A Dissociative Mechanism for Reactions of Nitric Oxide with Water Soluble Iron(III) Porphyrins

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Nitric oxide has significant roles in mammalian biology as an intercellular signaling agent and in cytotoxic immune response.[1] The principal targets for NO under bioregulatory conditions are metal centers, primarily iron.[2] Although the ferroheme enzyme soluble guanylyl cyclase (sGC) is the best characterized example,[3] various reports point to roles in inhibition of metalloenzymes such as cytochrome oxidase,[4] nitrile hydrase[5] and catalase[6] and in vasodilator properties of a salivary ferriheme protein of blood-sucking insects.[7] Concentrations generated for bioregulation are low ([NO] < 1 μM reported in endothelium cells for blood pressure control[8]); thus, reactions with targets such as sGC must have very high rate constants (k_{on}) to compete effectively with other physical and chemical processes leading to NO depletion.

Rates of NO reactions with various metal targets can be determined by laser flash techniques,[9,10] where NO is photolabilized from a M−NO precursor, and subsequent relaxation to equilibrium is followed spectroscopically. Indeed, reactions of NO with heme centers were thus investigated before the bioregulatory functions were postulated.[10] Despite this activity, the mechanisms by which NO reacts with heme iron, i.e., the "on" reaction for eq 1, (Por = a porphyrin moiety), have not

$$\mathrm{Fe(Por)} + \mathrm{NO} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{Fe(Por)(NO)} \qquad (1)$$

been systematically probed. While it is often assumed that reaction of a ligand with heme iron requires an open coordination site, the quantitative basis for such assumption, especially for reaction with NO, is more legendary than factual. Furthermore, the possibility remains that NO, a stable free radical, may react by pathway(s) different than other Lewis bases.[11] To address these mechanistic questions, we report the activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta V^{\ddagger}$ determined from temperature (T) and hydrostatic pressure (P) effects on reactions with the water soluble complexes $Fe^{III}(TPPS)(H_2O)_2^{3-}$ (**I**) and $Fe^{III}(TMPS)(H_2O)_2^{n-}$ (**II**) (TPPS = tetra(4-sulfonatophenyl)porphine; TMPS = tetra(sulfonatomesityl)porphine).[12] These point to a ligand dissociative pathway as dominating the "on" mechanism and offer insight into the nature of such pathways in bioregulatory signaling.

Laser flash photolysis[13] of aqueous solutions of **I** or **II** under defined NO pressures gave transient spectra consistent with the spectral differences between $Fe^{III}(Por)(H_2O)(NO)^{n-}$ and $Fe^{III}(Por)(H_2O)_2^{n-}$ (e.g., Figure 1). The transients decayed exponentially (k_{obsd}) to regenerate the equilibrium mixture of solvated and nitrosyl complexes (see Figure 1 inset). No permanent photoproducts were observed; thus the decay represents the relaxation of the $Fe^{III}(Por)/NO$ system according to eq 1 and $k_{obsd} = k_{on}[NO] + k_{off}$. Accordingly, a plot of k_{obsd} versus [NO] was linear with a slope (k_{on}) of 3.0×10^6 M^{-1} s^{-1} and a nonzero intercept (k_{off}) of 7.3×10^2 s^{-1} for Por = TMPS at 25 °C giving a k_{on}/k_{off} ratio (4.1×10^3 M^{-1}) within experimental uncertainty of the equilibrium constant (3.93×10^3 M^{-1}) determined spectroscopically.

Temperature and hydrostatic pressure effects were evaluated by determining k_{obsd} at several [NO] and extracting the k_{on} and k_{off} values at individual T or P over the respective ranges of 25−45 °C and 0.1−250 MPa. Eyring plots for k_{on} and k_{off} gave sizable activation enthalpies $\Delta H^{\ddagger}$ and, more dramatically, *very positive* activation entropies $\Delta S^{\ddagger}$ for both the "on" and "off" reactions for each complex. Similarly, plots of ln(k_{on}) and ln(k_{off}) vs P were found to be linear,[13] and the calculated $\Delta V_{on}^{\ddagger}$ and $\Delta V_{off}^{\ddagger}$ values are substantially positive (Table 1).

The large and positive $\Delta S^{\ddagger}$ and, more emphatically, $\Delta V^{\ddagger}$ values for k_{on} and k_{off} represent signatures for a substitution mechanism dominated by ligand dissociation,[14] i.e., eqs 2 and 3:

$$\mathrm{Fe^{III}(Por)(H_2O)_2} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Fe^{III}(Por)(H_2O)} + \mathrm{H_2O} \qquad (2)$$

$$\mathrm{Fe^{III}(Por)(H_2O)} + \mathrm{NO} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{Fe^{III}(Por)(H_2O)NO} \qquad (3)$$

Consistent with this mechanism is the report by Hunt et al.[15] that H_2O exchange between solvent and $Fe^{III}(TPPS)(H_2O)_2^{n-}$ occurs at a first-order rate ($k_{ex} = 1.4 \times 10^7$ s^{-1} in 25 °C water) far exceeding the k_{obsd} values determined here for any [NO]. If the steady-state approximation were taken with regard to intermediate $Fe^{III}(Por)(H_2O)$, the k_{obsd} for the exponential relaxation of the nonequilibrium mixture generated by the flash photolysis experiment would be

$$k_{obsd} = \frac{k_1 k_2[\mathrm{NO}] + k_{-1}k_{-2}[\mathrm{H_2O}]}{k_{-1}[\mathrm{H_2O}] + k_2[\mathrm{NO}]} \qquad (4)$$

Under the experimental conditions, one may conclude that $k_{-1}[H_2O] \gg k_2[NO]$ since both steps involve nearly diffusion-

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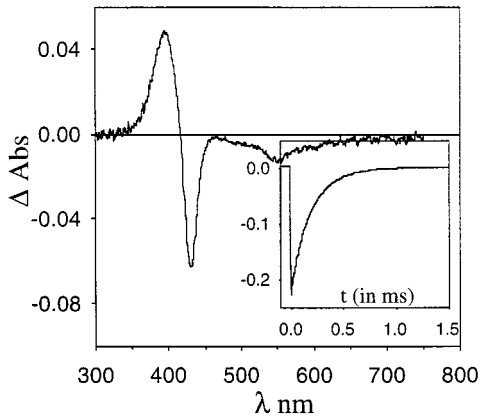

Figure 1. Transient difference spectrum 50 μs after 355 nm flash photolysis (15 mJ/pulse) of $Fe^{III}(TMPS)(H_2O)(NO)$ in pH 6.0 aqueous solution (25 °C). Inset: decay of transient bleaching at 426 nm ([NO] $= 1.7 \times 10^{-3}$ M).

Table 1. Activation Parameters for Reaction of NO with Fe^{III}(TMPS) and Fe^{III}(TPPS) (eq 1)

	$Fe^{III}(TMPS)(NO)$		$Fe^{III}(TPPS)(NO)$	
	k_{on}	k_{off}	k_{on}	k_{off}
$\Delta H^{\ddagger}$ (kJ mol^{-1})	62 ± 2	83 ± 3	70 ± 3	78 ± 4
$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	$+86 \pm 2$	$+89 \pm 3$	$+100 \pm 4$	$+67 \pm 3$
$\Delta V^{\ddagger}$ (cm^3 mol^{-1})	$+13 \pm 1$	$+18 \pm 4$	$+8.3 \pm 1.5$	$+17.9 \pm 1.4$

limited trapping of an unsaturated metal center and $[H_2O] \gg [NO]$. Accordingly, $k_{on} = k_1k_2/k_{-1}[H_2O]$ and $k_{off} = k_{-2}$. In this context, the apparent activation parameters for k_{on} would be sums of terms, e.g.,

$$\Delta S^{\ddagger}_{on} = \Delta S^{\ddagger}_{1} + \Delta S^{\ddagger}_{2} - \Delta S^{\ddagger}_{-1}$$

$$\Delta V^{\ddagger}_{on} = \Delta V^{\ddagger}_{1} + \Delta V^{\ddagger}_{2} - \Delta V^{\ddagger}_{-1} \qquad (5)$$

Since the k_2 and the k_{-1} steps represent similar (very fast) reactions of the unsaturated intermediate $Fe^{III}(Por)(H_2O)$ with an incoming ligand (NO and H_2O, respectively), the differences in their activation parameters (e.g., $\Delta S^{\ddagger}_2 - \Delta S^{\ddagger}_{-1}$ and $\Delta V^{\ddagger}_2 - \Delta V^{\ddagger}_{-1}$) should be small. In such a case the principal contributor to $\Delta S^{\ddagger}_{on}$ would be $\Delta S^{\ddagger}_1$, the activation entropy for the H_2O dissociative step. The k_1 step should thus display a positive $\Delta H_1^{\ddagger}$ reflecting the energy necessary to break the $Fe^{III}-OH_2$ bond, a large, positive $\Delta S_1^{\ddagger}$ owing to formation of two species from one without a significant change in solvation and a substantially positive $\Delta V_1^{\ddagger}$ for the same reason. These conditions are met for the k_{on} activation parameters for both **I** and **II** (Table 1). Furthermore, the values of $\Delta H^{\ddagger}_{ex}$ (57 kJ mol^{-1}) and $\Delta S^{\ddagger}_{ex}$ ($+84$ J K^{-1} mol^{-1}) for the H_2O exchange[15] on **I** are very similar to the respective k_{on} activation parameters. Thus, the factors that determine H_2O exchange kinetics for the di-aquo species apparently dominate the kinetics of the NO k_{on} step for the same species. We therefore conclude that the reaction parameters in this case are largely defined by a dissociative mechanism, the limiting example being eq 2. *The key point is that, for the "on" reaction with the $Fe^{III}(Por)(H_2O)_2^{n-}$ complexes, NO apparently displays reaction kinetics properties quite similar to those for H_2O owing to the dominance of ligand dissociation in determining the behavior of k_{on}.*

The principle of microscopic reversibility argues that the k_{off} pathway proceeds via the same intermediates. However, activation parameters similar to those of k_{on} are not required, since the energetically dominant k_{-2} step involves NO dissociation. Formation of the nitrosyl complex is accompanied by charge transfer to give a species often represented as $Fe^{II}(NO^+)$; furthermore, the iron is no longer high-spin Fe^{III} but (formally) low-spin Fe^{II}. Thus, the reaction coordinate of NO dissociation must incorporate the entropic and volume differences accompanying the solvation changes as charge redistributes and the spin crossover. This may explain why $\Delta V^{\ddagger}_{off}$ is more positive than $\Delta V^{\ddagger}_{on}$ in both cases, but regardless of such speculation, these activation parameters remain consistent with the limiting mechanism described by eqs 2 and 3.

What do these results signify with regard to the reactivity of NO with other heme centers? Hoshino et al.[9] have summarized the "on" rates for several Fe^{II} and Fe^{III} heme proteins and noted a range of more than 8 orders of magnitude in k_{on} values. These rates are in part a function of protein structure. For example, "on" rates are very slow with both Fe^{II} and Fe^{III} cytochrome *c*, where the protein limits the access of NO to the metal site, even though the equilibrium constants for complex formation are large. When such access is available, facile reaction requires either a very labile coordination site, such as the high-spin Fe^{III} heme centers **I** and **II** and catalase ($k_{on} = 3.0 \times 10^7$ M^{-1} s^{-1}),[9] or, better, a vacant coordination site such as high-spin Fe^{II} heme centers $Fe^{II}(TPPS)(H_2O)^{4-}$ (1.8×10^9),[9] myoglobin (1.7×10^7),[16] and, presumably, soluble guanylyl cyclase.[3,17] This suggests that the free radical nature of NO, which clearly has utmost importance in determining the stability and chemical properties of biologically relevant metal nitrosyl complexes, may have but minor influence on the reaction dynamics to form such complexes. Since the odd electron resides in a NO π^* orbital, its involvement with the metal center is unlikely to be significant except at short distances where coordination is largely accomplished. Thus, in terms of its "on" reactions with Fe^{II} and Fe^{III} hemes, NO acts as a normal two-electron donor ligand in the initial stages of its interaction with the metal center. We are currently extending these activation parameter measurements to NO reactions with relevant heme proteins.

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Supporting Information Available: Absorption spectra and k_{obs} plots (3 pages). See any current masthead page for ordering and Internet access instructions.

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