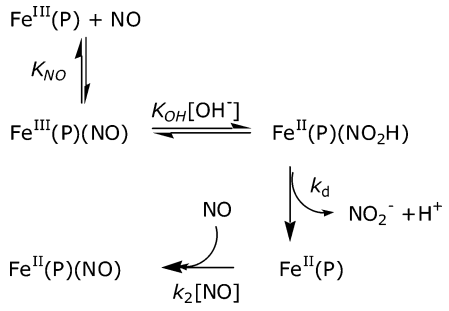


Figure 2. [NO] (A) and [OH^-] (B) dependences of Cyt^{III} reduction rate. (Reproduced with permission from ref 39. Copyright 1996 American Chemical Society.)

3.1. Base and NO Dependence

In subsequent studies, Hoshino and co-workers[39] reported the detailed kinetics for the NO reductions of the ferriheme proteins, ferricytochrome *c*, met-myoglobin, and met-hemoglobin in buffered aqueous media at various pH values. For example, the rate by which Cyt^{III} undergoes NO reduction to Cyt^{II} at pH > 6.5 displayed first-order dependence on [OH^-] and a complex dependence on [NO] (Figure 2). The NO dependence of k_{obs} proved to be cleanly consistent with the equilibrium formation of the nitrosyl complex Fe^{III}(P)(NO) followed by nucleophilic attack of OH^- at the coordinated nitrosyl. In other words, the NO is activated toward reaction with nucleophile (OH^-) by coordination to the oxidizing metal center as in the general mechanism described by Scheme 2. Scheme 5 describes a plausible mechanism that is

Scheme 5



based upon the kinetics studies by Hoshino et al. of the ligand substitution reactions of NO with heme proteins[30] and NO reduction of ferriheme proteins.[39] The rate law predicted by this scheme is presented in eq 13.

$$d[Fe^{II}(P)]_{Total}/dt = k_d \times \frac{K_{NO}[NO]}{1 + K_{NO}[NO]} \times \frac{K_{OH}[OH^-]}{1 + K_{OH}[OH]} \times [Fe^{III}(P)]_{Total} \quad (13)$$

The observed rates for the NO reduction of Cyt^{III}, metMb, and metHb proved to be functions of both [NO] and [OH^-] in accord with this analysis. Because the reaction of NO with Cyt^{II} to form $Cyt^{II}(NO)$ is very slow ($k_{NO} = 8.3\ M^{-1}\ s^{-1}$),[30] the formation of Cyt^{II} can be observed directly. The observed rates are functions of [NO] and [OH^-] as predicted by eq 13, $k_{obs} = k_{OH} \times K_{NO}[NO][OH^-]/(1 + K_{NO}[NO])$ at low pH (where $k_{OH} = k_d \times K_{OH}$), while $k_{obs} = k_{OH}[OH^-]$ at high [NO]. Figure 2A illustrates the response of k_{obs} to [OH^-] for the NO reduction of Cyt^{III}, a simple first-order dependence on [OH^-] at constant [NO]. No evidence for the N-bound nitrous acid complex $Fe^{II}(N(O)OH)$ was found for the three ferriheme proteins studied, so, either the formation of this intermediate is rate limiting or K_{OH} is very small in each case. Values of K_{NO} were determined from the spectroscopic titration of the respective ferriheme protein by NO, and kinetics studies gave the values for k_{OH} listed in Table 1.

Table 1. Reductive Nitrosylation of Ferriheme Proteins at 25.0 °C (Data from Ref 39)

	Cyt^{III}	metMb	metHb
K_{NO} (M^{-1})	1.6×10^4	1.3×10^4 [a]	1.3×10^4
k_{OH} ($M^{-1}\ s^{-1}$)	1.5×10^3	3.2×10^2	3.2×10^3
k_2 ($M^{-1}\ s^{-1}$)	8.3	1.7×10^7	2.5×10^7
pH	6.2−8.5	6.0−7.2	5.6−7.4

[a] K_{NO} for metMb is pH dependent, decreasing to 0.5×10^4 at higher pH (8.2).

The mechanisms for reductive nitrosylation of metMb and metHb are regarded to be similar to that for Cyt^{III}; however, because both Mb and Hb readily react with NO ($k_2 \approx 10^7\ M^{-1}$),[30] the only observable products were the ferroheme nitrosyls Mb(NO) and Hb(NO). For metMb, K_{NO} values decreased at the higher pH's, suggesting that pH change may bring forth protein conformation changes.

Another difference between metHb and metMb in this regard is that reductive nitrosylation of the former also occurs at lower pH values (<6), implying that metHb(NO) reacts with not only OH^- but also with H_2O (eq 14).[39] The pseudo-first-order rate constant, k_{H_2O}, was determined to be $1.1 \times 10^{-3}\ s^{-1}$ in 298 K aqueous solution, and one might propose that the metHb(NO) reaction with water is activated by general base catalysis. The analogous reactions of metMb(NO) and $Cyt^{III}(NO)$ were not observed at low pH; therefore, direct reactions of metMb(NO) and $Cyt^{III}(NO)$ with H_2O appear to be much slower than those for metHb.

$$metHb(NO) + H_2O + NO \xrightarrow{k_{H_2O}} Hb + NO_2^- + 2H^+ \quad (14)$$

One problem in the Hoshino et al. studies of the reductive nitrosylation of ferriheme proteins[39] is that attempts to quantify the nitrite formed fell 30−40% short of the amount predicted by Scheme 5. This was attributed to analytical difficulties, especially in light of competing processes that led to the formation of nitrate ion as well. However, Singel et al.[13c] have suggested that the low NO_2^- analysis may be an indicator of the formation of another product in the reductive nitrosylation, at least in the case of metHb, and have suggested that the product may be SNO-Hb (see below).

A subsequent investigation by Reichenbach et al.[40] reported the NO reduction of metMb in pH 7.4 phosphate buffer solution by the biological antioxidant glutathione (GSH). Optical spectral changes indicated the formation of Mb(NO) as one product, while amperometric sensor experiments were interpreted in terms of the nitrosoglutathione (GSNO) being the other product (eq 15). The second-order rate constant for reaction of GSH with metMb(NO) was determined to be 47 $M^{-1}\ s^{-1}$. This is a somewhat surprising result given that k_{OH} for the much smaller and more basic hydroxide ion is only an order of magnitude higher (Table 1).

$$metMb + 2NO + GSH \rightarrow Mb(NO) + GS{-}NO + H^+ \quad (15)$$

The kinetics behavior observed by Hoshino and co-workers for metHb carry over to FixL, a heme-based sensor protein in the signal transduction system responsible for regulating nitrogen fixation in *Rhizobium melitoti*.[41] Ferric FixL undergoes reduction by NO via a [OH^-]-dependent pathway to generate the reduced protein and nitrous acid. The rates are about an order of magnitude smaller than those for metMb(NO) under analogous conditions, and the difference was attributed to two features of FixL versus the globins, lower electrophilicity of the bound NO because of diminished H-bonding in the heme pocket and decreased OH^- accessibility to the coordinated NO. Notably, there is also a pathway independent of [OH^-] that was attributed to reaction with another nucleophile, most likely water.

3.2. General Base Catalysis

The observation that metHb was reduced by NO via a hydroxide independent pathway stimulated Fernandez et al.[35,42] to examine more closely the kinetics for the water-soluble ferriheme models $Fe^{III}(TPPS)(H_2O)_2$ and $Fe^{III}(TMPy)(H_2O)_2$[42c] (TMPy = *meso*-tetrakis(*N*-methyl-4-pyridyl)-porphyrinato) in moderately acidic buffered aqueous solutions (pH 4−6). Both complexes demonstrate the ready formation of the Fe(III) nitrosyl complexes as evidenced by shifts in the Soret and Q-band regions of the spectra characteristic of such iron prophyrinato complexes. For example, addition of NO to a solution of $Fe^{III}(TPPS)$ leads to the equilibrium formation of $Fe^{III}(TPPS)(NO)$ as evidenced by a shift of the Soret

band from a λ_{max} at 394 nm ($\epsilon = 9.7 \times 10^4$ M^{-1} cm^{-1}) to 422 nm ($\epsilon = 12.0 \times 10^4$ M^{-1} cm^{-1}), characteristic of the latter. At low pH, significant reduction of the Fe^{III} center was not observed because of the thermodynamic considerations discussed above. From the spectral changes as a function of the NO partial pressure (P_{NO}), the equilibrium constant K_{NO} for formation of the ferric nitrosyl complex (eq 16) was determined to be 1.32 ($\pm$0.09) $\times$ 10^3 M^{-1} for the anionic TPPS complex (μ = 0.10 M, 298 K) in agreement with the previously reported value (1.2×10^3 M^{-1} in acidic solution).[16] Similar spectral changes upon addition of NO to solutions of the cationic complex Fe^{III}(TMPy) (Figure 3) gave the K_{NO} value of $(4.6 \pm 1.7) \times 10^2$ M^{-1}.[35]

$$Fe^{III}(P) + NO \xrightarrow{K_{NO}} Fe^{III}(P)(NO) \qquad (16)$$

For both systems, there are progressive spectral changes at higher pH values consistent with the reduction of the ferric species to the ferrous analogue (e.g., for Fe^{II}(TPPS)(NO) λ_{max}(Soret) = 412 nm, $\epsilon = 10.0 \times 10^4$ M^{-1} cm^{-1}) as indicated by eq 11. For example, the observed rate constant k_{obs} for NO reduction of Fe^{III}(TPPS) in pH 5 acetate buffered solution (20 mM) under 1.0 atm NO was 2.36×10^{-4} s^{-1}. The k_{obs} for the cationic complex Fe^{III}(TMPy) was more than an order of magnitude higher (7×10^{-3} s^{-1}).

The effects of pH and of NO and buffer concentrations were probed in detail for the NO reduction of Fe^{III}(TPPS).[35] The rates were markedly sensitive to P_{NO} with curved k_{obs} versus [NO] plots approaching saturation at 1 atm in a manner consistent with the reaction occurring via the general mechanism described by Scheme 2. The curvature of these plots could be modeled accurately using eq 17 and the K_{NO} value described above.[35]

$$\frac{d[Fe^{III}]}{dt} = \frac{k_{red}K_{NO}[NO][Fe^{III}]}{1 + K_{NO}[NO]} = k_{obs}[Fe^{III}] \qquad (17)$$

The kinetics of this reaction were pH independent in moderately acidic solutions (pH 4−5); thus there was no specific base catalysis under these conditions. However, in the pH range 4−6, the k_{obs} values were dependent on the nature and concentration of the buffer. From the linear plot of k_{obs} versus [buffer] (buffer = NaOAc/HOAc at 0.1 M ionic strength), a slope of 1.73×10^{-3} M^{-1} s^{-1} and an intercept of 1.95×10^{-4} s^{-1} were found at $P_{NO} = 1$ atm (Figure 4). This indicates the relationship $k_{red} = k_o + k_{buffer}$[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} can be calculated for these conditions. At the same pH and P_{NO}, the buffer DESPEN exhibited a 5-fold greater slope. Similar behavior was noted for phosphate buffer at pH 6. In this context, one may propose a mechanism for the NO reduction of **1** under conditions of general base catalysis as illustrated by Scheme 6.

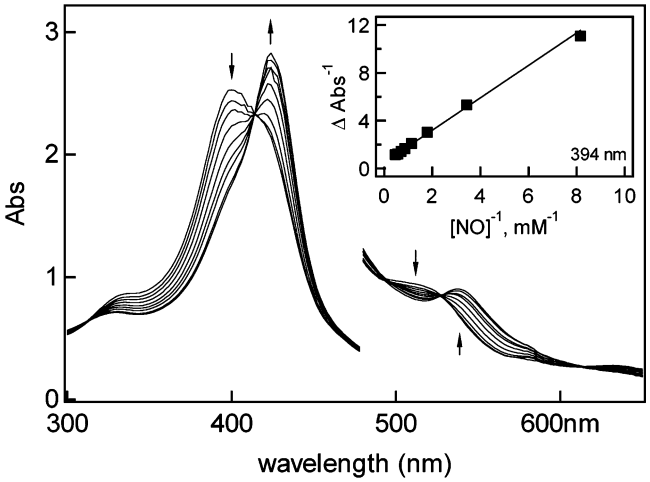


Figure 3. Spectral changes upon adding different [NO] to an aqueous solution of Fe^{III}(TMPy) with 100 mM triflic acid (μ_{tot} = 0.10 M) at 298 K. The inset is a linear plot of ΔAbs^{-1} (at 394 nm) versus $[NO]^{-1}$ from which the equilibrium constant $K_{NO} = (4.6 \pm 1.7) \times 10^2$ M^{-1} was calculated. (Reproduced with permission from ref 35. Copyright 2004 American Chemical Society.)

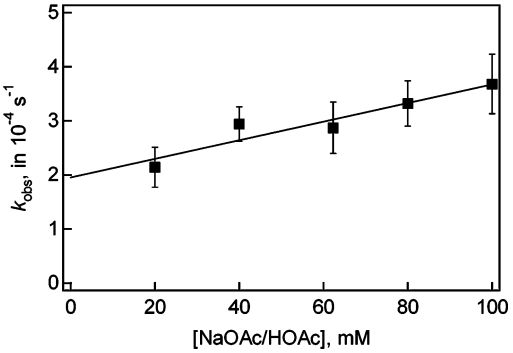
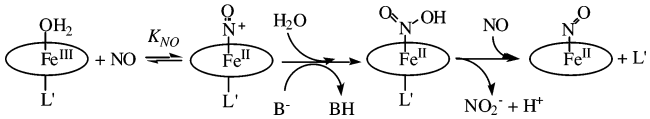


Figure 4. Effect of NaOAc/HOAc buffer concentration on the rate constant k_{obs} for NO reduction of Fe^{III}(TPPS) in pH 5.00 aqueous solution at 298 K (μ_{tot} = 0.10 M, [NO] = 1.9 mM). (Reproduced with permission from ref 47.)

Scheme 6



3.3. Catalysis by Nitrite

In the course of probing the buffer catalysis mechanism, Fernandez et al.[35,42] made the more remarkable discovery that Fe^{III}(TPPS) reduction by NO is also catalyzed by traces of nitrite ion in solution. Nitrite is not only the reaction product but is also a ubiquitous impurity in aqueous NO solutions, because it is the product of NO autoxidation in such environments.[43] When $NaNO_2$ (0−2.5 mM) was deliberately added to buffered (NaOAc/HOAc at pH 4.96, μ_{tot} = 0.1 M) solutions of Fe^{III}(TPPS) and NO (1.9 mM), the rates of Fe^{III}(TPPS) reduction were dramatically enhanced. The k_{obs} values proved to be a linear function of [NO_2^-], and a functional dependence on [NO] in accord with eq 17 was demonstrated. Thus, $k_{obs} = (k_{red} + k_{nitrite}[NO_2^-]) \times f(NO)$, where $f(NO) = (K_{NO}[NO]/(1 + K_{NO}[NO])$ and accounts for

the role of NO under a specified [NO]. For $P_{NO} = 1$ atm, the slope of this plot is 2.2 ± 0.1 M^{-1} s^{-1} (298 K), and once f(NO) is taken into account, the catalytic rate constant $k_{nitrite}$ can be calculated from this slope as 3.1 M^{-1} s^{-1}. This is more than 3 orders of magnitude larger than the effect of the acetate buffer alone. In contrast, when nitrite (0−100 mM) was added to Fe^{III}(TPPS) solutions in the absence of added NO, no reduction was observed and complexation of NO_2^- with the ferriheme was not seen under these conditions. Furthermore, $k_{nitrite}$ proved to be independent of the buffer solution. Therefore, nitrite is indeed a catalyst for NO reduction of Fe^{III}(TPPS).

Nitrite catalysis was also seen for the NO reduction of Fe^{III}(TMPy). Again, linear plots of k_{obs} versus [NO_2^-] with near zero intercepts were obtained (Figure 5), and, from these data, the $k_{nitrite}$ value of 83 ± 5 M^{-1} s^{-1} was obtained, nearly 30-fold larger than that seen with Fe^{III}(TPPS).

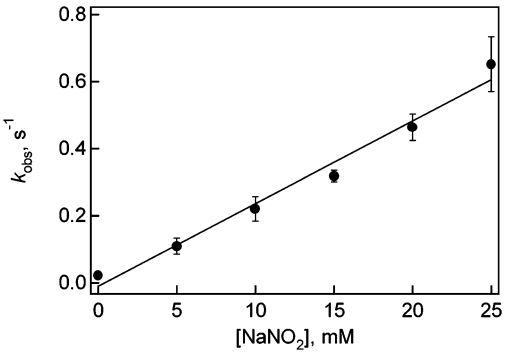


Figure 5. Plot of k_{obs} versus [NO_2^-] for the NO reduction of Fe^{III}(TMPy). The slopes of these plots reproducibly fell in the range 24.6 ± 1.8 M^{-1} s^{-1}. From these slopes and the relationship between k_{obs} and k_{red} described above, the $k_{nitrite}$ value of 83 ± 6 M^{-1} s^{-1} was obtained. (Reproduced with permission from ref 47.)

The observation of this nitrite catalysis also led Fernandez et al. to reexamine the NO reductions of the ferriheme proteins metHb and metMb in pH 7.0 aqueous phosphate buffer with various added $NaNO_2$ concentrations under NO (1.8 mM). Consistent with the earlier studies by Hoshino et al.,[39] reactions carried out in pH 7.0 solution in the absence of added nitrite showed that metHb and metMb react rapidly with NO to generate an equilibrium mixture of the Fe^{III}(P) and Fe^{III}(P)(NO) that underwent slow reductive nitrosylation with lifetimes of 10^3 and 10^4 s, respectively. Added $NaNO_2$ (0−20 mM for metHb; 0−80 mM for metMb) dramatically accelerated rates consistent with a catalytic role of NO_2^- in these systems as well, and values of $k_{nitrite}$ were calculated as 0.14 M^{-1} s^{-1} for metHb and 1.1 × 10^{-2} M^{-1} s^{-1} for metMb (Table 2).

Two limiting mechanisms were proposed to explain the nitrite catalysis. Both involve the initial reversible formation of the ferric nitrosyl (eq 16); however, the role of this species diverges for the two proposals. For example, as we have argued above for the generalized Scheme 2, the Fe(III)-coordinated NO is activated toward nucleophile addition. If NO_2^- were to serve as the primary nucleophile to give the adduct $Fe^{II}-N_2O_3$ as an intermediate, dissociation of this

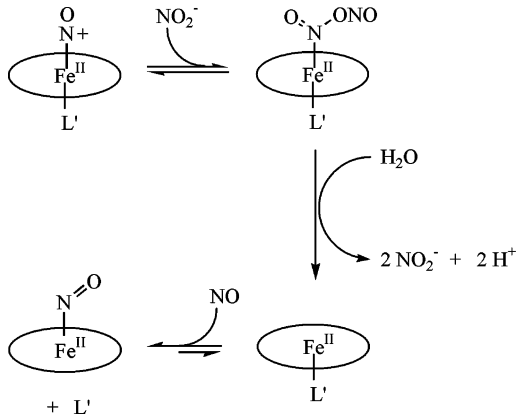
Table 2. Nitrite Catalysis of the Reductive Nitrosylation of Ferriheme Complexes (Data from Refs 35, 42)

	E (V)[a] (vs NHE)	$K_{nitrite}$ (M^{-1} s^{-1})
Fe^{III}(TmPy)(NO)	0.79	85
Fe^{III}(TPPS)(NO)	0.59	3.1
metHb(NO)	0.55	0.13
metMb(NO)	0.47	0.011

[a] To our knowledge, direct measurements of the metHb(NO)/Hb(NO) and metMb(NO)/Mb(NO) half cell reduction potentials have not been reported, but estimates of 0.49−0.57 and 0.47 V (vs NHE) were generated from known reduction potentials and equilibrium constants[39,104] using Born−Haber-type cycles (Scheme 6).

species would release the ferroheme protein plus N_2O_3. The latter species would undergo hydrolysis to 2 moles of nitrous acid,[44] while the Fe^{II}(P) is trapped by excess NO to give the Fe^{II}(P)(NO) final product (Scheme 7). This mechanism follows the

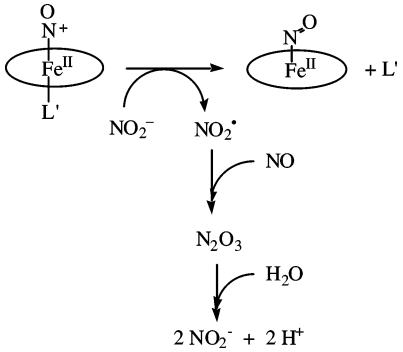
Scheme 7



theme discussed above with regard to specific and general base catalysis, but requires that NO_2^- be a much stronger nucleophile than the buffer anions present in much higher concentrations (see below).

A markedly different hypothetical mechanism would involve direct reduction of the ferriheme nitrosyl to Fe^{II}(P)(NO) by outer-sphere electron transfer from NO_2^- (Scheme 8). The nitrogen dioxide formed would

Scheme 8



be rapidly scavenged by excess NO (k = 1.1 × 10^9 M^{-1} s^{-1}) to give N_2O_3, which undergoes hydrolysis to the nitrite product. The key step here is the electron transfer, and while it is not thermodynamically favored (ΔE = −0.31 V), the fast trapping of one of

the initial products $NO_2^{\bullet}$ and subsequent N_2O_3 hydrolysis could make it viable.

The potential role of an outer-sphere electron transfer as the rate-limiting catalytic step may be evaluated by using the Marcus cross relation (eq 9) to estimate k_{OS}, the rate constant for the reaction between $Fe^{III}(P)(NO)$ and NO_2^- (P = TPPS). Such analysis concluded that for $k_{OS} = k_{nitrite}$, the rate constant for $Fe^{II}(P)(NO)/Fe^{III}(P)(NO)$ self-exchange would need to be $\sim 6 \times 10^6$ M^{-1} s^{-1}. While this estimate falls within the range of observed self-exchange rates for low-spin Fe porphyrin complexes,[46] it should be emphasized that this rate constant is undetermined. Qualitatively, the reactivity order of various ferriheme complexes (Table 2) also fits an outer-sphere electron-transfer mechanism, because the rate constant $k_{nitrite}$ should then be a function of the $\Delta E_{1/2}(Fe^{III}/Fe^{II})$ reduction potentials. This derives from the Marcus cross relation because k_{OS} should be proportional to $K_{OS}^{1/2}$, if self-exchange rate constants are approximately the same for different hemes.

A common aspect of the two proposed schemes for nitrite catalysis is the formation of N_2O_3 as a reactive intermediate. In aqueous solution, this would be expected to hydrolyze readily to nitrous acid; however, the potential of N_2O_3 formation in the hydrophobic pocket of a protein, where its hydrolysis should be slower, offers some interesting possibilities. For example, N_2O_3 is a nitrosating agent and could conceivably react with protein amine or thiols to generate *N*-nitrosoamines or *S*-nitrosothiols. For example, it has been speculated[35] that nitrite catalysis of metHb reduction by NO could be one explanation for formation of *S*-nitrosylated hemoglobin, which was mentioned above and will be discussed further in a subsequent section.

3.4. Reactions with Other Nucleophiles

Preliminary experiments were carried out to test the potential reactivity of other nucleophiles in the NO reduction of $Fe^{III}(TPPS)$.[47] The most dramatic effects were seen with added sodium azide. Although N_3^- and acetate ion have similar pK_a's, the former is considerably more nucleophilic. Addition of [NaN_3] (20−160 μM) greatly enhanced rates of $Fe^{III}(TPPS)$ reduction by NO (1.9 mM) in aqueous NaOAc/HOAc buffer at pH 5.0 and constant ionic strength (0.10). A linear plot of k_{obs} versus [N_3^-] gave a k_{azide} value of 67.2 ± 3.4 M^{-1} s^{-1} (298 K), which is 20-fold faster than the catalysis rate constant by NO_2^- described above. Although the products other than $Fe^{II}(TPPS)(NO)$ were not identified in this study, the expected reaction with azide is indicated in eq 18, and it is obvious that the stabilities of these products provide considerable driving force for the overall transformation.

$$Fe^{III}(TPPS) + 2NO + N_3^- \rightarrow Fe^{II}(TPPS)(NO) + N_2O + N_2 \quad (18)$$

The high reactivity of azide in this process suggests that the role of nitrite in catalyzing NO reduction of ferriheme proteins and models is likely to be due to its action as a nucleophile, for example, Scheme 7. This needs to be examined more thoroughly. Several scales of nucleophilicity have been proposed on the basis of the reactivity of these species with a particular common reactant. Two such scales used to compare a handful of nucleophiles are listed in Table 3. The n_{Pt} scale is based on relative reactivity with *trans*-[$Pt(py)_2Cl_2$] in 298 K MeOH, while the n_{CH_3I} scale is based on the relative reactivity with CH_3I in 298 K MeOH.[48] Qualitatively, one might expect the reactivity of a nucleophile Nuc toward an Fe(III) coordinated NO to be more reflective of its nucleophilicity toward the softer electrophile Pt(II) than perhaps with CH_3I, or with the pK_a. However, on both scales, nitrite and azide are much stronger nucleophiles than acetate so their greater reactivities are consistent with a scheme such as Scheme 2. However, pyridine has nucleophilic properties in this regard that are similar to NO_2^- yet apparently does not accelerate the reaction, while glutathione does with a second-order rate constant of 0.16 M^{-1} s^{-1}, nearly 2 orders of magnitude larger than that of acetate.[47]

Table 3. Second-Order Rate Constants Reflecting the Effect of Added Nucleophiles on the $Fe^{III}(TPPS)$ Reduction by NO (1.9 mM) in Aqueous NaOAc/HOAc Buffer at pH 5.0 and Constant Ionic Strength (0.10) As Compared to the Nucleophilicity Parameters n_{Pt} and n_{CH_3I} and Their pK_a's (Data from Ref 47)

	n_{Pt}[a]	n_{CH_3I}[b]	pK_a	k_{X^-} (M^{-1} s^{-1})
$CH_3CO_2^-$	<2.0	4.3	4.75	2.4×10^{-3}
pyridine	3.19	5.23	5.23	$<2 \times 10^{-3}$
NO_2^-	3.22	5.35	3.37	3.1
imidazole[c]	3.44	4.97	7.10	$<2 \times 10^{-3}$
N_3^-	3.58	5.78	4.74	67

[a] n_{Pt} based on relative reactivity with *trans*-[$Pt(py)_2Cl_2$] in 298 K MeOH.[48] [b] n_{CH_3I} based on relative reactivity with CH_3I in 298 K MeOH.[49] [c] In the case of imidazole, the reaction was run at a pK_a where the unprotonated form was of very low concentration, so the comparison may not be valid.

With the exception of the hypothetical outer-sphere electron transfer proposed as a possible explanation of nitrite catalysis (Scheme 8), the discussion above regarding the NO reduction of ferriheme models and proteins has focused on mechanisms that are derivatives of the pathways described in Scheme 2. These ferrihemes reversibly form nitrosyl complexes, and the kinetics behavior clearly points to the $Fe^{III}(P)(NO)$ species lying directly along the reaction coordinate. Furthermore, there is ample precedent for reactions of various bases with, for example, nitroprusside, to form nucleophile nitrosyl adducts, so the general expectation for these systems is that these will proceed via NO^+ transfer after nucleophilic addition at the coordinated nitrosyl. However, it should be emphasized that an $Fe^{II}(P)(N(O)Nuc)$ adduct formed in this manner has not yet been observed directly as an intermediate along the reaction coordinate for the ferriheme complexes.

4. Studies of Other Iron(III) Complexes

As noted in the Introduction, Caulton et al.[10] demonstrated clear evidence for the role of NO as a reducing agent in reactions with metal salts. In the

presence of methanol and amines (base), both $FeCl_2$ and $CoCl_2$ were shown to react with NO to form metal nitrosyls and alkyl nitrite (RONO). Among numerous examples of other non-heme iron(III) complexes displaying this reactivity are the dithiocarbamate complex (Fe^{III}(MGD)),[49] iron(III) (dithiocarboxy)sarcosine (Fe^{III}(DTCS)),[50] the antitumor agent iron(III) bleomycin (Fe^{III}(Blm)),[51] $[Fe(bpb)(py)_2]ClO_4$ (H_2bpb = 1,2-bis(picolinamido)benzene),[52] and $Fe(pyN_4)$ (pyN_4 = 2,6-$C_5H_3N[CMe(CH_2NH_2)_2]_2$.[53]

The most extensively investigated non-heme iron(III) system is nitroprusside, $Fe(CN)_5(NO)^{2-}$. Olabe and co-workers[24c,25] have systematically studied the mechanism of eq 19 for M = Fe, Ru, and Os. The equilibrium constant K_{19} is quite dependent on the nature of the metal center with values of 1.5×10^5, 4.4×10^6, and 42 M^{-2} for the Fe^{II}, Ru^{II}, and Os^{II} species, respectively. The lower K_{19} for the osmium complex is consistent with the relatively low ν_{NO} value for this species (ν_{NO} = 1897 cm^{-1}) as compared to higher values for M = Fe and Ru (1945 and 1926 cm^{-1}, respectively). The rationale for such a relationship is that ν_{NO} should be reflective of the π^*_{NO} electron density, the higher frequencies being seen with the more electron-accepting metal centers; a similar correlation has been described for activation of coordinated CO by nucleophiles.[28,54] However, the relative K_{19}'s for Fe and Ru do not fit this expectation, and it was argued that other factors such as the greater polarizability of the $Ru(CN)_5(NO)^{2-}$ may be counteracting the π-bonding differences between Fe and Ru in this case. The rates of forward reactions are first order in [OH^-], and the k_{OH} values (298 K) follow a pattern similar to the equilibrium constants with k_{OH} being, respectively, 0.55, 0.95, and 1.4×10^{-4} M^{-1} s^{-1} for M = Fe, Ru, and Os.[25] The difference lies largely in the activation enthalpies; $\Delta H^{\ddagger}$ is 23 kJ mol^{-1} higher for the slowest Os complex than for the fastest Ru complex.

$$M(CN)_5(NO)^{2-} + 2OH^- \rightleftharpoons M(CN)_5(NO_2)^{4-} + H_2O \quad (19)$$

There have been numerous other studies regarding reactions of various nucleophiles with nitroprusside. Reaction of HS^- with NP leads initially to a species interpreted to be the strongly colored thiol analogue of the nitro product, $Fe(CN)_5(N(O)S)^{4-}$; however, this is not stable and undergoes oligimerization, possibly via the formation of bridging disulfide bonds.[55] Notably, the reactions of the $M(CN)_5(NO)^{2-}$ ions with the SH^- ion are much faster than the analogous reactions with OH^-; the rate constants k_{SH}(M) are several orders of magnitude larger than the k_{OH}(M) values for the same complexes.[25] NP also reacts with mercaptans (RSH) and mercaptides (RS^-) to form deeply colored metal nitrosothiolato intermediates,[56] which are unstable and decay via formation of disulfides and reduced nitroprusside. Similar processes may be responsible for the biological activity of sodium nitroprusside, which is used as an intravenously administered vasodilator drug.[57]

With regard to nitrogen bases, NH_3 reacts with nitroprusside to give $Fe(CN)_5(H_2O)^{3-}$ plus N_2.[58] Likewise, primary amines RNH_2 are diazotized by aqueous NP to give the alcohols plus N_2, with the maximum rate occurring at about pH 10.5.[59] The rates of these reactions are first order in [NP] and [RNH_2] and increase with the basicity of the amines. The reaction of NP with hydrazine leads to the formation of NH_3 and nitrous oxide (eq 20) with the rate law: $-d[NP]/dt = k[NP][NH_2NH_2]$.[60] The hydrazinium ion $N_2H_5^+$ was inactive, so the rate dropped to near zero at pH 6 in accord with the pK_a of this species.

$$Fe(CN)_5(NO)^{2-} + NH_2NH_2 + H_2O \rightarrow Fe(CN)_5(H_2O)^{3-} + NH_3 + N_2O + H^+ \quad (20)$$

Reaction of metal nitrosyls with azide ion proceeds with formation of N_2 and N_2O.[61]

5. Investigations of Copper(II) Reductions

Although not as extensively studied as the reactions with ferriheme models and proteins, it has long been known that certain copper(II) models and proteins are reduced by NO.[62,63] For example, this has been demonstrated for cupric centers in cytochrome *c* oxidase and laccase, and, in the case of the former, the NO reduction of Cu(II) to Cu(I) has been suggested to play a role in regulating the electron transport activity of this protein.[64] Cu(II) has also been shown to promote the nitrosation of various thiolates (forming *S*-nitrosothiols),[65] and Cu(II) reduction was found to correlate with formation of *S*-nitroso bovine serum albumin (BSA) and *S*-nitroso glutathione. Such observations have been presented to fomulate a potential mechanism for the formation of RSNO compounds found in blood (eq 21).[67] However, Cu(I) also participates in side reactions leading to RSNO degradation to reform NO.[66]

$$Cu(II) + BSA{-}SH \overset{-H^+}{\underset{+H^+}{\rightleftharpoons}} [Cu(II) + BSA{-}S^-]^+ \overset{+NO}{\underset{-NO}{\rightleftharpoons}} BSA{-}SNO + Cu(I) \quad (21)$$

Among studies of model compounds, Cao and coworkers reported that the reaction of a series of copper(II) dithiocarbamates with NO in aqueous solution results in the formation of an air-stable copper-nitrosyl and dinitrosyl species.[68] Using ^1H NMR and ESR spectroscopy, they were able to observe the formal reduction of the copper center; however, the only nitrosylated complex that they were able to isolate and characterize was bis(morpholyldithio-carbamate)(nitrosyl)copper(II) (ν_{NO} = 1682 cm^{-1} in KBr).

Detailed kinetics studies of the Cu(II)/NO reactions are few. One involves the NO reduction of the cupric complex $Cu(dmp)_2(H_2O)^{2+}$ (dmp = 2,9-dimethyl-1,10-phenanthroline), which was studied by Tran et al. in aqueous solution and various mixed solvents.[6] The very positive reduction potential for $Cu(dmp)_2(H_2O)^{2+}$ (0.58 V vs NHE in water)[69] relative to most other cupric complexes can be attributed to steric repulsion between the 2,9-methyl substituents that favors the tetrahedral coordination of Cu(I) over the tetragonal pyramidal structure of Cu(II). For example, the less

distorted 1,10-phenanthroline analogue $Cu(phen)_2(H_2O)^{2+}$ is a weaker oxidant (0.18 V).[69] In methanol, the product of the $Cu(dmp)_2(H_2O)^{2+}$ oxidation of NO is CH_3ONO (eq 3); in water, it is NO_2^- (eq 22). The reaction did not occur in CH_2Cl_2 unless methanol was added, and in such solutions the reaction rate was linearly dependent on the concentration of alcohol added.[6]

$$Cu(dmp)_2(H_2O)^{2+} + NO \rightarrow Cu(dmp)_2^{+} + NO_2^{-} + 2H^{+} \quad (22)$$

The kinetics of this reaction were followed by tracking the appearance of $Cu(dmp)_2^+$, which displays a strong metal-to-ligand charge-transfer (MLCT) band at 455 nm using a stopped-flow kinetics spectrophotometer. Under these conditions, there was no spectroscopic evidence of intermediates; that is, a $Cu^{II}-NO$ complex of the type common to the ferriheme reactions with NO was not seen. At a fixed pH, the kinetics in aqueous solution followed the rate law:

$$\frac{d[Cu(dmp)_2^{+}]}{dt} = k_{NO}[NO][Cu(dmp)_2(H_2O)^{2+}] \quad (23)$$

Addition of a small amount of $NaNO_2$ (50 μM) with NO present had no effect on the kinetics, and no reaction was observed (on the time scale of the stopped flow experiment) when NO was absent. At higher concentrations, other anions, including the conjugate bases of various buffers (B^-), slowed the reductive nitrosylation kinetics. This was attributed to the competition between water and these anions for the labile fifth coordination site of $Cu(dmp)_2(H_2O)^{2+}$.[69] Similarly, the kinetic behavior with regard to pH was complicated by the acidity of the coordinated H_2O, which has a pK_a of 8.6.[6]

Tran et al.[6] considered two hypothetical reaction mechanisms for the Cu^{II} reduction by NO. One was based on the activation of NO by coordination to the Cu(II) center followed by nucleophilic attack as illustrated by Scheme 2. The alternative was based on the generalized electron-transfer mechanism suggested by Scheme 3.

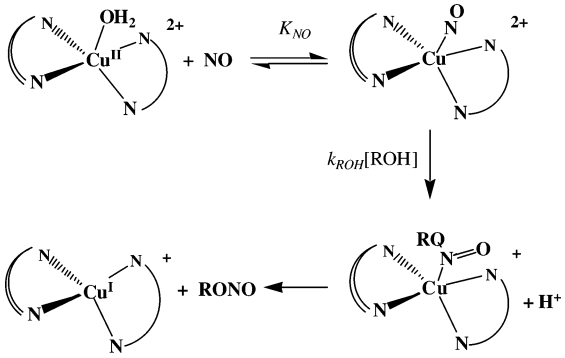
The latter would involve outer-sphere oxidation of NO (eq 24) followed by hydrolysis of NO^+. The two limiting cases are both first order in [NO]. One would involve a reversible equilibrium followed by rate-limiting hydrolysis of the nitrosonium ion, giving the second-order rate constant $k_{NO} = K_{OS}k_{hyd}$, where $K_{os} = k_{os}/k_{-os}$. In the mixed CH_2Cl_2/MeOH solution, the term k_{hyd} would equal k_{MeOH}[MeOH], thus explaining the rate dependence on [MeOH]. Alternatively, k_{os} could be rate limiting ($k_{NO} = k_{os}$), and electron transfer is effectively irreversible owing to rapid hydrolysis of NO^+. This seems more likely given that k_{hyd} should be quite large.[71]

In either limit for the outer sphere electron transfer mechanism, the inhibition by buffer anions could be explained by the (likely) lower reduction potential of a $Cu(dmp)_2(B)^+$ species. Regardless, k_{os} is the maximum rate constant by which NO reduction of Cu(II) would occur, and a value for this can be estimated from the Marcus cross relation (eq 9). This treatment estimated k_{os} as $\sim 3 \times 10^{-3}$ M^{-1} s^{-1}, a value 5 orders of magnitude smaller than the k_{NO} measured for eq 24 at lower pH's. On this basis, the outer-sphere reaction mechanism was concluded to be unlikely.[6]

$$Cu(dmp)_2^{2+} + NO \underset{k_{-os}}{\overset{k_{os}}{\rightleftharpoons}} Cu(dmp)_2^{+} + NO^{+} \quad (24)$$

Alternatively, the kinetics for NO reduction of aqueous $Cu(dmp)_2(H_2O)^{2+}$ can be rationalized in terms of the mechanism shown in Scheme 9, which

Scheme 9

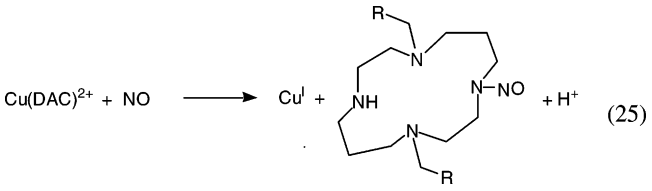


is equivalent for the generalized mechanism depicted in Scheme 3. The three steps would be (i) the reversible equilibrium displacement of solvent (H_2O or ROH) by NO to form the coordination complex Cu(II)−NO; the latter is activated toward nucleophilic attack by ROH (step ii) because of charge transfer from NO to the metal ($Cu^{II}-NO \leftrightarrow Cu^{I}-NO^+$). Dissociation of the RONO complex (step iii) would be rapid because of the preference of the cuprous complexes for tetrahedral coordination. This parallels the reductive nitrosylation discussed above for the ferriheme systems with the exception that the $Cu^{II}-NO$ complex is formed with a very low K_{NO}.

In recent studies designed to evaluate the viability of these two potential mechanisms, Lim[71,72] investigated the NO reduction of $Cu(dpp)_2^{2+}$ (where dpp = 2,9-diphenyl-1,10-phenanthroline) in methanol solution. The $Cu(dpp)_2^{2+}$ ion has a higher reduction potential (0.70 V) than does $Cu(dmp)_2^{2+}$, but the 2,9-phenyl groups are more bulky than the methyl groups in the same sites. Thus, one might expect that $Cu(dpp)_2^{2+}$ would be more reactive than $Cu(dmp)_2^{2+}$ via the outer-sphere electron-transfer mechanism but less so via the mechanism illustrated by Scheme 9. The latter scenario appears to be the case with the k_{NO} (298 K) measured in neat MeOH being 22 M^{-1} s^{-1} for $Cu(dpp)_2^{2+}$ and 38 M^{-1} s^{-1} for $Cu(dmp)_2^{2+}$. This result supports a reductive nitrosylation pathway similar to that seen for the ferriheme complexes.

A somewhat different mechanism has proved necessary to interpret the reaction of NO with the copper(II) complex $Cu(DAC)^{2+}$, where DAC is the 1,8-bis(9-anthracylmethyl)-derivative of the macrocyclic tetraamine cyclam (1,4,8,11-tetraazacyclotetradecane).[73] Although the free ligand is strongly fluorescent from the anthracene chromophores, analogous solutions of $[Cu(DAC)]^{2+}$ (various salts) display little or no luminescence at room temperature or at 77 K (MeOH/EtOH 1:4 frozen glass) because of intramolecular quenching by the paramagnetic Cu(II)

center. Introduction of NO to methanolic solution of $Cu(DAC)^{2+}$ resulted in the disappearance of the broad, weak d−d absorption band at $\lambda_{max} = 566$ nm ($\epsilon = 266\ M^{-1}\ cm^{-1}$), shifts in the anthrancene $\pi\pi^*$ absorption bands, and the appearance of anthracene-type fluorescence. Electrochemical analysis indicated the formation of Cu(I) species in solution. However, in marked contrast to the reaction with $Cu(dmp)_2(H_2O)^{2+}$ (eq 22), NO reduction of $Cu(DAC)^{2+}$ in aqueous methanol is accompanied by the nitrosation and release of the DAC ligand itself (eq 25) as evidenced by ESI-mass spectral and ^1H NMR analysis. Preliminary kinetic studies of this reaction showed that there was a first-order dependence on [NO] and that the reaction was accelerated at higher pH.

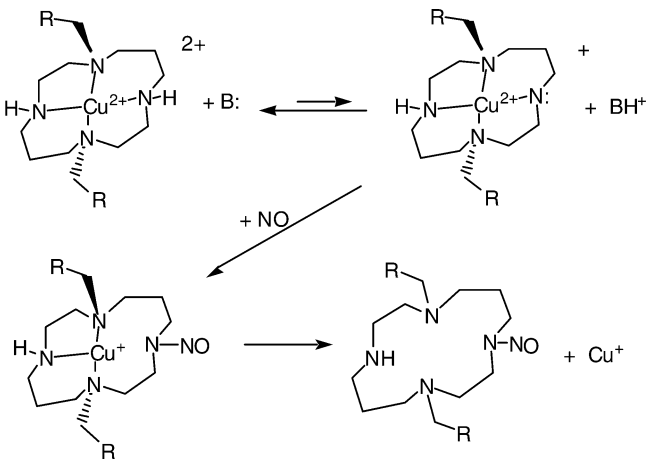


(25)

On the basis of these observations, two pathways consistent with the published data have been discussed in the context of the mechanism of this reaction.[73] The first would be that the NO initially reacts at the copper(II) site to form a $Cu^{II}-NO$ (or $Cu^{I}-NO^{+}$) complex. This would be followed by base-catalyzed deprotonation of one of the amines perhaps concerted with NO^+ migration to the resulting amide. Given that the DAC type ligand is well suited for square planar coordination to Cu(II) but not for tetrahedral coordination to Cu(I), the nitrosated ligand is then released.

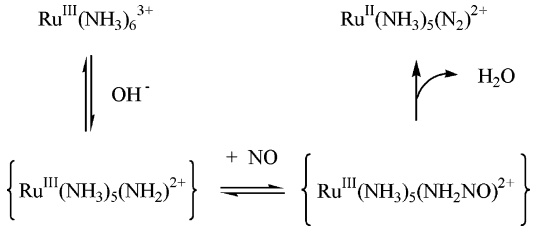
The alternative pathway is described in Scheme 10 and follows the theme presented in Scheme 4. The initial step would be reversible deprotonation of the coordinated secondary amine, and this would be followed by addition of NO at the amide site concomitant with electron transfer to reduce the copper center. Again, the resulting Cu^{I} dissociates from the ring because of its preference for tetrahedral geometry (which is constrained by the ring) and the less basic nature of the nitrosoamine. Although the two pathways are kinetically equivalent (if the equilibrium constants for $Cu^{II}-NO$ formation and amine deprotonation are both small), it can be argued that the NO to Cu charge transfer when the complex is formed in the first of these would depress the acidity of the coordinated amide. Therefore, Scheme 10 was favored, although far from proven.[73]

Scheme 10



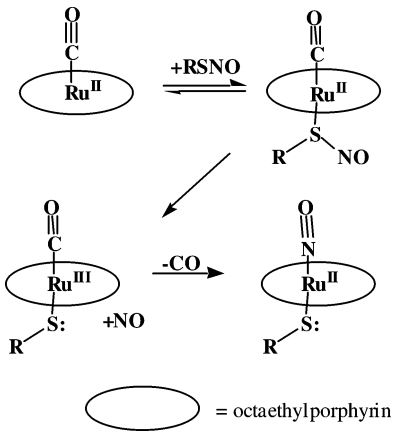
Although the nitrosation of a coordinated ligand according to Scheme 4 appears to be an unusual pathway for reductive nitrosylation, this pathway should receive wider scrutiny given its close analogy to the inner-sphere electron-transfer pathway first demonstrated by Henry Taube half a century ago.[74] One fairly clear precedent is the report by Armor and co-workers[18] that the alkaline solution reaction of hexaammineruthenium(III) $Ru(NH_3)_6^{3+}$ with nitric oxide leads to the formation of the Ru(II) dinitrogen complex $Ru(NH_3)_5(N_2)^{2+}$. The reaction is base catalyzed, and a pathway involving NO attack on a coordinated amide with concomitant electron transfer to the ruthenium (Scheme 11) appears to be the logical explanation.

Scheme 11



Furthermore, if one considers the microscopic reverse of ligand nitrosation, for example, copper(I)-catalyzed decompositions of S-nitrosothiols,[75,76] analogous intermediates/transition states are likely to be playing a role in these mechanisms. A related process is the reaction of Ru(II) complex Ru^{II}(OEP)(CO) (OEP = octaethylporphyrinate) with *S*-nitrosothiols to form the respective Ru^{II}(NO)(thiolate) complex. Stopped-flow spectrophotometric studies have shown that this reaction occurs via an *S*-coordinated Ru^{II}(OEP)-(RSNO)(CO) intermediate (Scheme 12).[77] This readily undergoes cleavage of the S−NO bond to release NO and form the Ru(III) species Ru^{III}(OEP)(RS)(CO) followed by NO replacement of the coordinated CO to give the final product. The Ru^{II}(OEP)(RSNO)(CO)

Scheme 12



cleavage is the microscopic reverse of the NO reaction with the coordinated thiolate ligand of $Ru^{III}(OEP)(RS)(CO)$ in analogy to the general mechanism suggested by Scheme 4.

6. Possible Biological Effects

As noted above, NO reductions ("autoreduction") of ferriheme and copper(II) proteins and models were observed long ago, and the more recent mechanistic studies provide an intellectual basis for interpreting these reactions. In this section, we will discuss several systems where the reductive nitrosylation pathways involving endogenous or exogenous NO may have relevance to mammalian systems.

6.1. Ferriheme Proteins

The β-cys-93 *S*-nitrosated residue of hemoglobin, generally referred to as "SNO-Hb", has drawn considerable attention since the controversial proposal by Stamler and co-workers that SNO−Hb had a significant role in the transport of NO in the cardiovascular system.[13a−c,78] Although this species has been isolated and identified, much of the controversy is concerned with whether SNO−Hb plays a natural role with regard to the transport of NO generated endogenously or is an artifact resulting from the introduction of exogenous NO.[13d,13e,79,80]

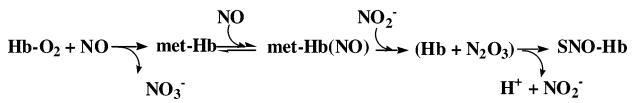
With regard to reductive nitrosylation, transfer of NO^+ from the heme iron to a β-cys-93 in hemoglobin has been proposed as a possible mechanism for the formation of SNO-Hb[13b] in analogy to the NO reaction with metMb and glutathione to give GSNO plus Mb(NO).[40] Singel et al.[13d] have suggested that Hoshino's model for the reductive nitrosylation of ferriheme proteins (Scheme 5)[39] was not sufficient to explain observations with the tetrameric hemoglobin model with inequivalent subunits. Use of the Saville assay and mass balance led them to conclude that the products include not only Hb(NO) and NO_2^-, but also some nitrosothiol derivatives, and that Fe(II)−NO and the sum of nitrite plus *S*-nitrosothiol each account for half of the reacted NO. With this finding, one can envision first the formation of $Fe^{III}(NO)$ by reaction of metHb with NO followed by competition between NO^+ transfer to OH^- (or H_2O) to give NO_2^- or to the −SH of β-cys-93 to give SNO-Hb. One concern, however, is that crystal structural data indicate the distance between the iron center and the β-cysteine-93 is quite large;[81] thus, direct reaction of the cysteine-SH with the coordinated NO does not appear to be likely.

Others have also demonstrated that *S*-nitrosothiol, presumably SNO-Hb, is a result of bolus addition of NO solution to normoxic solutions of Hb or red blood cells.[79] The major product under these conditions is metHb because of the rapid reaction of NO with oxy-hemoglobin[80] (eq 26), and it was argued that such formation of SNO-Hb may result from mixing artifacts, especially when dioxygen is present.[79,82] A related issue is the recent attention directed toward the possible role of nitrite ion (NO_2^-) in the cardiovascular system, because nitrite is reported to be a major vascular storage pool of NO_x species.[83]

$$Hb(O_2) + NO \rightarrow metHb + NO_3^- \qquad (26)$$

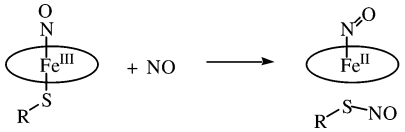
Clearly, the chemical interplay of ferriheme models and proteins with NO and nitrite continues to be an issue of major importance to cardiovascular biology. Because nitrite is the product of NO autoxidation in aqueous media,[43] it is a ubiquitous component of experiments when NO is added to an aerobic system. As described above, NO_2^- catalyzes reductive nitrosylation of ferriheme models and of metHb and metMb. Thus, the SNO-Hb observed in reactions of NO with red blood cells or with metHb might be the result of nitrite impurities. Both mechanisms described above for nitrite catalysis call for the intermediacy of N_2O_3, a known nitrosating agent. Although N_2O_3 undergoes rapid hydrolysis, this reaction is likely to have a longer lifetime in the hydrophobic pocket of a heme protein. Thus, N_2O_3 formation at the heme site could easily lead to protein modification such as the nitrosation of β-cys-93 as illustrated in the speculative Scheme 13.[35]

Scheme 13



The nitrophorins are ferriheme proteins that were first found in the saliva of the blood sucking insect *Rhodnius prolixus*.[84,85] They are nitric oxide carriers present in the saliva as $Fe^{III}(P)(NO)$ complexes that release the NO at various rates upon entering the bloodstream of a mammal and have anticoagulation and vasodilation properties to the advantage of the insect. Given that ferroheme centers are much less labile to NO release than are the ferriheme analogues, it is notable that the nitrophorins do not readily undergo autoreduction. This can be attributed in part due to the low pH of the insect saliva, which suppresses the reductive nitrosylation mechanism, and to negative charges in the heme pocket that apparently suppress the attack by hydroxide.

Recently, a nitrophorin from the bedbug *Cimex lectularius* was identified and characterized structurally to have a cysteine thiolate ligand in the axial site trans to the nitrosyl in the $Fe^{III}(P)(NO)$ complex. An interesting transformation was noted when crystals of this nitrophorin were infused with NO.[86] This involved reduction of the Fe(III) center to $Fe^{II}(P)(NO)$ and nitrosation of the trans thiolate to a *S*-nitroso thiol that is no longer coordinated (eq 27). This represents a biochemical example of the nitrosation of a coordinated ligand with concomitant reduction of the metal center as illustrated in Scheme 4 and described above for the copper(II) complex $Cu(DAC)^{2+}$.



(27)

Cys92 is a homodimeric hemoglobin from the mollusk *Scapharca inaequivalvis* that contains a single reactive cysteine residue in the vicinity of each heme group. Chiancone et al.[87] have found that NO reduction of the ferriheme form gives the ferroheme product and cysteine nitrosation. However, in a different twist, this reaction is not accompanied by detectable formation of the ferric iron nitrosyl intermediate, unlike the analogous reductive nitrosylations of other ferriheme models and proteins described above.

The monomeric globular heme protein neuroglobin has recently been discovered in the brain and in the retina of vertebrates and belongs to a class of hexacoordinate globins with a coordinated distal histidine in both its iron(II) (NGB) and iron(III) (metNGB) forms.[88] The physiological function(s) is unknown, but it may be an endogenous neuroprotective factor in cerebral ischemia (stroke) with the resulting hypoxic insult.[89] Under hypoxic conditions, NO production in the brain increases and reaction of NO with superoxide to form peroxynitrite would be expected. To evaluate the potential role of human neuroglobin as a scavenger of such species, Herold and co-workers[90] have reported kinetics investigations of metNGB with nitric oxide, peroxynitrite, and hydrogen peroxide. They demonstrated that in the presence of excess NO, metNGB is converted to $NGBFe^{II}(NO)$ by reductive nitrosylation in pH 7.2 solution, in analogy to reactions of NO with metmyoglobin and methemoglobin. This study showed the slow formation of NGB(NO) with the biexponential behavior displaying fast and slow components with the second-order rate constants of 21 and 0.4 M^{-1} s^{-1} (293 K), respectively, perhaps due to the protein having several dissimilar conformations.[90] No metNGB(NO) intermediate was observed, so the rate-limiting step is apparently the first substitution reaction to form this species. This is 3 orders of magnitude slower than the analogous formation of metMb(NO) or metHb(NO), but is much closer to the formation rate for $Cyt^{III}(NO)$ as described by Hoshino et al.[30] The common differences are that metMb and metHb have labile water molecules in the coordination site that NO eventually occupies, while metNGB and Cyt^{III} are bound in both ferriheme axial sites by the protein.

6.2. Copper Proteins

The interaction of NO with copper proteins is a topic of active research interest in mammalian biomedical science. For examples, copper deficiencies have been linked to elevated blood peroxynitrite concentrations and impaired endothelium-dependent responses,[91] while increased levels have been linked to artherosclerosis[92] and increased mortality from degenerative cardiovascular states.[93] Examples of copper proteins that interact with NO include the oxidases ascobate oxidase,[94] tyrosinase, and hemocyanin,[95] the cupredoxins azurin, and halocyanin.[96] Ceruplasmin, a major protein with seven copper atoms found in vertebrate blood plasma, has been shown to inhibit NO-induced vasodilation reversibly.[97] In these contexts, reductive nitrosylation of copper proteins may also serve the function of trapping and deactivating NO.[98] It has been demonstrated that NO interactions with copper(II) centers may play a role in regulating the functions of cytochrome *c* oxidase (CcOX) and various laccases, enzymes that are important mediators in biological electron transport.[99]

As noted above, the copper-based (and other) nitrite reductases (NIR) carry out the microscopic reverse of reductive nitrosylation.[15] A model system has demonstrated the reaction in both directions.[6] The reduction of nitrite by copper(I)-containing proteins may also be a source of NO. An interesting recent report described the η^2- or side-on coordination of NO to copper in the crystal structure of a NIR.[100]

Cytochrome *c* oxidase is an enzyme responsible for coupling the reduction of O_2 to the production of ATP. It acts as a mediator by transporting electrons from cytochrome *c* and contains four redox active metal centers, two heme iron and two copper-centered sites. Reduction of the Cu(II) centers by NO in cytochrome *c* oxidase concomitant with nitrite production has been proposed to be a possible mechanism for the downregulation of overall mitochondrial activity.[101] Because NO binding is competitive with O_2, the reductive nitrosylation of cytochrome *c* oxidase may also serve as a signal for low $[O_2]$ conditions. It has been shown by EPR studies that NO binds directly to the Cu(II) site of cytochrome oxidase (known as Cu_B) to form what has been characterized as a photolabile and diamagnetic $Cu(I)-NO^+$ adduct. Reaction with water or hydroxide gives the cuprous species plus nitrite.[64c] The stoichiometry for this reaction was confirmed by the use of a fluorescence dye, and it was observed that nitrite was formed inside the active site prior to dissociation from the enzyme. Similar chemistry has also been observed with the structurally similar cytochrome bo_3 of *Escherichia coli*.[98a]

Glypican-1 is a member of a class of protoglycans, which in conjunction with a co-receptor on the cell surface have been cited with the regulation of cell growth and development. The core protein of glypican-1 specifically binds metals at seven different sites, five contain Cu(II) and the other two contain Zn(II). The cysteine group inside the protein becomes *S*-nitrosylated in the presence of Cu(II) and NO, generating the reduced copper as the other product in a reversible process.[102] A series of glypican-1 proteins have shown similar reactivity, demonstrating the precedence for reductive nitrosylation with regard to regulatory functions.[103]

7. Summary

The best characterized reactions of nitric oxide in mammalian biochemistry are the formation of heme nitrosyl complexes (as in the activation of soluble guanylyl cyclase) and the oxidation by O_2, HbO_2, and superoxide to form nitrite, nitrate, or preoxynitrite, respectively, in aqueous media.[3] However, it is clear that the interplay between the various nitrogen oxide derivatives and other biological molecules has considerably greater complexity and physiological relevance than even these reactions and their conse-

quences. For example, it has been demonstrated that derivatives such as nitrosylated amines and thiols, heme nitrosyls, and nitrite ion are all present in mammalian fluids, membranes, and organs in various, but site-specific concencentrations.[83] Besides the likelihood that such species may have roles in signaling mechanisms, they also serve as a reservoir for bioavailable NO. For example, nitrite concentrations in the blood far exceed the steady-state concentrations of NO itself,[83b] and understanding the interplay between various NO_x species, especially those in the +2 (NO), +3 (NO_2^-, RSNO, R_2NNO, etc.), and +4 (NO_2) oxidation states, is of considerable importance to biomedical science. In the present Article, we have presented an overview of recent mechanistic investigations of reductive nitrosylation reactions involving several iron(III) and copper(II)-centered models and proteins. As defined here, reductive nitrosylation involves the reduction of the metal center coupled with the transfer of NO^+ to a nucleophile. These studies indicate that such pathways may be relevant to the in vivo synthesis of RSNO and R_2NNO species and may have other bioregulatory functions. For example, it has also been shown that NO reduction of a cupric center of CcOX may serve to downregulate overall mitochondrial activity.[101]

From a mechanistic perspective, studies of model compounds and more limited studies of metalloproteins suggest three general pathways for reductive nitrosylation. The most common appears to function via initial coordination of NO to the oxidizing metal center (Scheme 2). Studies of the water-soluble ferriheme model, Fe^{III}(TPPS),[35] demonstrate reaction with NO to form a characterizable metal nitrosyl complex Fe^{III}(TPPS)(NO). Coordination to Fe(III) activates the nitrosyl to nucleophilic attack to give an adduct that dissociates rapidly to give the reduced metal center Fe^{II}(TPPS) and the nitrosylated nucleophile product (NO_2^- if the nucleophile is H_2O or OH^-). Analogous mechanisms have been demonstrated for other ferriheme models and proteins and appear likely for the $Cu(dmp)_2^{2+}$-type complexes.[6] Among mechanistic variations noted with the ferriheme systems was catalysis by general base and by other nucleophiles, including nitrite, one of the reaction products. Catalysis by nitrite adds the interesting possibility that N_2O_3 might be a reaction intermediate, and it was suggested that N_2O_3 generation in a protein hydrophobic pocket might lead to nitrosation of amino acids within that protein.

Another hypothetical mechanism discussed for reductive nitrosylation was the outer-sphere reduction of the oxidizing metal center by NO to give free NO^+, which would be rapidly captured by solvent water or other nucleophiles in the medium (Scheme 3). There is no direct evidence for such a process, and Marcus analysis suggests this to be unlikely for any of the systems described here. However, such pathway might take precedent with metal centers that are much stronger oxidants.

A third potential mechanism would involve attack of NO at a nucleophile coordinated to the oxidizing metal centers (Scheme 4). As noted above, this is the equivalent of the inner-sphere electron/atom-transfer mechanism first described half a century ago for the redox reaction between the cobalt(III) complex $Co(NH_3)_5Cl^{2+}$ and aqueous Cr^{2+}.[74] Notably, this appears to be the pathway by which $Cu(DAC)^{2+}$ undergoes nitrosation of a coordinated polyamine DAC ligand. Other likely examples of this mechanism may be drawn from the reaction of $Ru(NH_3)_6^{3+}$ with NO in alkaline solution[18] and the recently reported nitrosation of the axial cysteinate ligand in an Fe(III) bedbug nitrophorin.[86]

There is clearly a need for further studies on reductive nitrosylation mechanisms, especially with regard to the reactions of other metalloproteins to evaluate the potential in vivo roles. There are other possible pathways for the generation of the RSNO and R_2NNO compounds found in tissue, for example, by reactions of thiols or amines with the N_2O_3 formed as an intermediate in NO autoxidation. However, autoxidation is a third-order process (second order in [NO]), and thus is dramatically dependent on the NO and O_2 concentrations. For this reason, it would seem that if the generation of such species from endogenously generated NO has relevance to signaling processes, then a nitrosation process mediated by metalloproteins would be likely. Determining the kinetics and thermodynamics of reductive nitrosylation pathways involving metal complexes or proteins that are logical models for such processes will provide further insight into what mechanisms involving endogenously generated NO are relevant to mammalian biochemistry.

8. Abbreviations

BSA	bovine serum albumin
CcOX	cytochrome *c* oxidase
cyclam	1,4,8,11-tetraazacyclotetradecane
Cyt^{II}	ferrocytochrome *c*
Cyt^{III}	ferricytochrome *c*
DAC	1,8-bis(9-anthracylmethyl)cyclam
dmp	2,9-dimethyl-1,10-phenanthroline
dpp	2,9-diphenyl-1,10-phenanthroline
FixL	Heme-based O_2 sensor in signal transduction system
GSH	glutathione
GSNO	*S*-nitrosoglutathione
Hb	hemoglobin (Fe^{II})
Hb_h	oligomeric *Glycera dibranchiate* hemoglobin
Hb_m	monomeric *Glycera dibranchiate* hemoglobin
Mb	myoglobin (Fe^{II})
metHb	met-hemoglobin (Fe^{III})
metMb	met-myoglobin (Fe^{III})
MGD	*N*-methyl-D-glutamine dithiocarbamate
MLCT	metal-to-ligand charge transfer
NGB	neuroglobin
NHE	normal hydrogen electrode
NIR	nitrite reductase
NP	nitroprusside, $[Fe(CN)_5(NO)]^{2-}$
nuc	nucleophile
OEP	octaethylporphyrin
phen	1,10-phenanthroline
P	porphyrinato ligand of heme protein
P_{NO}	partial pressure of NO
py	pyridine
RSH	protonated thiolate compound
RSNO	*S*-nitrosylated compound
SNO-Hb	*S*-nitrosohemoglobin

TMPy	*meso*-tetrakis(*N*-methyl-4-pyridyl)-porphyrinato)
TPP	tetraphenylporphyrin
TPPS	tetra(4-sulfonato-phenyl)porphinato

9. Acknowledgments

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(5) There is ambiguity in the ways that the terms "nitrosylation" and "nitrosation" are sometimes used, and we have probably not been totally consistent in this regard as well. We consider a molecule or ion to be "nitrosylated" when the equivalent of an NO has been added. We consider the molecule to be "nitrosated" when an equivalent of NO^+ has been added.
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