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Inorganica Chimica Acta 357 (2004) 2503–2509
Inorganica Chimica Acta
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Nitric oxide binding to cationic and anionic ferric porphyrins in aqueous solution: reversible formation of ferrous NO species of the cationic porphyrin

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Received 3 September 2003; accepted 21 January 2004
Available online 6 March 2004

Abstract

Two water-soluble ferric porphyrins, sodium 5α,10β,15α,20β-tetrakis(2-(sulfonatoacetamido)phenyl)porphyrinatoiron(III) (Fe^{III}TanP) and 5α,10β,15α,20β-tetrakis(2-(*N*,*N*,*N*-trimethylammoniumacetamido)phenyl)porphyrinatoiron(III) chloride (Fe^{III}TcatP), were synthesized. The pK_a values of the coordinated H_2O of Fe^{III}TanP and Fe^{III}TcatP were evaluated to be 8.0 and 4.1, respectively. Reactions of NO with the ferric porphyrins were examined spectrophotometrically in aqueous solution. Porphyrin Fe^{III}TanP binds NO reversibly to give the corresponding ferric NO species at pH 1.3 and pH 3.0, and Fe^{III}TcatP reacts similarly with NO at pH 1.3. The thermodynamic data for the NO binding were estimated from van't Hoff plots. At pH 3.0, visible and ESR spectral data indicated that Fe^{III}TcatP binds NO reversibly to produce ferrous NO species depending on NO partial pressures. These results were discussed based on through-space intramolecular interactions between the coordinated H_2O or NO and the ionic substituents of the porphyrins.

Keywords: Water-soluble porphyrin; Iron complex; Nitric oxide; Binding equilibrium

1. Introduction

Reactions of NO with iron porphyrins have received much attention because of their relevance to biological systems [1]. NO binding to ferric porphyrins (Fe^{III}Por) as well as to ferrous porphyrins (Fe^{II}Por) is thought to be physiologically important as exemplified in the vasodilation that occurs in response to the bite of blood sucking insects [2]. Ferric porphyrins in hemeproteins reversibly bind NO to form (NO)Fe^{III}Por species, as follows:

$$Fe^{III}Por + NO \rightleftharpoons (NO)Fe^{III}Por \quad (1)$$

Some of them further react irreversibly with NO to give (NO)Fe^{II}Por species [3–5].

$$(NO)Fe^{III}Por \xrightarrow{NO} (NO)Fe^{II}Por \quad (2)$$

For synthetic model porphyrins, treatment of 5,10,15,20-tetraphenylporphyrinatoiron(III) Fe^{III}TPP with NO in toluene containing a small amount of methanol irreversibly affords (NO)Fe^{II}TPP [6]. In aqueous solution, an analogous water-soluble porphyrin 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinatoiron (III) (Fe^{III}TPPS) reacts reversibly with NO to produce NO-bound ferric species, (NO)Fe^{III}TPPS, but the corresponding reduced species, (NO)Fe^{II}TPPS, was not observed [5]. A recent study has shown that the reaction of Fe^{III}TPPS and excess NO in aqueous solution gives (NO)Fe^{II}TPPS very slowly and this is catalyzed by nitrite ion [7]. We have also reported that the reaction in H_2O-organic mixed solvents reversibly yields (NO)-Fe^{II}TPPS as the dominant product [8]. These results, observed for both natural and model compounds, suggested that the microenvironments and/or solvents

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doi:10.1016/j.ica.2004.01.038

around the bound NO at the active site might play an important role in the reduction behavior of the ferric ion. Thus, studies using properly designed model compounds in aqueous solution could be expected to provide useful information on such effects on NO binding. However, as a result of the lipophilic nature of porphyrins, only limited metalloporphyrins for studying axial-ligand binding in aqueous solutions have been synthesized.

In earlier works, we reported [9,10] that microenvironments near the active site of synthetic metalloporphyrins can affect the thermodynamic parameters for the binding of axial ligands on the basis of non-covalent intramolecular interactions. In this report, to elucidate the microenvironmental effects on NO binding, we have designed and synthesized anionic (Fe^{III}TanP) and cationic (Fe^{III}TcatP) water-soluble ferric porphyrins (Fig. 1). Since these porphyrins have binding pockets that are sterically similar but electrostatically opposite, the microenvironments around a bound, polar axial ligand would be expected to affect the binding properties differently.

2. Experimental

2.1. Materials

All reagents and solvents were of commercial reagent quality and were used without further purification. Nitric oxide gas (99.9% purity) for measurements was purified by passage through KOH pellets. Precoated silica-gel plates (Merck Kieselgel $60F_{254}$) or reversed silica-gel plates (Merck Kieselgel PR-8 F_{254}) were used for TLC to determine *Rf* values.

2.2. Equilibrium procedures and instrumentation

Visible absorption spectra were obtained on a Hitachi U-2000 spectrophotometer. The pH values for spectral measurements were adjusted with $HClO_4$. Partial pressures of NO (P_{NO}) were adjusted by mixing NO with Ar while monitoring with a mass-flow controller (Kojima 2503F) and a gas-flow meter (Kojima 3810). The mixing gas was further purified by KOH pellets before introduction into a cell for visible spectral measurements. ESR spectra were measured on a JEOL JES-FE2X spectrometer.

The pK_a values of the coordinated H_2O of the ferric porphyrins were estimated from the mid-point pH in the visible absorption spectral changes under various pHs. The *K* values as the binding constant for reaction (1) were evaluated from the visible absorption spectral changes at various P_{NO}, on the basis of the published method by Collman et al. [11]. Thermodynamic data were estimated from the temperature dependence of *K* at temperatures ranging from 10 to 30 °C.

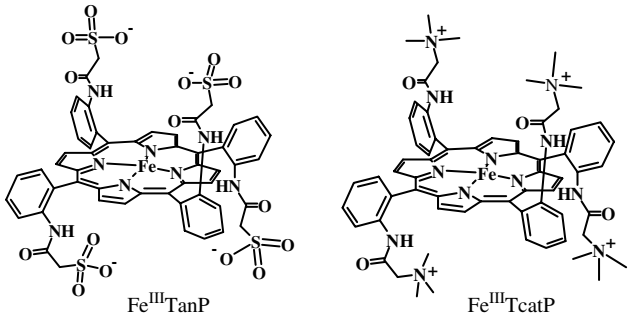


Fig. 1. Water-soluble ferric porphyrins. Axial ligands (H_2O) and counter ions Na^+ for Fe^{III}TanP and Cl^- for Fe^{III}TcatP are omitted for clarity.

2.3. 5α,10β,15α,20β-Tetrakis(2-aminophenyl)porphyrinatoiron(III) chloride (*1*)

5α,10β,15α,20β-Tetrakis(2-aminophenyl)porphyrin (H_2TamPP) [12] (121 mg, 0.179 mmol) was dissolved in tetrahydrofuran (50 ml) containing 2,6-dimethylpyridine (0.5 ml, 2.17 mol). After the solution was purged with N_2 over 30 min, iron(II) chloride (0.300 g, 2.37 mmol) was added and the mixture was stirred at room temperature for 3 h under N_2 atmosphere. The achievement of the metalation was confirmed by disappearance of red fluorescence with a UV lamp (365 nm). After evaporation of the mixture, the solid was dissolved in a small amount of $CHCl_3/CH_3OH$ (50/1), then purified on a dry silica-gel column (ca. 200 mesh, $\varnothing 2.5 \times 8$ cm) and eluted with $CHCl_3/CH_3OH$ (25/1). The eluate was evaporated to dryness and the solid was recrystallized from CH_3OH/ether, yielding 110 mg (86%). *Rf* value = 0.63 ($60F_{254}$; $CHCl_3/CH_3OH = 20/1$). UV–vis [λ_{max} nm in benzene]: 417, 508.

2.4. Sodium 5α,10β,15α,20β-tetrakis(2-(sulfonatoacetamido)phenyl)porphyrinatoiron(III) ($(H_2O)_2Fe^{III}$TanP)

To a solution of isobutyl chlorocarbonate (0.300 ml, 2.31 mmol) and triethylamine (0.600 ml, 4.30 mmol) was added sulfoacetic acid (0.410 g, 2.93 mmol). The mixture was stirred for 30 min at room temperature. To this solution was added a solution of **1** (90.0 mg, 0.126 mmol) in CH_2Cl_2 (70 ml). After the mixture had been stirred at room temperature for 24 h, the solution was evaporated to dryness. The resultant solid was dissolved in $CHCl_3$ (100 ml) and the organic layer was extracted with aqueous NaOH (0.1 $mol\,l^{-1}$, 50 ml). After the aqueous layer was washed with $CHCl_3$ (100 ml × 5), the solution was evaporated to dryness. The solid was dissolved in a small amount of ethanol and filtered off a salt solid, then the filtrate was evaporated. The residual solid

was purified by reversed phase chromatography (Cosmosil 75C_{18}-OPN (Nakarai), $\varnothing 3.5 \times 8$ cm, CH_3OH/H_2O (1/1)). The eluate was evaporated to dryness and the solid was recrystallized from CH_3OH/ether, yielding 120 mg (79%). *Rf* value = 0.37 (PR-8 F_{254}; $CHCl_3/CH_3COOH$/pyridine/CH_3OH = 2/2/1/1). UV–vis [λ_{max} nm in H_2O at pH 3 ($\log \varepsilon$)]: 395 (5.17), 526 (4.17). *Anal.* Calc. for $C_{52}H_{36}N_8O_{16}S_4Na_4Fe \cdot 3CH_3OH \cdot 6H_2O \cdot OH$: C, 43.28; H, 4.03; N, 7.34. Found: C, 43.30; H, 4.33; N, 7.22%. FAB-MS (magic bullet); *m*/*z* calculated most abundant parent mass (*M*): 1304. Observed: 1304 (M^+, 6%), 1305 ($M + H^+$, 7%), 1327 ($M + Na^+$, 9%).

2.5. 5α,10β,15α,20β-tetrakis(2-(*N*,*N*-dimethylaminoacetamido)phenyl)porphyrinatoiron(III) chloride (**2**)

To a suspended mixture of *N*,*N*-dimethylaminoacetic acid hydrogen chloride (1.60 g, 11.5 mmol) in CH_2Cl_2 (50 ml) was added dropwise oxalyl chloride (5.00 ml, 38.7 mmol). After stirring for 2 h at room temperature, the mixture was evaporated to dryness, then the solid was dissolved in CH_2Cl_2 (30 ml). To a cooled solution of **1** (500 mg, 0.659 mmol) in CH_2Cl_2 (150 ml) containing triethylamine (3.00 ml, 21.5 mmol) was added the acid chloride solution. After stirring for 5 h at room temperature, the solution was washed with aqueous NaOH (0.1 mol l^{-1}, 150 ml), dried over anhydrous Na_2SO_4, and evaporated to dryness. The solid was purified on a silica-gel column ($CHCl_3$, $\varnothing 4 \times 30$ cm) and eluted with $CHCl_3/CH_3OH$ (200/1). The eluate was evaporated and recrystallized from CH_3OH/ether, yielding 382 mg (53%). *Rf* value = 0.42 ($60F_{254}$; $CHCl_3/CH_3OH$ = 5/3). UV–vis [λ_{max} nm in CH_3OH]: 413, 507 (sh).

2.6. 5α,10β,15α,20β-tetrakis(2-(*N*,*N*,*N*-trimethylammoniumacetamido)phenyl)porphyrinatoiron(III) chloride ($(H_2O)_2Fe^{III}$TcatP)

To a solution of **2** (210 mg, 0.196 mmol) in *N*,*N*-dimethylformamide (100 ml) was added methyl iodide (0.250 ml, 3.21 mmol). After the solution was stirred for 24 h at room temperature, ether was added to precipitate a solid. The solid was dissolved in a small amount of CH_3OH and the porphyrin iodide was converted to chloride on an ion-exchange resin column (amberlist A-21 chloride form, $\varnothing 3 \times 30$ cm) then eluted with CH_3OH. The eluate was evaporated and recrystallized from CH_3OH/ether, yielding 127 mg (57%). *Rf* value = 0.40 ($60F_{254}$; $CHCl_3/CH_3OH/H_2O$ = 11/7/2). UV–vis [λ_{max} nm in H_2O at pH 3 ($\log \varepsilon$)]: 395 (5.08), 526 (4.02). *Anal.* Calc. for $C_{64}H_{72}N_{12}O_4Cl_5Fe \cdot 6H_2O$: C, 54.34; H, 5.99; N, 11.88. Found: C, 54.30; H, 6.00; N, 11.83%. FAB-MS (magic bullet); *m*/*z* calculated most abundant parent mass (*M*): 1305. Observed: 1235 (M^+–2Cl, 0.6%), 1198 (M^+–3Cl, 1.2%).

3. Results and discussion

3.1. Synthesis of porphyrins

Metal insertion to tetrakis(ortho-substituted-phenyl)porphyrins is usually not easy [13], then often requires a high reaction temperature that leads to atropisomerization. Contrary to this, iron was successfully inserted to H_2TamPP under mild conditions in the present work, in which the absence of isomerization was confirmed by silica-gel TLC. The easy metalation of H_2TamPP could be attributed to a catalytic function of the amino groups that present near the center of the porphyrin core and might act as a metal-ion provider [14].

To construct electrostatically different microenvironments around the central metal ion in ferric porphyrin, anionic sulfonato or cationic ammonium groups were introduced using methods similar to those described in the literature [9,10]. These ionic groups also make the porphyrins water-soluble. Since these porphyrins are the α,β,α,β-atropisomer, the substituents of the porphyrin prevent self-aggregation in aqueous solution and the porphyrins have no isomeric conformations for axial-ligand bound species.

3.2. Acidity of coordinated H_2O

In aqueous solutions, the central iron(III) ion of ferric porphyrins Fe^{III}Por is six-coordinated in which the axial ligands are two H_2O [$(H_2O)_2Fe^{III}$Por] or one H_2O and one OH^- [$(H_2O)(OH)Fe^{III}$Por], depending on pH. The two species are easily distinguishable by their visible spectra. From the spectral changes at various pHs as exemplified in Fig. 2, the pK_a values of the coordinated H_2O for the ferric porphyrins prepared were estimated (Table 1). The pK_a values for simple cationic and anionic water-soluble ferric porphyrins reported thus far have been in the range of 4.1 and 7.0 [15,16]. Interestingly, the pK_a values of 8.0 for $(H_2O)_2Fe^{III}$TanP and of 4.1 for $(H_2O)_2Fe^{III}$TcatP are out of and terminal

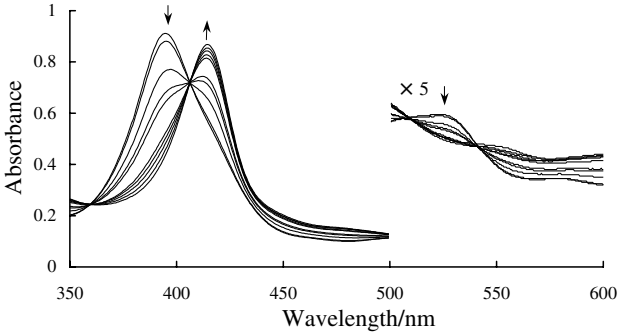


Fig. 2. Visible spectra of $(H_2O)_2Fe^{III}$TanP at various pHs (6.00, 6.75, 7.59, 7.92, 8.00, 8.50, 8.62, 8.80, 9.15, 11.06) at 25 °C.

Table 1
pK_a values of the coordinated H_2O of ferric porphyrins (25 °C)

Compound[a]	Type	pK_a	Reference
$(H_2O)_2Fe^{III}$TanP	Anion	8.0	This work[b]
$(H_2O)_2Fe^{III}$TPPS	Anion	7.0	[c]
$(H_2O)_2Fe^{III}$TCPP	Anion	6.72	[d]
$(H_2O)_2Fe^{III}TCl_2$PPS	Anion	4.1	[e]
$(H_2O)_2Fe^{III}$TMNP	Cation	6.09	[f]
$(H_2O)_2Fe^{III}$TMpyP	Cation	5.79	[f]
$(H_2O)_2Fe^{III}$TcatP	Cation	4.1	This work[b]

[a] The charge of the complexes is omitted. Abbreviations used for coordinated porphyrin dianions: TCPP: 5,10,15,20-tetrakis(4-carboxylatophenyl)porphyrinato; TCl_2PPS: 5,10,15,20-tetrakis((2,6-dichloro-3-sulfonato)phenyl)porphyrinato; TMNP: 5α,10β,15α,20 β-tetrakis(2-(*N*-methylnicotinamido)phenyl)porphyrinato; TmpyP: 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrinato.
[b] In 0.05 mol l^{-1} $NaClO_4$.
[c] A.D. El-Awady, P.C. Wilkins, R.G. Wilkins, Inorg. Chem. 24 (1985) 2053. In 0.1 mol l^{-1} $NaNO_3$.
[d] J.D. Strong, C.R. Hartzell, Bioinorg. Chem. 5 (1976) 219.
[e] S. Jeon, T.C. Bruice, Inorg. Chem. 31 (1992) 4843.
[f] Ref. [15].

for the range, respectively, in spite of the estimate that electronic effects of the ionic groups on the central metals through the many chemical bonds should be negligible. The estimate is also supported by the fact that the visible spectra of these ferric porphyrins are very similar (see Section 2). In natural proteins, variation in pK_a of amino acid residues resulting from differences in polar microenvironments is commonly observed [17]. In $(H_2O)_2Fe^{III}$TanP, predicting from the case of a similar zinc porphyrin, the coordinated H_2O must be stabilized by hydrogen bond(s) with the sulfonato groups of the porphyrin [9b]. This weakens the release of protons from $(H_2O)_2Fe^{III}$TanP, leading to a high pK_a value (Chart 1). Further, the electrostatic repulsion between the coordinated OH^- and the anionic sulfonato groups in $(H_2O)(OH)Fe^{III}$TanP must also increase the pK_a. In contrast to this, the cationic ammonium groups of $(H_2O)_2Fe^{III}$TcatP should stabilize the coordinated OH^- more than they stabilize the H_2O, based on Coulomb interaction as shown in Chart 1; hence, the pK_a value would become substantially small.

3.3. Binding of NO at pH 1.3

To examine NO binding to ferric porphyrins, the experimental conditions were adjusted to be acidic where the dominant species was determined from the estimated pK_a values to be $(H_2O)_2Fe^{III}$Por. Fig. 3 shows the spectral changes of $(H_2O)_2Fe^{III}$TcatP under various NO pressures (P_{NO}) at pH 1.3. The spectral changes were found to be reversible: bubbling of Ar gas through the solution under 708 Torr P_{NO} resulted in re-formation of the initial spectrum of $(H_2O)_2Fe^{III}$TcatP. These spectral changes with clear isosbestic points were similar to those for Fe^{III}TPPS [5] and corresponded to reaction (1). The thermodynamic data for reaction (1) are summarized in Table 2. It is generally accepted that NO-bound ferric porphyrin species can be formulated as linear Fe^{2+}–NO^+. In this case, the NO affinity of $(H_2O)_2Fe^{III}$TcatP is higher than that of $(H_2O)_2Fe^{III}$TanP, due to the increased binding energy ($-\Delta H$). This is in conflict with the prediction, based on simple electrostatic interactions, that anionic groups

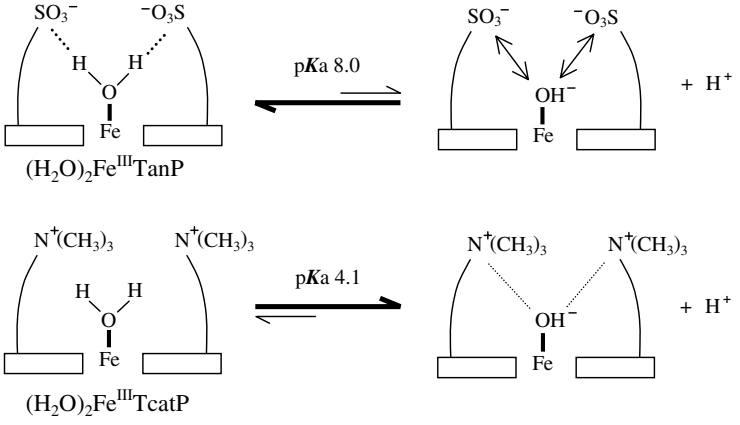


Chart 1.

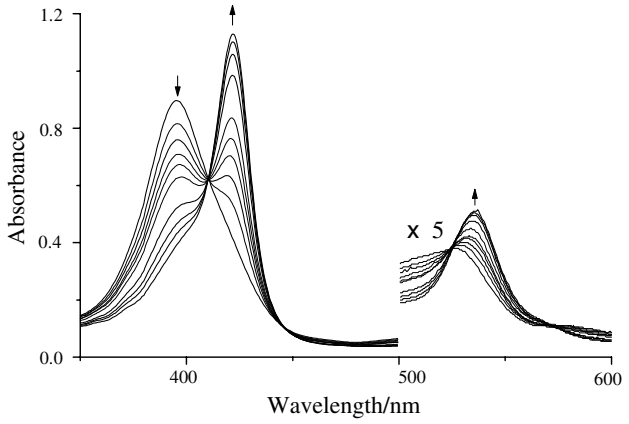


Fig. 3. Visible spectra of $(H_2O)_2Fe^{III}$TcatP under various P_{NO} (0, 15.3, 29.1, 44.8, 59.2, 79.0, 152, 297, 506, 708 Torr) at pH 1.3 (25 °C).

Table 2
Thermodynamic data for NO binding to ferric porphyrins

	K/atm^{-1}[c]	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J mol^{-1} K^{-1}
$(H_2O)_2Fe^{III}$TanP[a]	3.45	-55 ± 1	-175 ± 5
$(H_2O)_2Fe^{III}$TcatP[a]	6.16	-64 ± 1	-200 ± 4
$(H_2O)_2Fe^{III}$TPPS[b]	1.40	-62 ± 2	-206 ± 6

[a] At pH 1.3.
[b] At pH 6.0; Ref. [8].
[c] Calculated at 25 °C from van't Hoff plots.

should stabilize the bound NO^+ and cationic groups destabilize the NO^+. Our earlier study [9] on ligation of amines to anionic zinc porphyrins containing sulfonato groups indicated that upon amine binding, the release of the coordinated H_2O tightly bound by hydrogen bonding decreases the amine affinity with increases in both ΔH and ΔS. NO binding to the ferric porphyrins also accompanies the release of the bound H_2O. Therefore, the release of the tightly bound H_2O from $(H_2O)_2Fe^{III}$TanP provides some additional, positive ΔH and ΔS, which may lead to the decreased NO affinity of $(H_2O)_2Fe^{III}$TanP as compared with that of $(H_2O)_2Fe^{III}$TcatP (Chart 2). This explanation is also supported by a recent report in which NO binding to ferric porphyrins in aqueous solution was found to be accelerated by labilizing the ligated H_2O molecule [18].

3.4. Binding of NO at pH 3.0

The spectral changes of $(H_2O)_2Fe^{III}$TanP under various P_{NO} at pH 3.0 were almost identical to those at pH 1.3. The equilibrium constants were also the same, within the range of experimental errors, at pHs between 1.3 and 3.0. However, as shown in Fig. 4, the spectral changes of $(H_2O)_2Fe^{III}$TcatP at pH 3.0 are quite different from those at pH 1.3. The spectral changes were reversible, though isosbestic points were no longer observed. Although the spectral changes under low P_{NO} may be similar to those at pH 1.3, a new species as the major product is apparently formed at higher P_{NO}. The λ_{max} values of 412 and 539 nm under P_{NO} of 71.1 Torr are similar to those for ferrous NO species, $(NO)Fe^{II}$TPPS. Next, for purposes of comparison, we

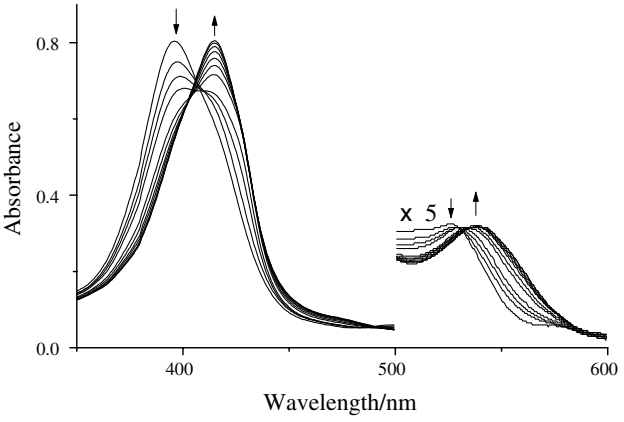


Fig. 4. Visible spectra of $(H_2O)_2Fe^{III}$TcatP under various P_{NO} (0, 6.6, 12.1, 17.3, 29.4, 36.6, 44.2, 51.4, 59.7, 67.2, 71.1 Torr) at pH 3.0 (25 °C).

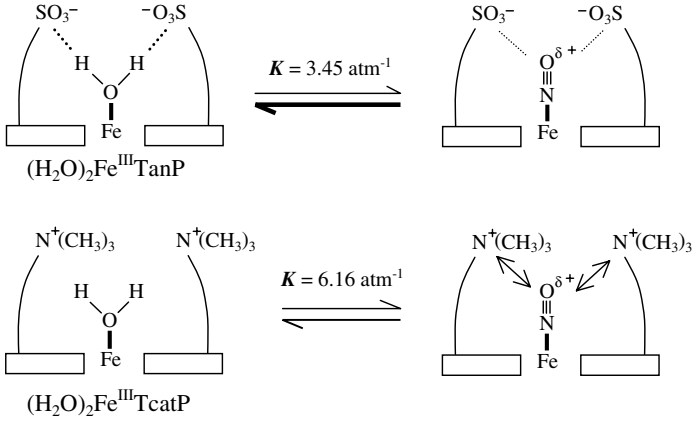


Chart 2.

Table 3
Visible spectral data for iron porphyrins

Compound	pH	λ_{max}/nm	
$(H_2O)_2Fe^{III}TanP$	1.3–3.0	395	526
$(H_2O)_2Fe^{III}TanP + NO$	1.3–3.0	422	535
$(H_2O)_2Fe^{III}TcatP$	1.3–3.0	395	526
$(H_2O)_2Fe^{III}TcatP + NO$	1.3	422	535
$(H_2O)_2Fe^{III}TcatP + NO$	3.0	412	539
$(H_2O)_2Fe^{II}TcatP$[a]	3.0	420	532
$(H_2O)_2Fe^{II}TcatP$[a] + NO	3.0	412	540

[a] Prepared from $(H_2O)_2Fe^{III}TcatP$ with $Na_2S_2O_4$ under Ar.

prepared $(NO)Fe^{II}TcatP$ from NO and $Fe^{II}TcatP$ that had been obtained reductively from $(H_2O)_2Fe^{III}TcatP$ with $Na_2S_2O_4$. The λ_{max} values for the porphyrins are summarized in Table 3. These data suggested that reaction of $(H_2O)_2Fe^{III}TcatP$ with NO at pH 3.0 affords $(NO)Fe^{II}TcatP$ reversibly.

Use of ESR spectroscopy allowed us to distinguish each possible compound of iron porphyrins (i.e., $(H_2O)_2Fe^{III}Por$, $(NO)Fe^{III}Por$, and $(NO)Fe^{II}Por$ species). It is generally accepted that $(NO)Fe^{III}Por$ is diamagnetic than ESR-silent, whereas $(NO)Fe^{II}Por$ shows a characteristic ESR spectrum [6,19]. The ESR spectra of the iron porphyrins were obtained at 77 K for frozen aqueous solutions after treatment with NO or Ar gas at room temperature. For $(H_2O)_2Fe^{III}TanP$, the d^5 high spin signal (not shown) disappeared upon NO binding and no signal was observed, which findings supported the formation of $(NO)Fe^{III}TanP$. In the case of $(H_2O)_2Fe^{III}TcatP$, as expected from visible spectral changes, the ESR spectrum of the porphyrin treated with NO at pH 3.0 was quite different from that of $(H_2O)_2Fe^{III}TanP$. Upon NO bubbling through $(H_2O)_2Fe^{III}TcatP$, the high spin spectrum disappeared and the characteristic spectrum shown in Fig. 5 ($g_1 = 2.10$, $g_2 = 2.01$ ($A = 23$ G), $g_3 = 2.00$) was observed. The spectral feature and the spectral parameters were very similar to those reported for five-coordinated $(NO)Fe^{II}Por$ [19]. Further, purging the NO gas with Ar

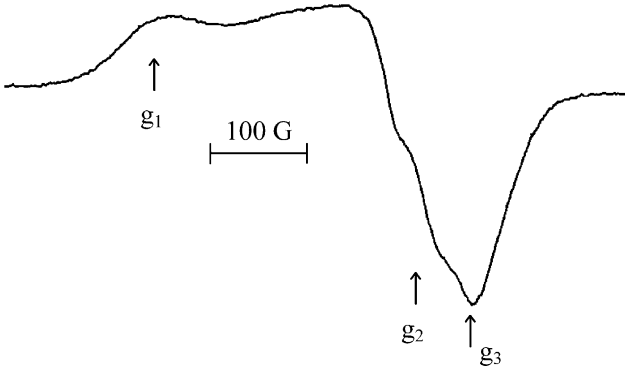


Fig. 5. ESR spectrum of the NO-reacting product from $(H_2O)_2Fe^{III}TcatP$ at 77 K in H_2O; $g_1 = 2.10$, $g_2 = 2.01$ ($A = 23$ G), $g_3 = 2.00$.

re-formed the initial high spin signal, indicating that the reaction with NO is reversible. Therefore, together with the visible spectral changes, the reaction of $(H_2O)_2Fe^{III}TcatP$ with NO at pH 3.0 generates $(NO)Fe^{II}TcatP$ reversibly. Notably, the oxidation state of the central iron reversibly changes depending on P_{NO}. The reactions of $(H_2O)_2Fe^{III}TcatP$ with NO are explained summary as follows:

$$(H_2O)_2Fe^{III}TcatP \overset{+NO}{\underset{-NO}{\rightleftharpoons}} (NO)Fe^{III}TcatP \overset{+NO}{\underset{-NO}{\rightleftharpoons}} (NO)Fe^{II}TcatP + NO^+ \quad (3)$$

Since the Fe(II)–NO bond is formulated as bent Fe^{3+}–NO^-, the positively charged groups near the bound NO should stabilize the $(NO)Fe^{II}Por$, as shown in Chart 3. The formation of $(NO)Fe^{II}Por$ in Eq. (3) is thought to occur through liberation of NO^+ from $(NO)Fe^{III}Por$ to produce labile $Fe^{II}Por$ species that successively react with NO to give $(NO)Fe^{II}Por$, where H_2O acts as the NO^+ acceptor as exemplified by the following equation [20]:

$$NO^+ + H_2O \rightleftharpoons HNO_2 + H^+ \quad (4)$$

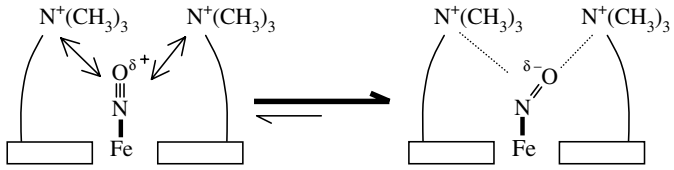
It is obvious that H_2O cannot thermodynamically act as the NO^+ acceptor at a low pH. This is very reasonably correlated to the observation that the reaction of $(H_2O)_2Fe^{III}TcatP$ with excess NO gives the reduced $(NO)Fe^{II}TcatP$ at pH 3.0 but not at pH 1.3. [2] This reduction behavior may also be explained in terms of the pH dependence of the half-cell potential between NO and NO_2^- (or HNO_2, NO^+); at low pH, the potential acts such that the ferric NO species is predominant.

The reason for oxidation from $(NO)Fe^{II}Por$ to $(NO)Fe^{III}Por$ observed for FeTcatP but not for natural hemeproteins is not yet clear. Taking into account that the microenvironments around the central iron in the hemeproteins must be hydrophobic, the oxidation will correlate with the incompletely protected NO of $(NO)Fe^{II}TcatP$ and/or with the acidic aqueous solution examined. One of the plausible routes of oxidation of $(NO)Fe^{II}Por$ is through an attack of H^+ or NO^+, where the latter may be formed in acidic aqueous solution as follows: [3]

$$NO + NO_2 + 2H^+ \rightarrow 2NO^+ + H_2O \quad (5)$$

[2] The pH dependence of NO-binding behavior of iron porphyrins has been reported [7]. For FeTcatP, unfortunately, reaction with NO at higher pH than 4 gave a precipitate that could not be examined spectrophotometrically.

[3] Although we further purified NO gas of high-purity grade, we could not completely exclude the possibility that the NO gas contained NO_2 as an impurity. The importance of a trace amount of NO_2 in NO gas in the observed chemistry of iron porphyrins has been previously pointed out [7,21].



linear form (NO)Fe^{III}TcatP bent form (NO)Fe^{II}TcatP

Chart 3.

4. Concluding remarks

The synthesized ferric porphyrins with negatively or positively charged microenvironments near the central metal provide some unique properties in equilibrium and redox behavior. Both the pK_a values of the coordinated H_2O and the NO affinities of these porphyrins are reasonably explained on the basis of intramolecular interactions. Further, it is likely that the positively charged microenvironments near the central metal stabilize the formed (NO)Fe^{II}Por species. Thus, the present work demonstrates that some change in microenvironment near the active sites of both model and natural compounds has the potential to give specific reactivity for exogenous ligands.

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