# Reactions of water soluble iron(II) and cobalt(II) porphyrins with nitric oxide. Implications for the reactivity of NO and biologically relevant metal centers

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**The entropies and volumes of activation ($\Delta S^{\ddagger}_{on}$ and $\Delta V^{\ddagger}_{on}$) for the fast reactions ($k_{on} > 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$) reaction of NO with the water soluble iron(II) porphyrin complexes Fe$^{II}$(TPPS) [TPPS = tetrakis(4-sulfonatophenyl)porphinato] and Fe$^{II}$(TMPS) [TMPS = tetrakis(sulfonatomesityl)porphinato] are small and positive indicating that these reactions are dominated by the diffusion processes as is the analogous formation of the cobalt(II) complex Co$^{II}$(TPPS)(NO).**

Nitric oxide has important and diverse roles in mammalian biology including cytotoxic immune response and intracellular signaling.[1] Under physiological conditions the principal targets for NO are metal centers, primarily iron, the best documented being the ferroheme center in soluble guanylyl cyclase (sGC).[2] NO may also have roles as an inhibitor of metalloenzymes such as catalase,[3] nitrile hydrase[4] and cytochrome oxidase,[5] as the vasodilator released from the salivary ferriheme protein of a blood sucking insect[6] and in blood pressure regulation by hemoglobin.[7] However, despite numerous rate measurements for NO reactions with ferro- and ferri-heme centers,[8] there has been little quantitative study to deduce the bimolecular mechanisms by which NO undergoes coordination to metal porphyrin centers [eqn. (1)].

$$\mathrm{M(por)_{aq} + NO} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{M(por)(NO)_{aq}} \qquad (1)$$

To address this deficiency, we have initiated studies on the temperature and hydrostatic pressure effects for the 'on' and 'off' reactions with water soluble iron(II) and iron(III) porphyrins in aqueous solutions as simple models for ferro- and ferri-heme proteins {M = Fe$^{II}$ or Fe$^{III}$; por = TPPS [tetrakis(4-sulfonatophenyl)porphinato anion] or TMPS [tetrakis(sulfonatomesityl)porphinato anion]}.[9] From these data, one can extract the activation parameters $\Delta H^{\ddagger}_{on}$, $\Delta S^{\ddagger}_{on}$ and $\Delta V^{\ddagger}_{on}$ in order to gain insight into the nature of the transition state(s) of the rate limiting step(s). Here we describe results for the 'on' reaction of the ferroheme complexes Fe$^{II}$(TPPS) **I** and Fe$^{II}$(TMPS) **II** in aqueous solutions and compare these to the analogous reaction for the cobalt(II) porphyrin Co$^{II}$(TPPS) **III**.

Fig. 1 shows the difference spectrum obtained by subtracting the absorbance spectrum of aqueous Fe$^{II}$(TPPS)(NO) and NO (1.7 mM) from that recorded subsequent to a 355 nm laser pulse recorded with a CCD camera (50 ns delay, 100 ns gate width).[10] This is in exact agreement with the difference between the spectra of **I** and Fe$^{II}$(TPPS)(NO) according to eqn. (1). The transient absorption changes decayed back to baseline and no permanent photoproducts were spectrally apparent (Fig. 1, inset).

The $k_{obs}$ values obtained by exponential fits of these data are independent of the observation wavelength. According to relaxation kinetics, $k_{obs} = k_{on}[\mathrm{NO}] + k_{off}$, and correspondingly plots of $k_{obs}$ *vs.* [NO] are linear with slopes equal to $k_{on}$ with values of (1.47 ± 0.05) × 10$^9$ and (1.04 ± 0.08) × 10$^9$ dm$^3$ mol$^{-1}$ s$^{-1}$ for **I** and **II**, respectively, in 298 K phosphate buffered (pH 7.0) aqueous solutions. The intercepts of such plots should equal $k_{off}$, but since equilibrium constants for Fe$^{II}$(por)(NO) formation ($K_1 = k_{on}/k_{off}$) are very large, $k_{off}$ values could not be determined by extrapolation. Independent measurements in this laboratory give the upper limit estimate $k_{off} < 2$ s$^{-1}$ for **I**.[11]

Temperature effects on the $k_{on}$ values for **I** and **II** were evaluated from linear plots of $k_{obs}$ *vs.* [NO] for at least six NO concentrations at each temperature. Eyring plots gave straight lines from which were extracted the values for $\Delta H^{\ddagger}_{on}$ and $\Delta S^{\ddagger}_{on}$. For **I** these were 24 ± 3 kJ mol$^{-1}$ and 12 ± 10 J mol$^{-1}$ K$^{-1}$ and for **II** these were 26 ± 6 kJ mol$^{-1}$ and 16 ± 20 J mol$^{-1}$ K$^{-1}$, respectively.

Hydrostatic pressure ($P$) effects were evaluated by determining $k_{on}$ from plots of $k_{obs}$ *vs.* [NO] for individual $P$ ranging from 0.1 to 250 MPa. Plots of ln($k_{on}$) *vs.* $P$ were linear and activation volumes were calculated according to $\Delta V^{\ddagger}_{on} = -RT[\mathrm{dln}(k_{on})/\mathrm{d}P]_T$.[12] The respective $\Delta V^{\ddagger}_{on}$ values obtained for **I** and **II** were 5.0 ± 1.0 and 2.2 ± 0.6 cm$^3$ mol$^{-1}$.

The measured rate constants $k_{on}$ for NO binding to ferrohemes range over many orders of magnitude, from 8.3 dm$^3$ mol$^{-1}$ s$^{-1}$ for Cyt$^{II}$ (Cyt$^{II}$ = Fe$^{II}$cytochrome *c*) to 1.5 × 10$^9$ dm$^3$ mol$^{-1}$ s$^{-1}$ for **I** at ambient temperature.[8] Ferrohemes may be six-coordinate in the presence of an excess of strong field ligands such as pyridine or imadazole but tend to be coordinatively unsaturated otherwise. In wet toluene or dichloromethane, the model ferroheme Fe$^{II}$(TtButPP) {TtButPP = [(N-*tert*-butylcarbamoyl)phenyl]porphyrin} was found to bind $H_2O$ only weakly.[13] Furthermore, ferroheme proteins tend to be five-coordinate unless a strong field ligand such as CO, $O_2$ or CN$^-$ occupy an axial site, in which case the Fe$^{II}$ is six-coordinate, although in the case of Cyt$^{II}$ the Fe$^{III}$ is six-coordinate with histidine and cysteine thiolate ligands in the axial site.[14] It does not appear coincidental that Cyt$^{II}$ is extremely slow in its reaction with NO. While the exact coordination environments of **I** and **II** have not been explicitly determined in buffered aqueous media, analogy to the above and other examples clearly suggests that water is a weak field ligand for these ferrohemes which are likely to be effectively five-coordinate in solution.

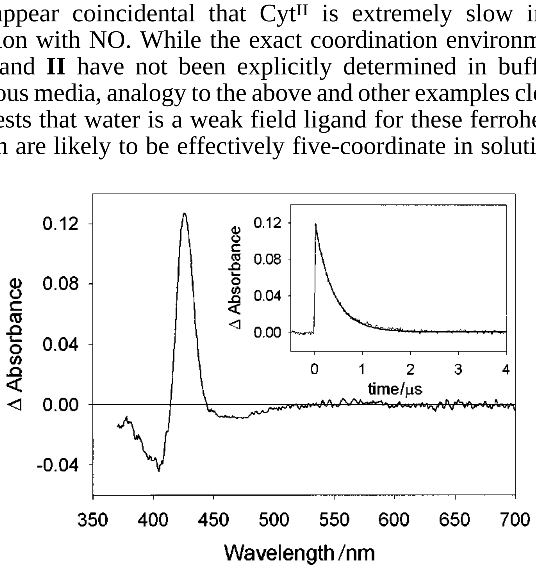

**Fig. 1** The transient absorbance spectrum of aqueous Fe$^{II}$(TPPS)(NO) recorded 50 ns after pulsed laser excitation at 355 nm. Inset: decay of absorbance at 425 nm following a laser pulse, $k_{obs} = 2.7 \times 10^6$ s$^{-1}$.

Our earlier study[9] of the temperature and hydrostatic pressure effects on the rates of NO binding with the ferriheme analogs $\mathrm{Fe^{III}(TPPS)}$ **III** and $\mathrm{Fe^{III}(TMPS)}$ **IV** demonstrated very large and positive values for both $\Delta V^{\ddagger}_{\mathrm{on}}$ and $\Delta S^{\ddagger}_{\mathrm{on}}$. Such behaviour is a signature for a mechanism where $\mathrm{Fe^{III}NO}$ bond formation is preceded by ligand dissociation, in this case from the hexacoordinate ferriheme complexes $\mathrm{Fe^{III}(por)(H_2O)_2}$. In contrast, the $\Delta S^{\ddagger}_{\mathrm{on}}$ and $\Delta V^{\ddagger}_{\mathrm{on}}$ values determined for the iron(II) species **I** and **II** are much smaller while, correspondingly, the $k_{\mathrm{on}}$ values are three orders of magnitude faster.

The kinetics of ligand binding to ferrohemes have led to a proposed scheme[15] in which an encounter pair is formed before bond formation occurs, *i.e.*

$$\mathrm{Fe^{II}(por)} + \mathrm{L} \underset{k_{-\mathrm{d}}}{\overset{k_{\mathrm{d}}}{\rightleftharpoons}} \{\mathrm{Fe^{II}(por)} \,\|\, \mathrm{L}\} \xrightarrow{k_{\mathrm{a}}} \mathrm{Fe^{II}(por)(L)} \qquad (2)$$

In this model, $k_{\mathrm{d}}$ is the rate constant for formation of the encounter pair by diffusion, $k_{-\mathrm{d}}$ is that for diffusion of the reactants apart, and $k_{\mathrm{a}}$ represents that for formation of the Fe–L bond. If the steady state approximation is made with respect to the encounter pair $\{\mathrm{Fe(por)}\| \mathrm{L}\}$, then,

$$k_{\mathrm{on}} = \frac{k_{\mathrm{d}} k_{\mathrm{a}}}{k_{-\mathrm{d}} + k_{\mathrm{a}}} \qquad (3)$$

and to a first approximation,

$$\Delta V^{\ddagger}{}_{\mathrm{on}} = \Delta V^{\ddagger}{}_{\mathrm{d}} + \Delta V^{\ddagger}{}_{\mathrm{a}} - RT\left(\frac{\mathrm{d}\ln(k_{\mathrm{a}} + k_{-\mathrm{d}})}{\mathrm{d}P}\right)_T \qquad (4)$$

The two limiting cases are ones in which ligand binding is activation controlled or in which the reaction is diffusion controlled. In the former $k_{\mathrm{d}} \gg k_{\mathrm{a}}$, thus

$$\Delta V^{\ddagger}_{\mathrm{on}} = \Delta V^{\ddagger}_{\mathrm{d}} + \Delta V^{\ddagger}_{\mathrm{a}} - \Delta V^{\ddagger}_{-\mathrm{d}} \qquad (5)$$

The difference $\Delta V^{\ddagger}_{\mathrm{d}} - \Delta V^{\ddagger}_{-\mathrm{d}}$ is the volume difference between a contact pair and a solvent separated pair. While unknown, this can be assumed to be small when L is uncharged. The principal contributor to $\Delta V^{\ddagger}_{\mathrm{on}}$ thus becomes $\Delta V^{\ddagger}_{\mathrm{a}}$, which would be expected to be negative if $\mathrm{Fe^{II}(por)}$ is five coordinate (since this step involves bond formation).[12]

In the other limiting case, $k_{\mathrm{a}} \gg k_{-\mathrm{d}}$ and $\Delta V^{\ddagger}_{\mathrm{on}} \cong \Delta V^{\ddagger}_{\mathrm{d}}$. Activation volumes for diffusion limited reactions ($\Delta V^{\ddagger}_{\mathrm{d}}$) in various solvents are generally positive owing to pressure induced increases in viscosity. In the present case, the $\Delta V^{\ddagger}_{\mathrm{on}}$ values measured for **I** and **II** (5.0 and 2.2 $\mathrm{cm^3\ mol^{-1}}$, respectively) are somewhat larger than expected from a diffusion limited rate in water[16] but are much smaller than found for the ferriheme analogs.[9] This suggests that $k_{\mathrm{on}}$ is largely dominated by the diffusional terms. Indeed the rates for reaction of NO with **I** and **II** are within an order of magnitude of the diffusion limit in water ($k_{\mathrm{d}} \approx 10^{10}\ \mathrm{dm^3\ mol^{-1}\ s^{-1}}$ at 298 K).[17]

A similar analysis of $\Delta S^{\ddagger}$ gives $\Delta S^{\ddagger}_{\mathrm{on}} \cong \Delta S^{\ddagger}_{\mathrm{d}}$, if $k_{\mathrm{a}} \gg k_{-\mathrm{d}}$. The activation entropy $\Delta S^{\ddagger}_{\mathrm{d}}$ for diffusion in water is small and positive; a value of *ca.* 34 $\mathrm{J\ mol^{-1}\ K^{-1}}$ can be calculated according to Eyring and coworkers.[18] In this context, the measured $\Delta S^{\ddagger}_{\mathrm{on}}$ values for **I** and **II** (12 and 16 $\mathrm{J\ mol^{-1}\ K^{-1}}$, respectively) are further argument for a diffusion dominated process.

We have also determined the temperature effects on the kinetics of the reaction of $\mathrm{Co^{II}(TPPS)}$ with NO to give $\mathrm{Co^{II}(TPPS)(NO)}$. Notably the behavior of this system is quite similar to that of the $\mathrm{Fe^{II}}$ analog with $k_{\mathrm{on}} = 2.3 \pm 0.1 \times 10^9\ (\mathrm{dm^3\ mol^{-1}\ s^{-1}})$ at 298 K, $\Delta H^{\ddagger}_{\mathrm{on}} = 28 \pm 2\ \mathrm{kJ\ mol^{-1}}$ and $\Delta S^{\ddagger}_{\mathrm{on}} = 28 \pm 7\ \mathrm{J\ mol^{-1}\ K^{-1}}$. Hence, the kinetics of the 'on' reaction for $\mathrm{Co^{II}(TPPS)}$ can also be concluded to be dominated by diffusion processes.

For nitric oxide to be important in intracellular signaling at the sub-μM concentrations generated *in vivo*,[19] the reactions of NO with ferroheme proteins must have very high second order rate constants in order to compete with other processes leading to NO depletion. The present study demonstrates that activation parameters for the reactions of NO with the water soluble Fe(II) porphyrin complexes **I** and **II** are consistent with the small and positive values of $\Delta V^{\ddagger}_{\mathrm{on}}$ and $\Delta S^{\ddagger}_{\mathrm{on}}$ expected for diffusion limited (or nearly limited) processes in solution. These results experimentally support the intuitive notion that the nearly diffusion limited rates for NO reaction with iron(II) hemes is due to vacant or extraordinarily labile coordination sites at those metal centers.

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## Notes and references

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