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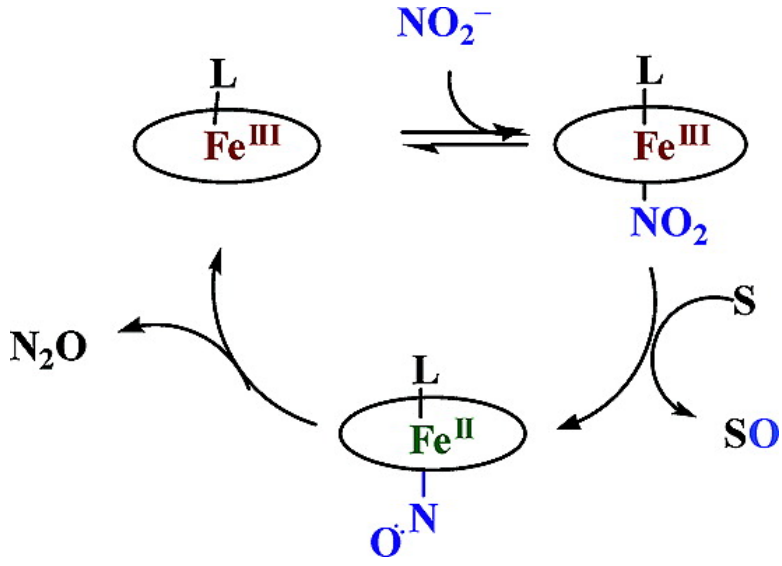

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Oxygen Atom Transfer from Nitrite Mediated by Fe(III) Porphyrins in Aqueous Solution

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Nitrite ion is present throughout the mammalian organism[1,2] and serves as an intravascular and tissue storage form of nitric oxide equivalents.[3] In the absence of dioxygen, NO_2^- is converted to NO via reaction with hemoglobin[4] and by blood-free heart and liver tissues.[5] Thus, nitrite may help regulate vasodilation during hypoxia and modulate ischemia-reperfusion tissue injury.[6] Another nitrite reaction of potential biological relevance is oxygen atom transfer (OAT) to substrates (**S**) as depicted in eq 1 and observed in organic solvents.[7,8] Here we describe OAT reactions from nitrite in near-neutral aqueous solutions catalyzed by the water-soluble heme model $Fe^{III}(TPPS)(H_2O)_2^{3-}$ (**1**) (Na^+ salt, TPPS = tetra(sulfonatophenyl)porphyrinato anion)).

$$Fe^{III}(Por)(NO_2^-) + \mathbf{S} \rightarrow Fe^{II}(Por)(NO) + \mathbf{S}O \qquad (1)$$

Substrates oxidized by the $Fe^{III}(TPPS)/NO_2^-$ mixture in buffered solutions include the Na^+ salt of tris(3-sulfonatophenyl)phosphine (tppts) and dimethyl sulfide (DMS). The ferrous nitrosyl Fe^{II}-(TPPS)(NO) (**2**) is formed rapidly in accord with eq 1, and product analyses demonstrate these substrates to give the corresponding monoxides tpptsO and DMSO. Addition of air regenerates **1** and suggests the possibility of a catalytic autoxidation cycle mediated by the nitrogen oxides and the heme iron. More surprising, however, is the slow spontaneous regeneration of **1** observed once the substrate is depleted. Careful analysis of the tppts oxidation demonstrates that this is accompanied by nitrous oxide formation according to eq 2.

$$2\mathbf{S} + 2NO_2^- + 2H^+ \xrightarrow{Fe(TPPS)} 2\mathbf{S}O + N_2O + H_2O \qquad (2)$$

Figure 1 displays the spectrum of **1** (10 μM) in deaerated, pH 5.8 aqueous phosphate buffer (10 mM) solution also containing 10 mM $NaNO_2$. The characteristic Soret and Q-band absorptions (λ_{max} 394 and 528 nm) are little different than those seen under analogous conditions in the absence of added nitrite (Figure S-1, Supporting Information). This is attributed to the small equilibrium constant for association of **1** with nitrite (eq 3, $K = \sim 3\ M^{-1}$).[9]

$$\mathbf{1} + NO_2^- \overset{K}{\rightleftharpoons} Fe^{III}(TPPS)(H_2O)(NO_2)^{4-} + H_2O \qquad (3)$$

Such solutions were stable in the dark indefinitely. However, adding tppts (1.0 mM) generated over a few minutes shifts of the Soret band to 413 nm and the Q-band to 543 nm, consistent with complete transformation of **1** to **2**.[10] These spectral changes persisted for several hours then slowly decayed back to the spectrum of **1** (Figures 1 and S-2 in Supporting Information). When more substrate was added, the ferrous nitrosyl **2** was again generated. The system was recycled three times, and spectral changes indicated that re-formation of **1** was at least 95% complete after each. This behavior suggests a catalysis cycle, where the resting state of the metalloporphyrin is **2**, but the thermodynamically favored form is

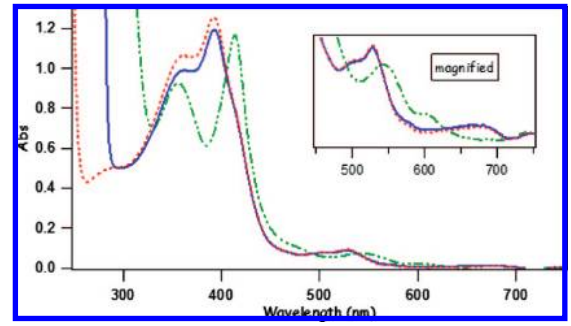

Figure 1. Spectral changes indicating regeneration of **1** under Ar (296 K). Red line: initial pH 5.8 solution of $Fe^{III}(TPPS)(H_2O)_2^{3-}$ (10 μM) and $NaNO_2$ (10 mM). Green dashed line: formation of **2** after adding tppts (1.0 mM). (Band at ~350 nm is due to the aq NO_2^-; λ_{max} 354 nm, $\epsilon = 23\ M^{-1}\ cm^{-1}$.) Solid blue line: re-formation of **1** overnight.

1, once the substrate is consumed. If, however, the system in the resting state is interrupted by adding a small amount of air, ferric complexes, principally **1**, are immediately reformed (Figure S-1 in Supporting Information). Adding more substrate after deaeration regenerated **2**.

The phosphine oxide product was characterized by in situ ^{31}P NMR spectroscopy and showed complete conversion in the presence of excess NO_2^-. An analogous solution of $NaNO_2$ and tppts not containing **1** showed no significant increase in phosphine oxide even after a week. Mass spectral product studies for **S** = DMS demonstrated complete conversion of DMS to DMSO, but no reaction with nitrite was seen in the absence of **1**. For tppts, the resting state of the catalyst was **2** when excess **S** was present, but **1** was restored once **S** was consumed.

Temporal absorbance changes representing formation of **2** in the first stage of reaction with tppts did not follow the exponential decay kinetics expected for a reaction first order in [**1**] (Figure S-3 in Supporting Information). Accordingly, the dynamics of the first stage were examined by using the initial rates method. Table S-1 (Supporting Information) lists initial rate data at varied concentrations of **1**, NO_2^-, and tppts. These data suggest the reaction to be approximately first order in all three components as would be expected if the rate-limiting step were oxidation of tppts by $Fe^{III}(TPPS)(H_2O)(NO_2^-)$.

When the headspace gas in a cell containing **1**, $NaNO_2$, and tppts in pH 5.8 solution (5 mL) was examined by FTIR spectroscopy, the spectrum displayed the characteristic P and R branches for the N_2O stretching vibration at 2211 and 2235 cm^{-1} (Supporting Information Figure S-4).[11] With the initial parameters, [**1**] = 6.1 μM, [NO_2^-] = 9.8 mM, and tppts = 2.33 mM in pH 5.8 buffer solution (5.1 mL), 6.4 ± 0.6 μmol of N_2O was generated (average of two trials) as calculated from the molar absorbances (redetermined in the same IR cell). A control reaction with tppts and nitrite (but not **1**) showed traces of N_2O, but these were at experimental detection limits.

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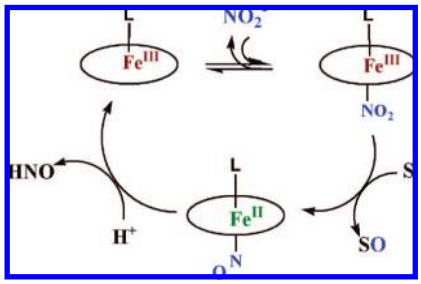
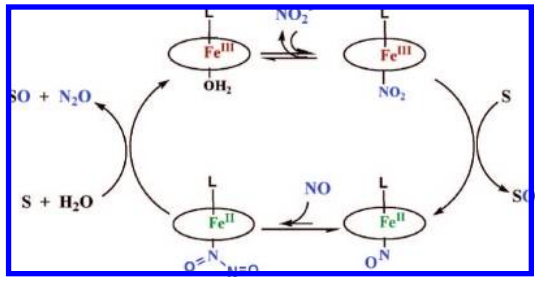
Scheme 1. Proposed Catalytic Cycle



Scheme 2



The nitrous oxide thus formed is 200 times the equivalents of **1** (3.2×10^{-8}) and half the tppts initially present (1.2×10^{-5}). In other words, N_2O formation is clearly catalytic in Fe(TPPS) and corresponds to the stoichiometry indicated by eq 2.

Scheme 1 postulates a pathway to the spontaneous regeneration of **1**. This involves HNO formation from **2** to give Fe^{III}(TPPS), a reaction that has been speculated for heme proteins.[12] The ferrous nitrosyl can be viewed in terms of the resonance forms Fe^{II}(TPPS)(NO) ↔ Fe^{III}(TPPS)(NO^-), as proposed for the pentaaquo complex $Fe(H_2O)_5(NO)^{2+}$.[13] Protonation would give Fe^{III}(TPPS)(HNO), although the pK_a would be considerably smaller than that of free HNO (~11.4).[14] Subsequent HNO dissociation and dimerization (2 HNO → $N_2O + H_2O$, $k_d = 8 \times 10^6$ M^{-1} s^{-1})[14a] or perhaps HNO reaction with another **2** would give nitrous oxide. Consistent with this is the observation that re-formation of **1** is much slower at pH 6.3.

Bimolecular coupling of two Fe^{II}(TPPS)(NO) (eq 4) is another possible route to N_2O. This finds analogy in certain NO reductases having two iron centers in the active site, one being a heme.[15] However, when the reaction was carried out under identical [NO_2^-], [tppts], and pH, but [Fe^{III}(TPPS)] differing by a factor of 2, there was no obvious difference in the rate of re-formation of **1** once the substrate was expended. This suggests a first order, not second order, decay of [**2**].

$$2Fe^{II}(TPPS)(NO) + 2H^+ \rightarrow 2Fe^{III}(TPPS) + N_2O + H_2O \quad (4)$$

Scheme 2 describes an alternative pathway to nitrous oxide formation based on a proposal regarding Fe(TPP)-catalyzed nitric oxide oxidation of PPh_3 in toluene.[16] A key step would be the [NO]-dependent formation of the putative $Fe^{II}-N_2O_2$ intermediate. This seems unlikely here since NO was not added and given the very low NO dissociation constant for **2** (3×10^{-13} M^{-1}).[10] Similarly, the equilibrium NO concentration from the acid disproportionation of nitrite would also be low under the reaction conditions. Another NO source might be NO_2^- reduction by free Fe^{II}(TPPS) (**3**), but this must be minimal given that **3** would derive from **2** for which the dissociation constant is very small.

In summary, oxygen atom transfer from nitrite to the substrates tppts and DMS is mediated by the model heme complex $Fe^{III}(TPPS)(H_2O)_2^{3-}$ in aqueous media. This reaction is accompanied by formation of the ferrous nitrosyl Fe^{II}(TPPS)(NO). Over time, the latter species spontaneously returns to **1** with formation of N_2O, the overall reaction being that described by eq 2 catalyzed by Fe(TPPS). The aqueous Fe^{III}(TPPS)/NO_2^- system also rapidly oxidizes the biological thiols cysteine and glutathione to give **2**. The other products were the respective disulfides, but it is yet unknown whether these are formed via OAT-generated intermediate sulfenic acids, which react with excess thiols to give disulfides.[17] Notably, N_2O was not a product with these substrates, and while this might signify a different mechanism for catalyst recycling, such thiols are also known to trap HNO.[18] Clearly, these studies further illustrate that the redox chemistry of nitrite with hemes is very rich and may have significant biological consequences.

Ongoing studies are focused on elucidating the mechanisms of the oxygen atom transfers and of pathway(s) responsible for N_2O release closing the catalytic cycle. We are also working to develop a quantitative model for the fast regeneration of ferric species when small quantities of air have been added to the system.

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Supporting Information Available: Table S-1: Initial rates for tppts reaction with **1** and nitrite in pH 5.8 solution. Figures S-1: Spectral change when air is added to reaction solution. Figures S-2 and S-3: Temporal spectral changes. Figure S-4: FTIR spectrum of reaction headspace. This material is available free of charge via the Internet at http://pubs.acs.org.

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