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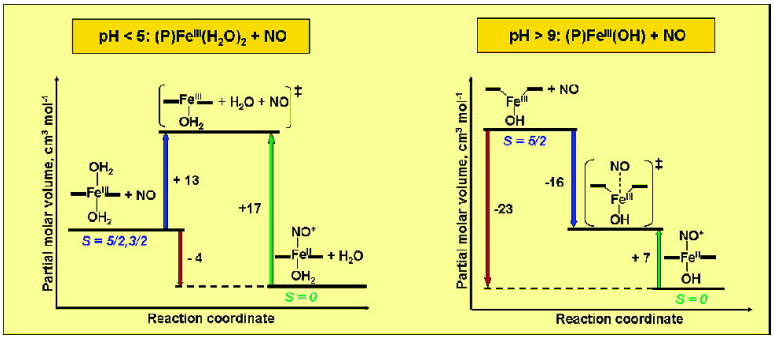

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pH Controls the Rate and Mechanism of Nitrosylation of Water-Soluble Fe^{III} Porphyrin Complexes

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Abstract: In-depth kinetic and mechanistic studies on the reversible binding of NO to water-soluble iron(III) porphyrins as a function of pH revealed unexpected reaction kinetics for monohydroxo-ligated (P)Fe^{III}(OH) species formed by deprotonation of coordinated water in diaqua-ligated (P)$Fe^{III}(H_2O)_2$. The observed significant decrease in the rate of NO binding to (P)Fe(OH) as compared to that of (P)$Fe(H_2O)_2$ does not conform with expectations based on previous mechanistic work on NO−heme interactions, which would point to a diffusion-limited reaction for the five-coordinate Fe^{III} center in (P)Fe(OH). The decrease in rate and an associatively activated mode of NO binding observed at high pH is ascribed to an increase in the activation barrier related to spin state and structural changes accompanying NO coordination to the high-spin (P)Fe^{III}(OH) complex. The existence of such a barrier has previously been observed in the reactions of five-coordinate iron(II) hemes with CO and is evidenced for the first time for the process involving coordination of NO to the iron heme complex. The observed reactivity pattern, relevant in the context of studies on NO interactions with synthetic and biologically important hemes (in particular, hemoproteins), is reported here for an example of a simple water-soluble iron(III) porphyrin [*meso*-tetrakis(sulfonatomesityl)-porphinato]−iron(III), (TMPS)Fe^{III}.

Introduction

Nitric oxide, which is recognized to have important bioregulatory roles, can readily bind to Fe(II) and Fe(III) hemes to form (P)Fe(NO) nitrosyls of the $\{FeNO\}^7$ and $\{FeNO\}^6$ type,[1] respectively. Since in vivo interactions of NO with the (P)Fe group of heme proteins form the basis of several pathways in which NO affects physiological and pathophysiological actions, numerous investigations focus on the elucidation of the mechanism of NO binding to hemoproteins and synthetic (P)Fe models, as well as on the stability and properties of the resulting heme nitrosyls.[2−8] A close examination of experimental data available to date shows, however, that our current understanding of factors which govern interaction of NO with hemes is far from complete.[2] This results, on one hand, from experimental difficulties in the use of NO in aqueous solutions (which may easily lead to inaccurate or erroneous results and complicate analysis of data reported for various biological systems), and on the other, from the rich diversity of structural and electronic features of (P)Fe centers. In the latter context, a variety of factors (e.g., oxidation state of the iron center, identity, and number of iron axial ligands, type of the heme ligand, and protein superstructure) may influence the dynamics of formation and stability of heme nitrosyls.

The interplay of these various factors in a given heme protein or a model (P)Fe complex can be better understood when the spectroscopic, kinetic, and thermodynamic studies are supplemented by mechanistic data, which reveal the details of molecular pathways followed during coordination and release of NO. Such mechanistic studies are, however, rather rare. Ford and Hoshino studied the mechanism of NO coordination to selected iron(II) porphyrins as synthetic models for active centers in ferroheme proteins.[2,4,6,7] With respect to iron(III) hemes, detailed mechanistic investigations of reversible NO binding to cytochrome P450, methemoglobin, and to model water-soluble iron(III) porphyrins were performed.[2−5,8] A general conclusion drawn from these studies was that the free radical nature of NO has little (if any) influence on the kinetics and mechanism of the NO−heme interactions, and the observed reaction dynamics is mainly controlled by lability of the iron center and its accessibility for the NO ligand.[2−8]

We have undertaken systematic studies on the influence of porphyrin substituents in model water-soluble iron(III) porphyrins on the properties of the central iron(III) ion reflected by its reactivity toward NO.[5,9,10] Previously reported studies of that

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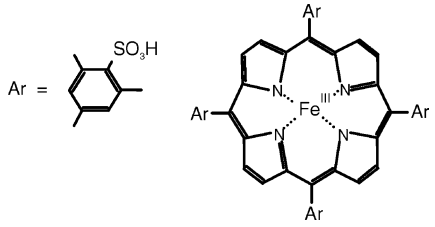

Figure 1. Structure of (TMPS)Fe^{III}.

kind were limited to the pH range in which the studied complexes exist as six-coordinate (P)$Fe^{III}(H_2O)_2$ species.[2,4,5,10] Our present studies included, for the first time, reactions with the monohydroxo complex (P)Fe^{III}(OH) (formed via deprotonation of coordinated water in (P)$Fe^{III}(H_2O)_2$), for which an unexpected kinetic behavior was observed. Since the observed reactivity and its mechanistic implications are of fundamental importance for studies on NO interactions with heme proteins, we report it here for a selected example, *meso*-tetrakis(sulfonatomesityl)porphinato]−iron(III), (TMPS)Fe^{III}, **1** (see Figure 1).

Experimental Section

Materials. The iron porphyrin, [*meso*-tetrakis(2,4,6-trimethyl-3-sulfonatophenyl)porphinato]−iron(III) hydrate (sodium salt), was purchased from Frontier Scientific Ltd. Fine Chemicals, Utah, USA, and used as received. NO gas (Messer Griesheim, ≥99.5 vol %) was cleaned from trace amounts of higher nitrogen oxides by passing it through concentrated KOH solution and an Ascarite II column (NaOH on silica gel, Sigma-Aldrich). All other chemicals used in this study were of analytical reagent grade.

Solution Preparation. All solutions were prepared from deionized water. Argon or nitrogen and gastight glassware were used for preparation and handling of deoxygenated solutions. The ionic strength of the solutions (0.15 M) was adjusted with $NaNO_3$. Further details of experimental conditions used in the performed experiments are specified in the figure captions.

Measurements. pH measurements were performed on a Methrom 623 pH meter calibrated with standard buffer solutions. UV−vis spectra were recorded in gastight cuvettes on a Shimadzu UV-2100 spectrophotometer. All kinetic experiments were performed under pseudo-first-order conditions, that is, with at least a 10-fold excess of NO over the iron porphyrin.

Stopped-flow kinetic measurements were performed on an SX 18.MV (Applied Photophysics) stopped-flow apparatus. In a typical experiment, a deoxygenated buffer solution was mixed in varying volume ratios with a saturated NO solution in a gastight syringe to obtain the appropriate NO concentration (0.2−1.8 mM). The NO solution was then rapidly mixed with a deoxygenated solution of iron(III) porphyrin in a 1:1 volume ratio in a stopped-flow apparatus. The kinetics of the reaction was monitored at 426 or 432 nm (at low and high pH, respectively). The rates of NO binding and release (k_{on} and k_{off}) were determined from slopes and intercepts of linear plots of k_{obs} versus [NO]. The NO dissociation rates at pH 11 were also measured directly by an NO-trapping method. This involved rapid mixing of a (TMPS)$Fe^{II}(NO^+)$(OH) solution (1.5×10^{-5} M) containing a small excess of NO with aqueous solutions of $[Ru^{III}(edta)(H_2O)]^-$ (1 mM) to give $[Ru^{III}(edta)NO]^-$ and (TMPS)Fe^{III}(OH), as evidenced by the accompanying spectral change. The kinetics of NO release was followed in a stopped-flow spectrophotometer at 432 nm. The first-order rate constants determined from the kinetic traces were in good agreement with those determined at pH 11 from the intercepts of the plots of k_{obs} versus [NO]. High-pressure stopped-flow experiments were performed at pressures up to 130 MPa on a custom-built instrument described previously.[11] The kinetic traces were analyzed with the use of the OLIS KINFIT (Bogart, GA, 1989) set of programs.

^{17}O NMR water exchange studies were performed on 20 mM solution of (TMPS)Fe^{III} at pH 11 (0.07 M CAPS buffer); 10% of the total sample volume of enriched ^{17}O-labeled water (normalized 19.2% ^{17}O H_2O, D-Chem Ltd.) was added to the solution. A sample containing the same components, except for iron porphyrin, was used as a reference. Variable-temperature FT ^{17}O NMR spectra were recorded at a frequency of 54.24 MHz on a Bruker Advance DRX 400WB spectrometer. The temperature dependence of ^{17}O line broadening was studied in the temperature range of 278−353 K.

Table 1. Selected Properties of (TMPS)$Fe^{III}(L)_n$

(P)Fe^{III}	β-py[a] (ppm)	Int%[b]	k_{ex} (s^{-1})	λ_{max} (nm) 1	λ_{max} (nm) 1-NO
1-H_2O	43[c]	26	2.1×10^6 [d]	396, 527	426, 540
1-OH[c]	82	HS[e]	ND[f]	416, 590	432, 549

[a] Chemical shift of β-pyrrole ^1H. [b] Ref 12. [c] This work. [d] From ref 10. [e] Pure high-spin complex, $S = 5/2$. [f] ND − not determined (compare Supporting Information, Figure S3).

Results and Discussion

UV−vis, ^1H NMR, and ^{17}O NMR studies performed on **1** (compare Table 1 and Figures S1−S3 in Supporting Information) indicated that (TMPS)Fe^{III} exists in aqueous solution at pH 1−12 as monomeric diaqua-ligated $[(TMPS)Fe^{III}(H_2O)_2]^{3-}$ (**1-H_2O**) and monohydroxo-ligated $[(TMPS)Fe^{III}(OH)]^{4-}$ species (**1-OH**) (eq 1).

$$[(TMPS)Fe^{III}(H_2O)_2]^{3-} \rightleftarrows [(TMPS)Fe^{III}(OH)]^{4-} + H_3O^+ \quad pK_a = 6.9 \quad (1)$$

1-H_2O and **1-OH** represent the six-coordinate spin-admixed ($S = 3/2$, 5/2, Int% ≈ 26%)[12] and predominantly five-coordinate[13] high-spin ($S = 5/2$) complexes, respectively. As previously reported,[4] reversible binding of NO to **1-H_2O** leads to formation of the linearly bonded diamagnetic $\{FeNO\}^6$ porphyrin nitrosyl, which can be formally represented as (TMPS)$Fe^{II}(NO^+)(H_2O)$[14] (**1-NO**). The product exhibits Soret and Q-bands at 426 and 540 nm, respectively. When **1-OH** reacts with NO at pH > 7, the resulting nitrosyl complex has a noticeably different UV−vis spectrum, with Soret and Q-bands shifted to longer wavelengths (see Table 1).

The $pK_{a(NO)} = 6.5 \pm 0.2$ estimated from the UV−vis spectra of **1-NO** in the pH range of 4−11 (Supporting Figure S4) is

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(12) The percentage of 3/2 spin admixture is estimated by the empirical equation Int% = [(80 − δ)/140] × 100 (%), where δ is the chemical shift of β-pyrrole ^1H resonance, compare: Ikezaki, A.; Nakamura, M. *Inorg. Chem.* **2002**, *41*, 6225.

(13) In purely five-coordinate high-spin (P)Fe^{III}(OH) complexes formed in noncoordinating solvents, the iron(III) center is displaced from the porphyrin plane, towards the OH^- ligand. This leads to splitting of the *meta* phenyl ^1H resonances into a characteristic doublet appearing at ca. 13 ppm. A broad poorly resolved *meta* ^1H signal observed at ∼13 ppm in the ^1H NMR spectrum of **1-OH** (Figure S2 in Supporting Information) and detectable broadening of the bulk $^{17}OH_2$ signal observed in water exchange study of **1-OH** at pH 11 (Figure S3) suggest that (TMPS)Fe(OH) has a weakly bound water molecule *trans* to OH^- ligand or exists in a dynamic equilibrium with a small fraction of a six-coordinate form, (TMPS)Fe(OH)(H_2O). In either case, a very labile Fe(III) center can be expected for **1-OH**.

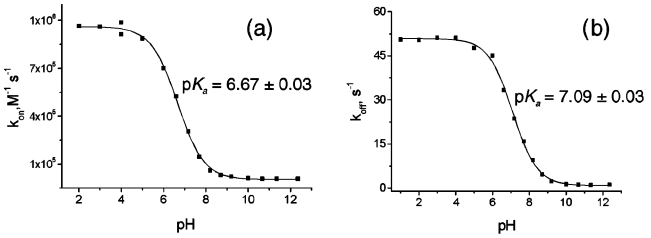
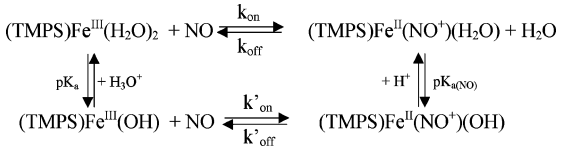


Figure 2. The pH dependence of k_{on} (a) and k_{off} (b) determined for **1** in the pH range of 1−13. Experimental conditions: [**1**] = 1×10^{-5} M, I = 0.15 M ($NaNO_3$), T = 9 °C; pH 1 adjusted with HNO_3, pH 2−3 $ClCH_2COOH/ClCH_2COONa$, pH 4−5 CH_3COOH/CH_3COONa, pH 6−7 Bis-Tris, pH 7.2−8.8, Tris, pH 9.2, borate, pH 10−11.4, CAPS, pH 12.5 adjusted with NaOH; [Buffer] = 0.07 M (except for borate buffer, 0.05 M).

Scheme 1



ascribed to deprotonation of water in (TMPS)$Fe^{II}(NO^+)(H_2O)$, as shown in Scheme 1, which outlines the reactivity of **1** toward NO in the pH range of 1−12.

Stopped-flow kinetic measurements performed under pseudo-first-order conditions ([NO] > 10 × [**1**]) indicated that the observed rate constants show a linear dependence on [NO] under all pH conditions (viz., $k_{obs} = k_{on}[NO] + k_{off}$). The rate constants $k_{on} = (9.6 \pm 0.2) \times 10^5$ M^{-1} s^{-1} and $k_{off} = 51 \pm 6$ s^{-1} (I = 0.15 M, 9 °C) determined for the binding and release of NO by **1-H_2O** at pH 3 agree well with the corresponding values reported from flash photolysis studies.[4]

Systematic measurements of k_{obs} versus [NO] performed in the pH range of 1−13 allowed determination of k_{on} and k_{off} as a function of pH, as shown in Figure 2.

As indicated by the data in Figure 2a, the five-coordinate **1-OH** binds nitric oxide $>10^2$ times slower in comparison to the six-coordinate **1-H_2O** form (compare also Table 2). This observation is surprising in view of the known *trans*-labilizing effect of the hydroxo ligand, which usually leads to a significant *increase* in the lability of the coordination site *trans* to OH^- in hydroxo-ligated metal complexes. In fact, ^{17}O NMR water exchange data and ^1H NMR spectra obtained for **1** at high pH suggest the presence of a vacant (or very labile)[13] coordination site in **1-OH**. Thus, if the NO coordination step remains to be controlled by the lability of the metal center in **1-OH** (as is the case at low pH, vide infra),[4] reaction rates close to the diffusion-controlled limit in water (ca. 10^8-10^9 M^{-1} s^{-1}) would be expected for NO coordination at high pH, that is, a significant *increase* in the reaction rate should occur on going from **1-H_2O** to **1-OH**. An unexpected opposite effect observed experimentally clearly indicates significant contributions of processes other than diffusion to the activation barrier associated with the binding of NO to **1-OH**. It is also evident from the data in Figure 2b and Table 2 that the rate of NO release decreases considerably on going from L = H_2O to L = OH^- in (TMPS)$Fe^{II}(NO^+)$(L).

Table 2. Rate Constants and Activation Parameters for Reversible NO Binding to **1-H_2O** and **1-OH**

rate constant/ activation parameter	NO binding		NO release	
	1-H_2O	**1-OH**[a]	**1-H_2O**	**1-OH**[a]
k[b]	9.6×10^5[5a,c]	7.4×10^3[3d]	51 ± 6[c]	1.5 ± 0.1[d]
$\Delta H^{\ddagger}$ (kJ/mol)	57 ± 3[e]	28 ± 1	76 ± 6[e]	90 ± 1
$\Delta S^{\ddagger}$ (J/mol·K)	$+95 \pm 10$[e]	-71 ± 2	$+60 \pm 11$[e]	$+76 \pm 3$
$\Delta V^{\ddagger}$ (cm^3/mol)	$+9 \pm 1$[e]	-16.2 ± 0.4	$+18 \pm 2$[e]	$+7.4 \pm 1.0$

[a] This work. [b] k_{on} in M^{-1} s^{-1}, k_{off} in s^{-1}. [c] At 9 °C. [d] At 10 °C. [e] Ref. 4.

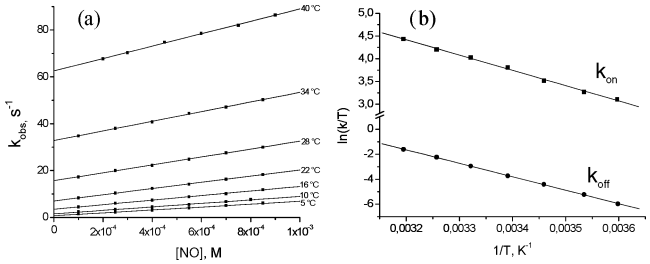


Figure 3. (a) Plots of k_{obs} versus [NO] as a function of temperature for the reaction of NO with **1-OH** at pH 11; (b) Eyring plots of $\ln(k_{on}/T)$ and $\ln(k_{off}/T)$ versus $1/T$. Experimental conditions: [**1**] = 1×10^{-5} M, I = 0.15 M ($NaNO_3$), pH = 11 (CAPS 0.07 M).

Stopped-flow kinetic measurements performed under pseudo-first-order conditions ([NO] > 10 × [**1**]) indicated that the observed rate constants show a linear dependence on [NO] under all pH conditions (viz., $k_{obs} = k_{on}[NO] + k_{off}$). The rate constants $k_{on} = (9.6 \pm 0.2) \times 10^5$ M^{-1} s^{-1} and $k_{off} = 51 \pm 6$ s^{-1} (I = 0.15 M, 9 °C) determined for the binding and release of NO by **1-H_2O** at pH 3 agree well with the corresponding values reported from flash photolysis studies.[4]

As indicated by previous mechanistic studies on diaqua-ligated iron(III) porphyrins, the rate of NO binding at low pH is controlled by rate-limiting dissociation of water from (P)$Fe^{III}(H_2O)_2$. The subsequent fast Fe−NO bond formation is accompanied by a spin state change and reorganization of electron density within the Fe−NO unit. An analogous dissociatively activated mechanism was also observed for NO release from (P)$Fe^{II}(NO^+)(H_2O)$.[2,4,5] Our mechanistic studies performed for **1-OH** at pH 11 involved measurements of k_{on} and k_{off} as a function of temperature and hydrostatic pressure (compare Figures 3 and 4 and Table S1 in Supporting Information).

The resulting rate constants and activation parameters for reversible NO binding to **1-OH** are summarized in Table 2.

As can be seen from these data, negative $\Delta S^{\ddagger}_{on}$ and $\Delta V^{\ddagger}_{on}$ values found for the NO binding to **1-OH** reveal a changeover in the mechanism of NO coordination from dissociative in **1-H_2O** to associatively activated in **1-OH**. This mechanistic difference is particularly evident on comparison of volume profiles constructed on the basis of $\Delta V^{\ddagger}_{on}$ and $\Delta V^{\ddagger}_{off}$ values determined for **1-H_2O**[4] and **1-OH**, respectively, as depicted in Figure 5.

We propose that NO binding to five-coordinate (P)Fe^{III}(OH) involves diffusion-controlled formation of an encounter complex, {(P)Fe^{III}(OH)||NO}, and subsequent rate-determining $Fe^{II}-NO^+$ bond formation. A similar mechanism was previously suggested for NO binding to five-coordinate high-spin iron centers in model (P)Fe^{II} porphyrins and in substrate-bound

(14) (a) This notation (assuming coordination of a strongly π-accepting NO^+ ligand to a formally Fe^{II} ion) overestimates to some degree the shift in electron density from the NO ligand to the Fe center in the {FeNO}^{6} porphyrin nitrosyls formed by (P)Fe^{III} porphyrins. However, since it reflects the partial NO^+ character of the coordinated nitrosyl ligand (which is clearly evidenced by spectroscopic and structural data on {FeNO}^{6} porphyrin nitrosyls, as well as their characteristic reactivity, in particular, facile reductive nitrosylation[5,14b,c]), this notation can be useful in discussing the properties and reactivity of such complexes. Although not mentioned in the text, reductive nitrosylation (leading to slow conversion of the {FeNO}^{6} nitrosyl to the corresponding {FeNO}^{7} complex, (TMPS)Fe^{II}NO) does occur for the product formed by the binding of NO to (TMPS)Fe^{III}. An analogous process is also observed for {FeNO}^{6} nitrosyls formed by other water-soluble iron(III) porphyrins studied by us, as will be described in detail in a subsequent publication.[14c] For this reason, from the two possible formula for the {FeNO}^{6} nitrosyl formed by (P)Fe^{III} (i.e., (P)Fe^{III}(NO)(L) and (P)-$Fe^{II}(NO^+)$(L)), the latter is preferentially used in the present report, as well as in subsequent papers from our group dealing with the reactivity of model Fe^{III} porphyrins toward NO.[9,14c] (b) Fernandez, B. O.; Lorkovic, I. M.; Ford, P. C. *Inorg. Chem.* **2004**, *43*, 5393 and references therein. (c) Jee, J.-E.; Jux, N.; van Eldik, R. Manuscript in preparation.

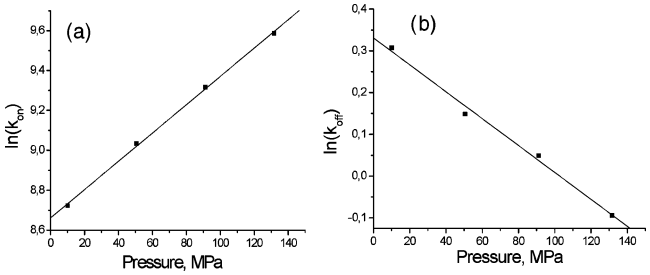


Figure 4. (a) Plot of $\ln(k_{obs})$ versus pressure determined for the reaction of NO with **1-OH** at [NO] = 0.8 mM, $T = 5$ °C and pH = 11 (CAPS, 0.07 M). Since the contribution of k_{off} to k_{obs} is small under the employed experimental conditions ($k_{off} = 0.71\ s^{-1} \approx 10\%\ k_{obs}$), the observed activation volume $\Delta V^{\ddagger}_{obs} \approx \Delta V^{\ddagger}_{on} = -16.2 \pm 0.4\ cm^3\ mol^{-1}$. (b) Plot of $\ln(k_{off})$ versus pressure determined at pH 11 using $[Ru(edta)(H_2O)]^-$ as NO scavenger.

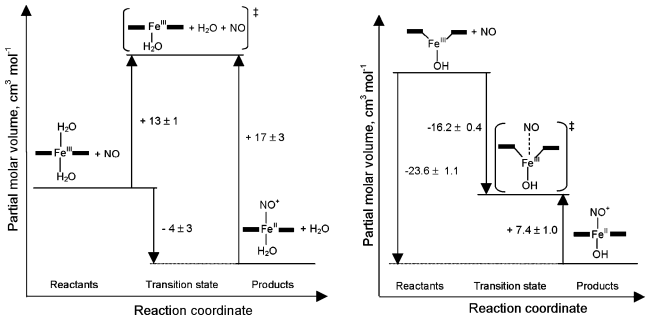


Figure 5. Volume profiles for reversible NO binding to **1-H_2O** and **1-OH**.

cytochrome P450.[2−4] In the present case, however, the rate of NO binding is several orders of magnitude slower in comparison to that observed for (P)Fe^{II} (10^8−$10^9\ M^{-1}\ s^{-1}$).[2] *This implies that NO coordination to* ***1-OH*** *is not controlled by the lability/accessibility of the iron(III) center (as in this case a diffusion-limited reaction would be expected) but rather by the Fe−NO bond formation step*, which apparently exhibits a considerably higher activation barrier than the corresponding process in (P)Fe^{II}. Notably, the activation barrier for the formation of the $Fe^{II}-NO^+$ bond in (P)Fe^{III}(OH) is evidently also higher than that encountered in (P)$Fe^{III}(H_2O)_2$. In the latter case, the activation barrier is assumed to mainly reflect the free energy change upon water dissociation, the subsequent $Fe^{II}-NO^+$ bond formation being energetically less demanding and therefore fast. This is not the case at high pH, where formation of the $Fe^{II}-NO^+$ bond becomes slow and rate-limiting. Since this process involves a spin reorganization at both low and high pH, we suggest that the observed decrease in NO binding rate reflects differences in the activation barrier related to the overall spin state change upon binding of NO to **1-H_2O** and **1-OH**, respectively. The overall spin state change accompanying formation of low-spin (TMPS)$Fe^{II}(NO^+)$(OH) from purely high-spin **1-OH** ($S = 5/2 \rightarrow S = 0$) is larger than that occurring upon binding of NO to the spin-admixed complex **1-H_2O** ($S = 5/2, 3/2 \rightarrow S = 0$). We suggest that this difference contributes to the observed ca. 100-fold decrease in k_{on} at high pH.

Influence of the overall spin state changes on the activation barrier associated with ligand coordination has previously been invoked to account for the differences in the dynamics of NO and CO coordination to Fe^{II} heme centers,[4] as well as in the kinetic studies on the NO binding to model (P)M^{II} complexes (M = Mn, Fe, and Co).[7] The results of the present study provide first experimental evidence for the involvement of spin state changes in determination of the rate and mechanism of NO coordination to a ferriheme complex. A crucial role played by solution pH in reversible binding of NO to the studied porphyrin is further indicated. By tuning the nature of axial ligands and spin state of the metal center in (TMPS)Fe^{III}, it controls not only the rate but also the mechanism of nitrosylation. Consequently, the model water-soluble porphyrin complex with a pK_a value close to 7 exhibits a typical dissociative NO binding mechanism at low pH compared to an associative binding mode for the five-coordinate **1-OH** form at high pH.

Our preliminary studies on NO reactions with other water-soluble iron(III) porphyrins indicate that the decreased rate of NO binding and release at high pH is a typical feature characterizing the reactivity of diaqua- and monohydroxo-ligated forms of (P)Fe^{III}. The magnitude of change in k_{on} and k_{off} values on going from diaqua- to monohydroxo-ligated species and the rates of NO binding and release for different porphyrins at low and high pH were observed to vary considerably among the complexes studied. The role of various factors (e.g., the overall charge of the porphyrin ligand, spin state, and lability of the iron center, etc.) on the observed kinetic and mechanistic variations will be discussed in detail in forthcoming reports.[9]

Conclusions

The reactivity pattern reported here for a simple model water-soluble complex (TMPS)Fe^{III} clearly shows that NO binding to hemes is not always controlled by the lability of the iron center, but can be determined by electronic and structural factors (i.e., reorganization of spin density and accompanying structural changes) in selected reaction systems. This conclusion is important in view of earlier mechanistic studies on NO interactions with heme proteins and model iron porphyrins. In the presently reported case, electronic and structural features of the iron(III) center, which influence the dynamics of reversible NO binding to (TMPS)Fe^{III}, are tuned by the pH of the solution. In the case of heme proteins, a number of other factors (in addition to acid−base equilibria) can determine the electronic and structural properties of a heme prosthetic group. Apart from the number and nature of axial ligands (which are of primary importance in this respect), these may involve hydrogen-bonding interactions, polarity of protein surrounding, accessibility of heme to the solvent, and others. We suggest that, in addition to lability of the iron center, the possible role of spin state and structural changes should always be considered in the interpretation of reactivity patterns observed in the interactions on NO with various heme systems.

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Note Added after ASAP Publication. The version of this article published ASAP on August 31, 2005, contained an error in Figure 5. The corrected version was posted on September 6, 2005.

Supporting Information Available: A total of four figures and a table reporting details of the experimental results. This material is available free of charge via the Internet at http://pubs.acs.org.

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