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# Nitrite Catalyzes Reductive Nitrosylation of the Water-Soluble Ferri-Heme Model $Fe^{III}$(TPPS) to $Fe^{II}$(TPPS)(NO)

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Quantitative investigation of the reaction of the ferri-heme model compound $Fe^{III}(TPPS)(H_2O)_2$ (**1**) to give $Fe^{II}$(TPPS)(NO) (**2**) (TPPS = tetra(4-sulfonato-phenyl)porphinato) in buffered aqueous solution demonstrates a slow pH-independent reductive nitrosylation pathway in the pH range 4–6. The rate of this reaction is subject to modest general base catalysis. In the course of this study, a surprising catalytic pathway whereby nitrite ion ($NO_2^-$) strongly catalyzes the reduction of **1** to **2** under reductive nitrosylation conditions was demonstrated.

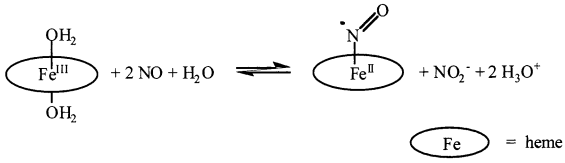
Nitric oxide (nitrogen monoxide) serves important roles in mammalian biology involving interactions with metals.[1] Notably, the reaction of NO with an oxidizing metal center can lead to reductive nitrosylation, that is, reduction of the metal concomitant with nitrosylation of a nucleophile $X^-$ (eq 1). This behavior has been demonstrated for various biologically relevant metals including the iron(III) of certain ferri-heme proteins[2,3] as well as the copper(II) centers in model complexes[4] and the metalloproteins cytochrome *c* oxidase and laccase.[5] Recent reports have also suggested that reductive nitrosylation is playing roles in the thiolnitrosylation of hemoglobin and in the heterogeneous reaction of red blood cells with NO released by the endothelium for blood pressure control.[6]

In this context, Hoshino et al.[3] studied the reductive nitrosylation kinetics of several ferri-heme proteins, including met-hemoglobin (met-Hb) and met-myoglobin, when exposed to NO at solution pHs >7 (eq 2). The reaction rates

$$L_xM^{n+} + NO + X^- \text{ (or XH)} \rightarrow L_xM^{(n-1)+} + X{-}NO\ (+H^+) \quad (1)$$

displayed first-order dependence on the (very low) hydroxide concentration; thus, nucleophilic attack of $OH^-$ at the ferri-heme nitrosyl (formally, $Fe^{II}(NO^+)$) was proposed as a key step in the reductive nitrosylation. Coordination of NO leads to charge transfer to the $Fe^{III}$ center;[7] thus, the ligand is more electrophilic than free NO.

Notably, met-Hb also undergoes reductive nitrosylation via a *pH-independent* pathway.[3] The present quantitative investigation of the reaction of the ferri-heme model compound $Fe^{III}(TPPS)(H_2O)_2$ (**1**) to give $Fe^{II}$(TPPS)(NO) (**2**) (TPPS = tetra(4-sulfonato-phenyl)porphinato) (eq 2) was initiated with the goal of a more complete elucidation of possible pH independent mechanisms.[8] In the course of this study, we have demonstrated a surprising catalytic pathway whereby nitrite ion ($NO_2^-$) strongly catalyzes this reaction.




(2)

The kinetics for the reductive nitrosylation of $Fe^{III}(TPPS)(H_2O)_2$ were evaluated over the pH range 3−6 using various buffers at 298 K, under which conditions $\mu$-oxo dimerization of $Fe^{III}$(TPPS) is minimal.[9] Equilibrating a buffered aqueous solution of **1** with NO (760 Torr, [NO] = 1.9 mM) led to

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(1) (a) *Nitric Oxide: Biology and Pathobiology*; Ignarro, L. J., Ed.; Academic Press: San Diego, 2000. (a) *Nitric Oxide and Infection*; Fang, F. C., Ed.; Kluwer Academic/Plenum Publishers: New York, 1999.
(2) (a) Wayland, B. B.; Olson, L. W. *J. Am. Chem. Soc.* **1974**, *96*, 6037. (b) Ford, P. C.; Lorkovic, I. *Chem. Rev.* **2002**, *102*, 993−1017.
(3) Hoshino, M.; Maeda, M.; Konishi, R.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc.* **1996**, *118*, 5702−5707.
(4) Tran, D.; Skelton, B. W.; White, A. H.; Laverman, L. E.; Ford, P. C. *Inorg. Chem.* **1998**, *37*, 2505−2511.
(5) (a) Torres, J.; Svistunenko, D.; Karlsson, B.; Cooper, C. E.; Wilson, M. T. *J. Am. Chem. Soc.* **2002**, *124*, 963−967. (b) Torres, J.; Cooper, C. E.; Wilson, M. T. *J. Biol. Chem.* **1998**, *273*, 8756−8766.
(6) (a) Han, T. H.; Hyduke, D. R.; Vaughn, M. W.; Fukuto, J. M.; Liao, J. C. *Proc. Nat. Acad. Sci. U.S.A.* **2002**, *99*, 7763−7768. (b) Gow, A. J.; Luchsinger, B. P.; Powloski, J. R.; Singel, D. J.; Stamler, J. S. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96*, 9027−9032.
(7) Scheidt, W. R.; Lee, Y. J.; Hatano, K. *J. Am. Chem. Soc.* **1984**, *106*, 3191−3198.
(8) (a) According to measured reduction potentials,[8b−d] the reaction of **1** with NO to give **2** is favorable at pH > ∼3. (b) Barley, M. H.; Takeuchi, K. J.; Meyer, T. J. *J. Am. Chem. Soc.* **1986**, *108*, 5876−5885. (c) *Standard Potentials in Aqueous Solutions*; Bard, A. J.; Parsons, R.; Jordan, J. Eds.; Marcel Dekker: New York, 1985; pp 127−139. (d) Pearson, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 6109−6114.
(9) (a) El-Awady, A. A.; Wilkins, P. C.; Wilkins, R. G. *Inorg. Chem.* **1985**, *24*, 2053−2057. (b) At higher pH, dimerization of **1** to the $\mu$-oxo dimer interferes with kinetics studies of reductive nitrosylation.



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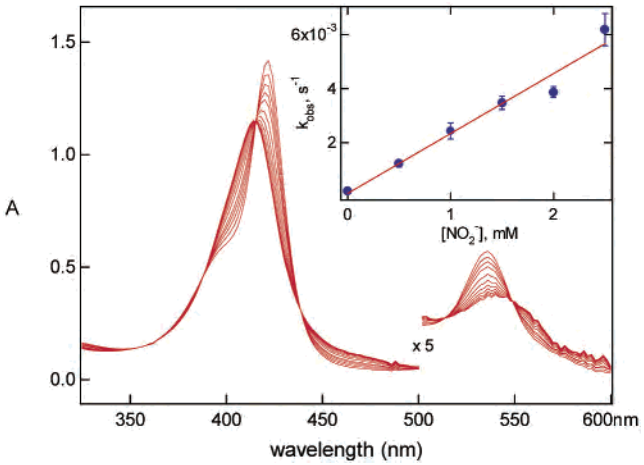



**Figure 1.** (a) Optical absorbance changes for reduction of an equilibrium mixture of **1** and **3** to $Fe^{II}$(TPPS)(NO) in pH 5.00 HOAc/NaOAc buffered (20 mM) aqueous solution at 298 K ($\mu_{tot}$ = 0.10 M, [NO] = 1.9 mM). The first four spectra were taken at 600 s intervals; subsequent scans were recorded every 1200 s. (b) The inset is a plot of $k_{obs}$ (for the reduction of **3** to **2**) versus the concentration of added $NO_2^-$ in HOAc/NaOAc buffer (16 mM) at pH 4.96 with $\mu$ = 0.1 M and [NO] = 1.9 mM. The catalysis rate constant $k_{nitrite} = 3.1 \pm 0.1\ M^{-1}\ s^{-1}$ (1.4 × slope) according to the relationship between $k_{red}$ and $k_{obs}$ (see text).

the rapid formation of an equilibrium mixture of **1** and the ferric nitrosyl complex $Fe^{III}$(TPPS)(NO) (**3**) ($K_1 = (1.32 \pm 0.09) \times 10^3\ M^{-1}$)[10] followed by slower reduction to the ferrous analogue **2** (eq 3). The spectral evolution in such an experiment (Figure 1) shows the shift of the Soret band from that of **3** ($\lambda_{max}$ 422 nm) to that of **2** (412 nm) as well as characteristic temporal changes in the Q-band region (500−650 nm). Modest ionic strength ($\mu$) dependence was found for the reductive nitrosylation kinetics (the rate is 45% faster at $\mu$ = 0.10 M than at 0.26 M), so $\mu$ was maintained at 0.1 M.

$$\mathbf{1} + \mathrm{NO} \overset{K_1}{\rightleftharpoons} \underset{(\mathbf{3})}{\mathrm{Fe^{III}(TPPS)(NO)}} \xrightarrow{k_{red}} \mathbf{2} \qquad (3)$$

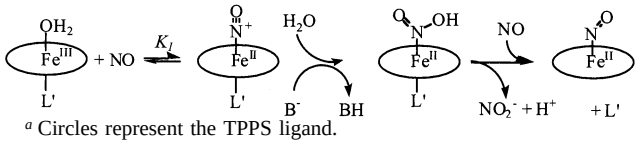
According to this model, the kinetics should follow the behavior indicated by eq 4

$$\frac{\mathrm{d}(\mathrm{NO_2^-})}{\mathrm{d}t} = \frac{k_{red}K_1[\mathrm{NO}][\mathrm{Fe^{III}}]}{1 + K_1[\mathrm{NO}]} = k_{obs}[\mathrm{Fe^{III}}] \qquad (4)$$

where $k_{red}$ may represent the sum of several terms (see a following discussion) and [$Fe^{III}$] is the sum of [**1**] and [**3**]. This predicts that (with other parameters held constant), $k_{obs}$ will increase linearly with [NO] at low concentrations but will approach the limiting value $k_{red}$ at high [NO]. This behavior was indeed evident when [NO] was varied in a manner consistent with the value of $K_1$ described. The experiments reported here were largely carried out at [NO] = 1.9 mM; thus, under these conditions, $k_{red} = \{(1 + K_1[\mathrm{NO}])/K_1[\mathrm{NO}]\} \times k_{obs} = 1.40\ k_{obs}$ (see eq 4).

(10) (a) $K_1$ was determined by spectroscopic titration in pH 5, buffered solution (298 K $\mu$ = 0.10 M) and agrees with the reported values 1.2 ± 0.2 × $10^3\ M^{-1}$ and 1.1 × $10^3\ M^{-1}$ in acidic solution.[10b,c] (b) Laverman, L. E.; Ford, P. C. *J. Am. Chem. Soc.* **2001**, *123*, 11614−11622. (c) Hoshino, M.; Ozawa, K.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc.* **1993**, *115*, 9568−9575.

**Scheme 1**[a]




[a] Circles represent the TPPS ligand.

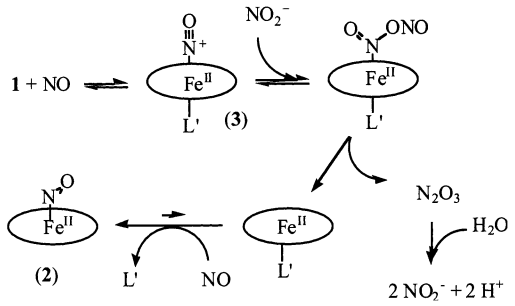
In the pH range 4−6, $k_{red}$ proved to be dependent on the identity of the buffer used as well as linearly dependent on the buffer concentration. Similar behavior was noted previously with regard to NO reduction of the copper(II) complex $Cu(dmp)_2^{2+}$ (dmp = 2,9-dimethyl-1,10-phenanthroline) and was attributed to general base catalysis.[4] At constant and near limiting [NO], $k_{red} = k_0 + k_{buffer}$[buffer], where $k_{buffer}$ is derived from the slopes of plots of $k_{obs}$ versus [buffer] and $k_0$ from the intercept at [buffer] = 0. For example, the value of $k_{buffer}$ for the acetic acid/sodium acetate (HOAc/NaOAc) buffer at pH 5.0 was $2.4 \times 10^{-3}\ M^{-1}\ s^{-1}$. The $k_0$ term would be the sum of contributions due to reaction with water ($k_{H_2O}$) and with hydroxide ($k_{OH}[OH^-]$). However, for these low pH solutions, direct reaction with $OH^-$ appears to be insignificant, because $k_{obs}$ values measured in 20 mM HOAc/NaOAc buffers were nearly identical at pH 4.0 ($(2.36 \pm 0.30) \times 10^{-4}\ s^{-1}$) and pH 5.0 ($(2.14 \pm 0.37) \times 10^{-4}\ s^{-1}$). This behavior is consistent with the mechanism indicated by Scheme 1 (L′ = $H_2O$), where the role of general base catalysis is suggested to activate $H_2O$ toward reaction with the coordinated NO.

Nitrite ion is not only a reaction product but is also a common impurity in aqueous solutions of nitric oxide.[11,12] In this context, we decided to probe the potential effects of trace amounts of $NO_2^-$ by examining the kinetics behavior in the presence of deliberately added $NaNO_2$. Catalysis of eq 2 by $NO_2^-$ was readily apparent as illustrated by the linear dependence of $k_{obs}$ on the concentration of added $NaNO_2$ (insert in Figure 1). The slope gives the catalytic rate constant ($k_{nitrite} = 3.1 \pm 0.1\ M^{-1}\ s^{-1}$) for the reductive nitrosylation of **1** in 16 mM aqueous HOAc/NaOAc buffer at pH 4.96 (298 K). A similar value ($3.4 \pm 0.1\ M^{-1}\ s^{-1}$) was found in DESPEN buffer at pH 5.0. In the absence of NO, there was no reduction of **1** by added $NO_2^-$ alone. *Hence, nitrite is indeed a catalyst for the reduction of $Fe^{III}$(TPPS) by NO*; given that $NO_2^-$ is a reaction product, the system is, in principle, autocatalytic.

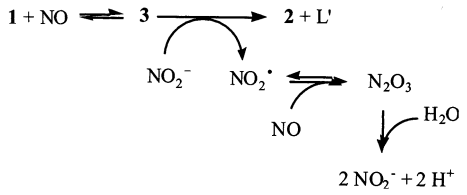
(11) (a) In the present study, multiple techniques were implemented to ensure the integrity of the NO source. Solution degassing techniques include boiling under reduced pressures and freeze−pump−thaw cycle(s). Removal of higher $NO_x$ was accomplished by passing NO(g) through Ascarite II scrubbers, or freezing the NO at 77 K and then distilling into the reaction flask. Even so, [$NO_2^-$] levels below 50 $\mu$M proved difficult to achieve,[12] the likely source being NO autoxidation[11b] by trace air combined with the tendency of $NO_x$ impurities to concentrate in the aqueous phase. Our early studies suffered from reproducibility problems despite considerable effort to ensure consistency of reaction conditions, etc., leading us to suspect a possible role for the nitrite ion. (b) Ford, P. C.; Wink, D. A.; Stanbury, D. M.; *FEBS Lett.* **1993**, *326*, 1−3.

(12) (a) Spectrophotometric detection of $NO_2^-$ (at $\lambda_{max}$ = 354, $\epsilon$ = 22.6 $M^{-1}\ cm^{-1}$) suggests that $NO_2^-$ levels in our aqueous NO solutions were in the range 40−180 $\mu$M (generally <100 $\mu$M). Others have reported difficulty attaining even these levels. (b) Wolak, M.; Stochel, G.; Hamza, M.; van Eldik, R. *Inorg. Chem.* **2000**, *39*, 2018−2019. (c) Nemes, A.; Pestovsky, O.; Bakac, A. *J. Am. Chem. Soc.* **2002**, *124*, 421−427.

**Scheme 2**




**Scheme 3**




Scheme 2 illustrates a prospective inner sphere mechanism for the catalytic role of nitrite with $NO_2^-$ acting as the primary nucleophile toward the coordinated nitrosyl. A key intermediate would be the $Fe^{II}$ complex of the reactive nitrogen species $N_2O_3$. Dissociation of $N_2O_3$ followed by hydrolysis to nitrous acid would give the reaction products and regenerate the catalytic nitrite.

A very different potential mechanism is represented by Scheme 3, in which it is proposed that nitrite catalysis proceeds via the outer sphere electron transfer from $NO_2^-$ to **1** to give **2** plus $NO_2$. This would be followed by rapid scavenging of $NO_2$ by NO to give $N_2O_3$, which undergoes hydrolysis.[13]

At this stage, it is difficult to differentiate these two mechanisms. There is considerable precedent for Scheme 2, for example, in the mechanisms explaining the role of hydroxide in certain ferri-heme protein reductive nitrosylations. On the other hand, it is not clear why $NO_2^-$ would be a much more effective nucleophile toward coordinated NO than, for example, acetate ion. With regard to catalysis via Scheme 3, a negative argument would be that the electron-transfer step is unfavorable ($\Delta E = -0.31$ V);[8] however, Marcus treatment of potential rates does not unequivocally exclude this possibility.[14]

In summary, $Fe^{III}$(TPPS) undergoes spontaneous reductive nitrosylation in pH 4−6 solution with rates subject to general base catalysis but exhibiting no pH dependence. Furthermore, $NO_2^-$ catalyzes the reductive nitrosylation. Nitrite is a common impurity in aqueous NO solutions, and biomedical experiments in which aqueous NO is added to an aerobic system to study biological effects must include undetermined concentrations of $NO_2^-$. The present study emphasizes that the net effect cannot be assumed to be innocuous. For example, in the reaction of NO with red blood cells,[6] nitrite may affect both the kinetics and products resulting from the reductive nitrosylation of met-Hb. Of particular concern would be the possible role of $N_2O_3$, a key intermediate in both proposed mechanisms (Schemes 2 and 3). Subsequent reactions of the strong oxidant and nitrosating agent $N_2O_3$ might include nitrosylation of a Hb thiol in competition with the hydrolysis to nitrite. The present results underscore the general importance of $N_2O_3$ and other reactive $NO_x$ intermediates and their potential roles in biological transformations attributed to NO itself.

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(13) (a) Graetzel, M.; Henglein, A.; Lilie, J.; Beck, G. *Ber. Bunsen-Ges. Phys. Chem.* **1969**, *73*, 646−653. (b) Graetzel, M.; Taniguchi S.; Henglein, A. *Ber. Bunsen-Ges. Phys. Chem.* **1970**, *74*, 488−493. (c) Treinin, A.; Hayon, E. *J. Am. Chem. Soc.* **1970**, *92*, 5821−5828.

(14) (a) The limit for Scheme 3 would be the rate of outer sphere electron transfer from $NO_2^-$ to **3**, which can be estimated from the Marcus cross relation[14b] $k_{os} \sim (k_{11}k_{22}K_{os})^{1/2}$, where $k_{11}$ is the rate constant for $Fe^{II}$(TPPS)(NO)/$Fe^{III}$(TPPS)(NO) self-exchange, $k_{22}$ is that for $NO_2^-/NO_2$ self-exchange via outer sphere electron transfer (0.3 $M^{-1}$ $s^{-1}$),[14c] and $K_{os}$ is the equilibrium constant for the first step ($5.7 \times 10^{-6}$). Accordingly, if $k_{os} = k_{nitrite}$, then the calculated value for $k_{11}$ would be $\sim 6 \times 10^6$ $M^{-1}$ $s^{-1}$. Although within the range of self-exchange rates for low spin Fe porphyrin complexes,[14d] the $Fe^{II/III}$(TPPS)(NO) self-exchange may show a large reorganization energy making it slower. (b) Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891−899. (c) Stanbury, D. M. In *Electron Transfer Reactions*; Isied, S. S., Ed.; Advances in Chemistry 253; American Chemical Society: Washington, DC, 1997; pp 165−182. (d) Shirazi, A.; Barbush, M.; Ghosh, S.; Dixon, D. W. *Inorg. Chem.* **1985**, *24*, 2495−2502; Pasternack, R. F.; Spiro, E. G. *J. Am. Chem. Soc.* **1978**, *100*, 968−972.