Inorg. Chem. **2003**, *42*, 1945–1951

Electrochemical and Spectral Studies on the Reductive Nitrosylation of Water-Soluble Iron Porphyrin

Natalia S. Trofimova,*,† Alexander Y. Safronov‡, and Osamu Ikeda†

Departments of Chemistry, Faculty of Science, Kanazawa University, Kakuma, Kanazawa, Ishikawa, Japan 920-1192, and Irkutsk State University, 1 Marks str., Irkutsk, Russia 664003

Received June 28, 2002

The reaction of iron(III) (*meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (Fe^{III}TMPyP) with nitric oxide (NO) was studied by electronic absorption spectroscopy, ESR, and electrochemical and spectroelectrochemical techniques in aqueous solutions with pH from 2.2 to 12.0. Fe^{III}TMPyP has been found to undergo a reductive nitrosylation in all pHs, and the product of nitric oxide binding to the porphyrin has been determined as iron(II) porphyrin nitrosyl complex ([Fe^{II}(NO)TMPyP]). The rate of the reductive nitrosylation exhibits a tendency to get faster with increase in pH. An intermediate species was observed around neutral pH by spectroelectrochemical technique and was proposed to be the iron(II) nitrosyl complex of the μ-oxo dimeric form of FeTMPyP, which is known to be a predominant in neutral solutions.

Introduction

Nitric oxide interactions with iron are by far the most important biological reactions in which NO participates.[1,2] Activation of guanylate cyclase,[3] inhibition of cytochrome oxidase[4] and catalase,[5] and blood pressure regulation by interactions with hemoglobin[6] are among them. Reaction of NO with oxygenated hemoglobin and myoglobin is known to be the main pathway of NO metabolism in the human body.[1] Because of their high biological importance, interactions of nitric oxide and related species with hemoproteins have been intensively studied by use of either natural or model species.[1b,7−13] Studies on NO binding to low molecular weight model iron complexes demonstrated that some of them could have a significant affinity for nitric oxide comparable with that of hemoproteins.[1,9,12] Binding properties of different hemes toward NO can vary significantly depending on their structure and iron site oxidation state. Ferric forms in aqueous media usually bind nitric oxide reversibly producing iron(III) nitrosyl complexes.[1,10,12] An alternative pathway, known as a reductive nitrosylation, has been described in aqueous solutions for some hemoproteins,[12b] such as hemoglobin,[1b,7,14a] myoglobin,[14] and cytochrome P450.[14b] Such a reductive nitrosylation in synthetic iron(III) porphyrins have been reported to occur only in solutions containing methanol.[15] Thus, reaction of NO with Fe^{III}TPP in mixed toluene/methanol solutions is exploited as Fe^{II}(NO)TPP

* To whom correspondence should be addressed. E-mail: natalia@sgkit.ge.kanazawa-u.ac.jp. Tel.: (81)-90-8261-7021. Fax: (81)-76-264-5988.
† Kanazawa University.
‡ Irkutsk State University.

(1) (a) Cooper, C. E. *Biochim. Biophys. Acta* **1999**, *1411*, 290−309. (b) Ford, P. C.; Lorkovic, I. M. *Chem. Rev.* **2002**, *102*, 993−1018.
(2) (a) Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. *Nature* **1987**, *327*, 524−526. (b) Moncada, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Rev.* **1991**, *43*, 109−142. (c) Butler, A. R.; Williams, D. L. H. *Chem. Soc. Rev.* **1993**, 233−241. (d) Lewis, R. S.; Tamir, S.; Tannebaum, S. R.; Deen, W. M. *J. Biol. Chem.* **1995**, *270*, 29350−29355.
(3) (a) Stone, J. R.; Marletta, M. A. *Biochemistry* **1994**, *33*, 5636−5640. (b) Feldman, P. L.; Griffith, O. W.; Struehr, D. J. *Chem. Eng. News* **1993**, *71* (26), 6−37.
(4) (a) Brown, G. C. *FEBS Lett.* **1995**, *369*, 136−139. (b) Cleeter, M. W. J.; Cooper, J. M.; Darley-Usmar, V. M.; Moncada, S.; Schapira, A. H. V. *FEBS Lett.* **1994**, *345*, 50−54.
(5) Brown, G. C. *Eur. J. Biochem.* **1995**, *232*, 188−191.
(6) (a) Stamler, J. S.; Jia, L.; Eu, J. P.; McMahon, T. J.; Demchenko, I. T.; Bonaventura, J.; Gernert, K.; Piantadosi, C. A. *Science* **1997**, *276*, 2034−2037.
(7) Hoshino, M.; Ozawa, K.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc.* **1993**, *115*, 9568−9575.
(8) Clarke, M. J.; Gaul, J. B. *Struct. Bonding* **1993**, *81*, 147−181.
(9) Kharitonov, V. G.; Sharma, V. S.; Magde, D.; Koesling, D. *Biochemistry* **1997**, *36*, 6814−6818.
(10) Laverman, L. E.; Hoshino, M.; Ford, P. C. *J. Am. Chem. Soc.* **1997**, *119*, 12663−12664.
(11) Rovira, C.; Kunc, K.; Hutter, J.; Ballone, P.; Parrinello, M. *J. Phys. Chem. A* **1997**, *101*, 8914−8925.
(12) (a) Ford, P. C.; Bourassa, J.; Lee, B.; Lorkovic, I. M.; Boggs, S.; Kudo, S.; Laverman, L. E. *Coord. Chem. Rev.* **1998**, *171*, 185−202. (b) Hoshino, M.; Laverman L.; Ford, P. C. *Coord. Chem. Rev.* **1997**, *187*, 75−102.
(13) Laverman, L. E.; Wanat, A.; Oszajca, J.; Stochel, G.; Ford, P. C.; van Eldik, R. *J. Am. Chem. Soc.* **2001**, *123*, 285−293.
(14) (a) Addison, A. W.; Stephanos, J. J. *Biochemistry* **1986**, *25*, 4104−4113. (b) Hoshino, M.; Maeda, M.; Konishi, R.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc.* **1996**, *118*, 5702−5707.
(15) Wayland, B. B.; Olson, L. W. *J. Am. Chem. Soc.* **1974**, *96*, 6037.

10.1021/ic025830n CCC: $25.00
Published on Web 02/19/2003

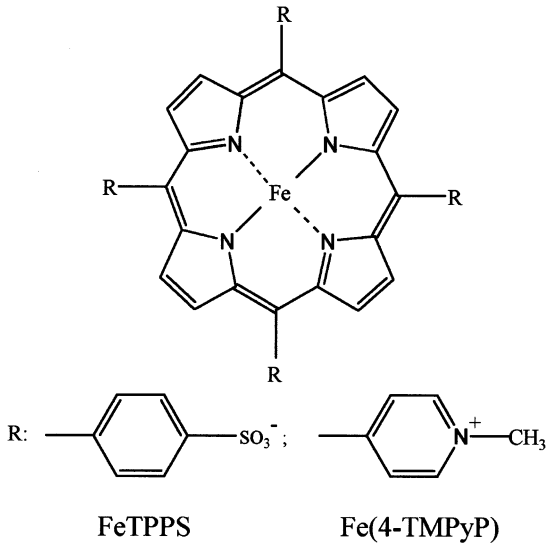


Figure 1. Chemical structures of FeTMPyP and FeTPPS.

preparation method. Studies on NO binding to synthetic water-soluble iron porphyrins have been mostly limited to FeTPPS (Figure 1) and some other sulfonated porphyrins.[7,10] Since it has been reported[7] that FeTPPS does not undergo a reductive nitrosylation in aqueous media, the same behavior seems to be assumed for other water-soluble iron porphyrins. The data obtained in the present research surprisingly demonstrated that the reactivity of iron *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (FeTMPyP, Figure 1), another widely exploited synthetic iron porphyrin, toward nitric oxide was different from that of FeTPPS.

In the present research, formation of porphyrin nitrosyl complexes was followed by electronic absorption spectroscopy and their electrochemical transformations were controlled by means of spectroelectrochemistry. The oxidation state of iron in nitrosyl complexes was clarified by ESR measurements.

Experimental Section

Fe^{III}TMPyP was prepared by the method of Pasternack et al.[16] starting from free base *meso*-tetrakis(*N*-methylpyridinium-4-yl)-porphyrin (H_2(4-TMPyP), Aldrich). Phosphate buffer solution (PBS, 0.05 M) with pH 7.4 and 8.6 were prepared by mixing 0.05 M solutions of analytical grade NaH_2PO_4 and Na_2HPO_4 (Nacalai Tesque). H_3PO_4 (Wako) was added to NaH_2PO_4 solutions to obtain pH 2.2 and 4.0, while pH 12.0 was obtained by adding NaOH (Nacalai Tesque) to Na_2HPO_4 solutions. The pH was adjusted by using digital pH-meter (TOA, HM-30V). All aqueous solutions were prepared from twice distilled water. Methanol (Dojindo) was also used for some absorption spectral measurements. All solutions were deaerated before each measurement by bubbling ultrapure argon gas (99.9999% minimum, Nippon Sanso).

Solutions saturated with nitric oxide were prepared by bubbling either pure 100% nitric oxide or 5% NO in an argon gas mixture (Nippon Sanso) into solutions settled in gastight electrochemical or spectral cells before each measurement. Both 100% and 5% NO gas samples were purified from possible traces of high-valent nitrogen oxides and dioxygen by passing through three successive vials containing a 10% solution of potassium hydroxide, an alkaline solution of pyrogalol, and pure water finally. All solutions were deaerated first by bubbling ultrapure argon gas. Molar concentrations of NO in the resulting solutions were estimated from the reported solubility of about 2 mM for nitric oxide in pH 7.4 PBS[17] and Ostwald's solubility coefficient for a given pressure of NO. As the result, NO concentrations in the solutions were estimated to be 2 mM for pure nitric oxide and 0.1 mM for 5% NO gas mixture, respectively.

Table 1. Electronic Absorption Spectral Data for Iron Porphyrins and Their Nitrosyl Complexes[a]

porphyrin complexes	λ_{max}, nm
Fe^{III}TPPS[7,10]	393, 528
Fe^{III}(NO)TPPS[7,10]	420, 533
Fe^{II}(NO)TPPS[20]	412, 542
Hb^{III}(NO)[7]	417, 532, 563
Hb^{II}(NO)[7,14]	417, 542, 570
Fe^{III}TMPyP	423, 595, 630
Fe^{II}TMPyP	441, 562
Fe^{II}(NO)TMPyP	423, 552;[b] 423, 552;[c] 424, 552[d]

[a] Hb = hemoglobin. [b–d] Since the final spectral data obtained by bubbling NO into Fe^{III}TMPyP(aq),[b] Fe^{II}TMPyP(aq),[c] and Fe^{III}TMPyP[d] in methanol were almost the same, all the products were assigned to Fe^{II}(NO)TMPyP.

Electrochemical data were collected by a BioAnalytical Systems (BAS) model BAS 100B electrochemical workstation. Cyclic voltammetry (CV) experiments were carried out in a one-compartment three-electrode cell protected against air penetration and equipped with a glassy carbon (GC) disk (0.071 cm^2) working electrode, an Ag|AgCl (3 M NaCl) reference (all potential values are given against this electrode), and a Pt-plate counter electrode. The GC electrode was polished by alumina powder (0.06 μm), thoroughly washed, and then ultrasonicated in twice distilled water for 5 min before each series of measurements. The working electrode was then electrochemically preconditioned in PBS by CV in a potential range from −0.5 to 1.2 V until a stable voltammetric response has been observed. In the experiments on bulk electrolysis, a Pt-mesh working electrode with large surface area was used instead of the GC electrode.

Electronic absorption spectra were obtained by Otsuka MCPD-1000 spectrophotometer in a spectroelectrochemical cell with 0.4 mm light path length. The cell was equipped with a Pt-mesh working electrode, an Ag|AgCl (3 M NaCl) reference, and a Pt-plate counter electrode. Both CV with a slow scan rate of 0.5 mV s^{-1} and potentiostatic electrolysis were carried out in the above cell by using a Yanaco P-1100 polarographic analyzer.

ESR spectral measurements were carried out at JEOL JES-ER1X ESR-spectrometer under 77 K.

Results

Electronic Absorption Spectral Studies in Neutral Solutions. The electronic absorption spectrum of Fe^{III}TMPyP in aqueous solutions has a Soret band at 423 nm and two Q-bands at 595 and 630 nm (Table 1).[18] During 5% NO gas bubbling into a 0.1 mM solution of iron(III) porphyrin for 2.5 h, irreversible spectral changes (Figure 2) were observed such as a new Q-band appearance at 552 nm instead of those at 595 and 630 nm and an increase in the absorbance at the Soret band without a significant shift in λ_{max}.

(16) Pasternack, R. F.; Lee, H.; Malek, P.; Spencer, C. *J. Inorg. Nucl. Chem.* **1997**, *39*, 1865.
(17) (a) Lantoine, F.; Trevin, S.; Devynck, J. *J. Electroanal. Chem.* **1995**, *392*, 85. (b) Mori, V.; Bertotti, M. *Analyst* **2000**, *125*, 1629−1632.
(18) Chen, J.; Ikeda, O.; Hatasa, T.; Katajima, A.; Miyake, M.; Yamatodani, A. *Electrochem. Commun.* **1999**, *1*, 274−277.

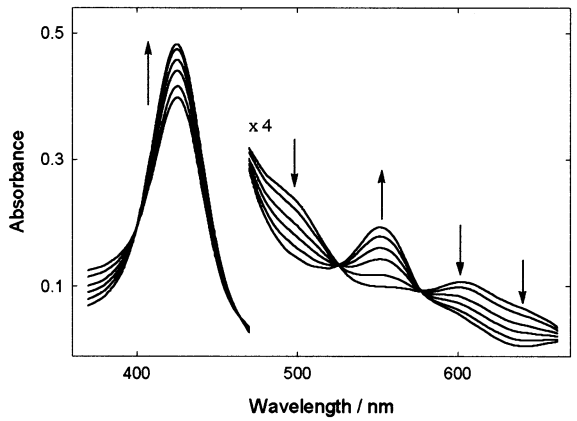


Figure 2. Electronic absorption spectral changes obtained during 5% NO bubbling into 0.1 mM Fe^{III}TMPyP in pH 7.4 PBS solution for 2.5 h (every 0.5 h).

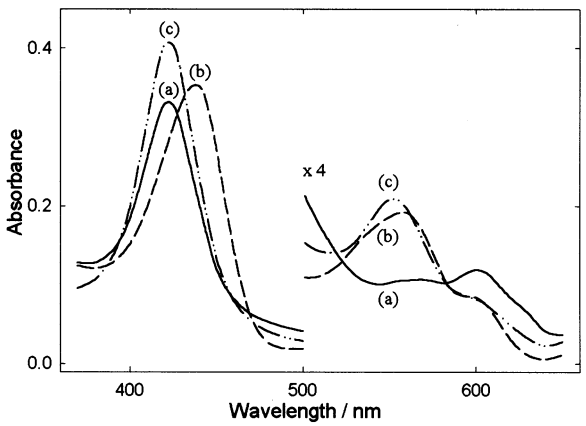


Figure 3. Electronic absorption spectra of 0.1 mM Fe^{III}TMPyP in pH 7.4 PBS: (a) before and (b) after electrolysis at −0.4 V; (c) after 5% NO bubbling into electrolyzed solution.

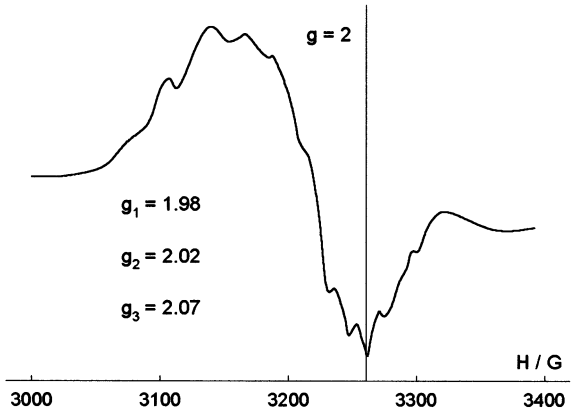


Figure 4. ESR spectrum of Fe^{III}TMPyP/NO adduct in pH 7.4 PBS at 77 K.

Argon gas was then bubbled into resulting solution to remove NO. No spectral change was detected during the bubbling. This shows the porphyrin/NO adduct was stable even in the absence of dissolved nitric oxide. Therefore, spectral changes shown in Figure 2 could hardly be assigned to the formation of iron(III) porphyrin nitrosyl complex, because they conflict with those observed for ferrihemoproteins and highly negatively charged water-soluble porphyrin, namely FeTPPS (Table 1): (a) red shift in Soret band;[7] (b) reversible character of changes.[7,10,12] According to our spectral data, Fe^{III}TMPyP seems to react with NO in different way, and we suppose a reductive nitrosylation as a probable pathway (eq 1).[19] A similar reaction has been described for NO binding to some hemoproteins (hemoglobin for instance).[7,14]

$$[Fe^{III}TMPyP]^{5+} + 2NO + H_2O \rightarrow [Fe^{II}(NO)TMPyP]^{4+} + NO_2^- + 2H^+ \quad (1)$$

To confirm an oxidation state of iron in porphyrin/NO adduct, [Fe^{II}(NO)TMPyP] has been prepared by an introduction of NO gas into an aqueous solution of Fe^{II}TMPyP, which was generated by bulk electrolysis of iron(III) porphyrin at −0.4 V (this potential has been chosen on the basis of CV studies). Absorption spectra were measured during the electrolysis and after introduction of NO gas (Figure 3). The spectrum of Fe^{II}TMPyP exhibits λ_{max} at 441 and 562 nm. These λ_{max} values have been blue-shifted to 423 and 552 nm, respectively, when NO gas was introduced into the solution. Such a blue shift at the Soret band is known to be characteristic of nitric oxide binding to iron(II) porphyrins.[7] Therefore, this result suggests the formation of [Fe^{II}(NO)-TMPyP].

As the reductive nitrosylation of iron porphyrins is known to occur in solutions containing alcohols,[15] nitrosylation of Fe^{III}TMPyP has been performed in methanolic solution and the spectrum of the product was compared with that in aqueous solution. The product, which is believed to be iron(II) nitrosyl complex, showed a spectrum with λ_{max} at 424 and 552 nm, being very similar to those obtained for NO bubbling into both Fe^{III}TMPyP and Fe^{II}TMPyP solutions (Table 1). This proves that the product is the same in all three cases and should be [Fe^{II}(NO)TMPyP].

ESR Studies. The reductive pathway in nitrosylation has been verified by ESR measurements. Nitric oxide is widely exploited as a probe of the structure of hemes.[21] Ferrohemoproteins react with NO and give paramagnetic complexes. The diamagnetic [Fe^{III}(NO)(TMPyP)] complex would give no ESR signal. Therefore, ESR spectroscopy can be utilized as another method to confirm the formation of [Fe^{II}(NO)-(TMPyP)] in a solution.

The fine structure and g values of the ESR spectrum obtained at 77 K for the Fe(II) porphyrin−NO adduct (Figure 4), which was prepared using Fe(III) porphyrin as starting material, are in good agreement with those reported on NO adducts of different synthetic and natural Fe(II) porphyrins and hemoproteins.[14a,21] In fact, the ESR spectrum of the

(19) Here and in subsequent reactions, the structures of the porphyrin species including their charges have been estimated without taking into account axial ligation of the iron cation by water or/and hydroxo anion if not specified. For more details, see ref 22.

(20) Laverman, L. E.; Ford, P. C. *Chem. Commun.* **1999**, 1843−1844.
(21) (a) Palmer, G. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 4, Chapter 6. (b) Migita, C. T.; Salerno, J. C.; Masters, B. S. S.; Martasek, P.; McMillan, K.; Ikeda-Saito, M. *Biochemistry* **1997**, *36*, 10987−10992.

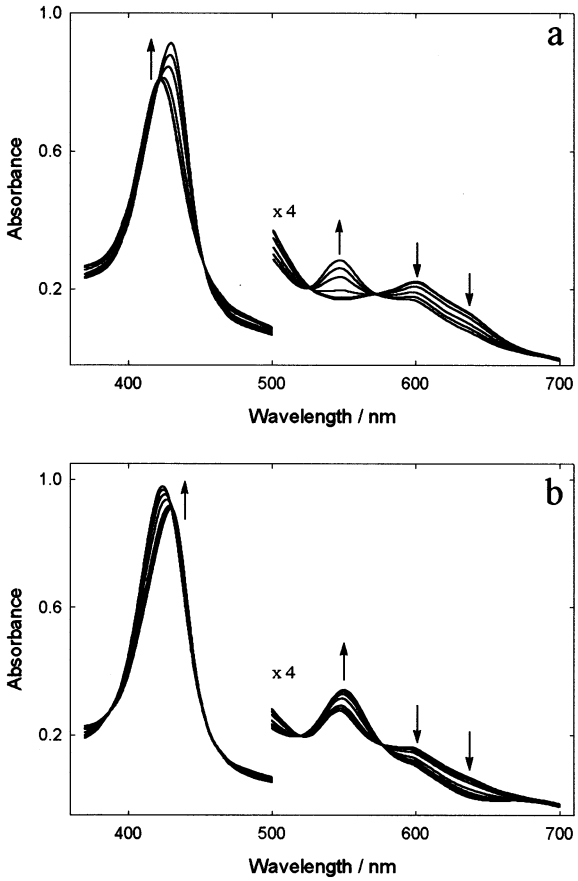
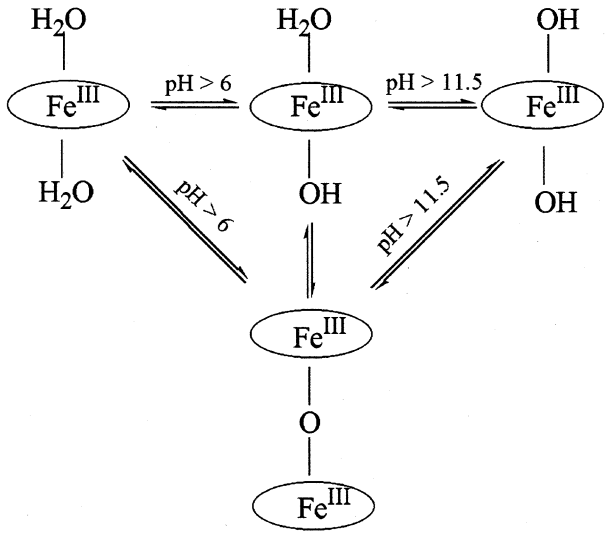


Figure 5. Electronic absorption spectral changes obtained during 100% NO bubbling into 0.2 mM Fe^{III}TMPyP at pH 7.4 for the following times (min): (a) 1, 3, 5, 7, 9, 11; (b) 11, 15, 20, 30, 40, 50, 60 min.

product of reductive nitrosylation of hemoglobin[14a] was very similar to that in Figure 4. Although both Fe^{III}(TMPyP) and NO are paramagnetic species, measurements of their ESR spectra confirmed that neither could be responsible for the spectrum in Figure 4. A similar spectrum was obtained for [Fe^{II}(NO)(TMPyP)], which was prepared through the electrolysis of Fe^{III}TMPyP and then introduction of NO.

Reductive Nitrosylation at High Concentration of NO. All the data described in previous sections have been obtained by using a 5% NO gas mixture and at concentrations of dissolved nitric oxide comparable to that of porphyrin (0.1 mM). Consequently, to elevate the rate of the reductive nitrosylation, more concentrated nitric oxide solutions were prepared by using 100% NO gas, and Fe^{III}TMPyP concentration was increased until 0.2 mM. The contact of the pH 7.4 solution containing 0.2 mM Fe^{III}TMPyP with 100% NO gas led to spectral changes which were different from those in the case of 5% NO gas. Two distinct processes were observed during 100% NO bubbling into solution of the porphyrin. The first 10 min was accompanied by a red shift in Soret band from 423 to 431 nm (Figure 5a). This might indicate the formation of iron(III) porphyrin nitrosyl complex by analogy with Fe^{III}TPPS and some hemoproteins.[7] In the Q region, bands at 595 and 630 nm were displaced by one at 545 nm. Further incubation (up to 1 h) of the solution in the presence of NO resulted in a shift in Soret band to 423 nm and in Q-band to 552 nm (Figure 5b). The final spectrum

Scheme 1. Structure of Fe^{III}TMPyP Species in Solutions with Different pH Values



was identical to that assigned previously to [Fe^{II}(NO)-TMPyP].

Effect of Solution pH on the Reductive Nitrosylation. As can be seen from eq 1, reductive nitrosylation is a pH dependent reaction. It has been reported that generally high pH values favor the process.[14b,12b] Thus, Cyt *c* and myoglobin do not undergo the reductive nitrosylation at pH < 6.5. However, hemoglobin forms a Hb^{II}−NO complex even at lower pH. The range of solution pH where natural hemoproteins can be studied is limited by protein stability. Some conformational changes in a protein structure that occur at extremely low or high pH (or even loss of original structure) may affect the behavior of the heme site and the reductive nitrosylation process as the result. Synthetic iron porphyrins may help to solve the ambiguity, because their pH behavior has been understood rather well.[22]

Depending on the solution pH and porphyrin concentration, Fe^{III}TMPyP can be represented by four different structures: a μ-oxo dimer and three monomeric forms (Scheme 1).[22c] The reactivity of monomeric and dimeric porphyrin species toward nitric oxide seems to be different. A series of Fe^{III}TMPyP solutions with different pH values were prepared to get a certain predominant form of Fe^{III}TMPyP in the solution (Table 2).

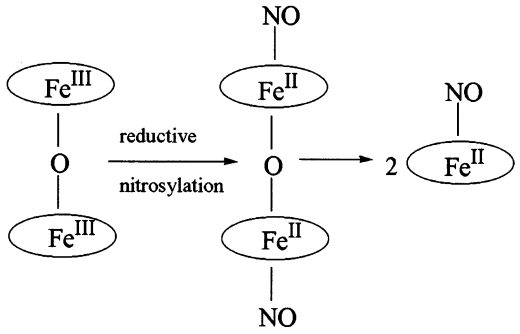
Bubbling 100% NO led to Fe^{II}(NO)TMPyP formation at all examined pH values. The time required for reductive nitrosylation to completion is summarized in Table 2. It is clearly seen that monomeric species produce Fe^{II}(NO)TMPyP more readily than μ-oxo dimeric species. Furthermore, formation of an intermediate was observed only at a pH where dimeric porphyrin species exist. The nature of the

(22) (a) Blom, N.; Odo, J.; Nakamoto, K.; Strommen, D. P. *J. Phys. Chem.* **1986**, *90*, 2847−2852. (b) Miskelly, G. M.; Webley, W. S.; Clark, C. R.; Buckingham, D. A. *Inorg. Chem.* **1988**, *27*, 3773−3781. (c) Bell, S. E. J.; Hester, R. E.; Hill, J. N.; Shawcross, D. R.; Lindsay Smith, J. R. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 4017−4023. (d) Ivanca, M. A.; Lappin, A. G.; Scheidt, W. R. *Inorg. Chem.* **1991**, *30*, 711−718. (e) Bell, S. E. J.; Cooke, P. R.; Inchley, P.; Leanord, D. R.; Lindsay Smith, J. R.; Robbins, A. *J. Chem. Soc., Perkin Trans. 2* **1991**, 549−559.

Table 2. Characteristics of the Reductive Nitrosylation Achieved by 100% NO Bubbling into 0.2 mM Fe^{III}TMPyP Solution with Different pH Values

pH	form of Fe^{III}TMPyP	time required to complete reductive nitrosylation	intermediate
2.2	diaqua monomer	10 min	not obsd
4.0	diaqua monomer	6 min	not obsd
7.4	μ-oxo dimer	8 min (intermediate was obsd) + about 1 h (complete reacn)	obsd
8.6	μ-oxo dimer	8 min (intermediate was obsd) + about 1 h (complete reacn)	obsd
12.0	dihydroxy monomer	<2 min	not obsd

Scheme 2. Role of μ-Oxo Dimeric Species in Reductive Nitrosylation



intermediate species is not obvious. Reductive nitrosylation of some hemoproteins has been reported to proceed through a three-step mechanism,[14b,12b] including the formation of iron(III) nitrosyl complex followed by nucleophilic attack by water (or hydroxide) and nitrosylation by another NO molecule finally. Considering the same scheme for Fe^{III}TMPyP (eqs 2−4), two possible intermediates are expected: iron(III) nitrosyl complex or/and iron(II) porphyrin.

$$[Fe^{III}TMPyP]^{5+} + NO \leftrightarrow [Fe^{III}(NO)TMPyP]^{5+} \quad (2)$$

$$[Fe^{III}(NO)TMPyP]^{5+} + H_2O \text{ (or } HO^-) \rightarrow [Fe^{II}TMPyP]^{4+} + NO_2^- + 2H^+ \text{ (or } H^+) \quad (3)$$

$$[Fe^{II}TMPyP]^{4+} + NO \rightarrow [Fe^{II}(NO)TMPyP]^{4+} \quad (4)$$

However, the latter spectrum (Figure 2b, Table 1) was different from that of the observed intermediate. [Fe^{III}(NO)-TMPyP], on the other hand, is expected to live longer in acidic pH rather than in neutral and especially in basic, according to eq 3. No intermediate was observed in acidic media, however. This allows us to propose a crucial role of μ-oxo dimeric form in reductive nitrosylation of Fe^{III}TMPyP around neutral pH. We suppose reductive nitrosylation take place at one or both iron cations of the dimer before breaking the oxygen bridge (Scheme 2, considering nitrosylation taking place at both iron sites).

From this point, time entries at pH 7.4 and 8.6 in Table 2 mean that reductive nitrosylation completes in about 8 min and splitting of the oxygen bridge took place gradually. If so, the rate of the reductive nitrosylation exhibits a tendency to get faster with increasing in pH in a good agreement with eqs 2−4. The precise mechanism of the reductive nitrosylation could be more complicated, however, because the axial ligation of the iron site by water or hydroxo anion (Scheme 1) is not taken into account in these equations. It has been reported that the formation of iron(III) nitrosyl complex of FeTPPS occurs through dissociation of the sixth ligand, namely water.[10] However, it is not so easy to accept ligand exchange before NO coordination in highly alkaline solutions, because electron-withdrawing peripheral groups in FeTMPyP can stabilize OH^- coordination to Fe^{III} site. In this sense, we cannot neglect the possibility of indirect reductive nitrosylation via ligand, namely a sort of synchronous electron transfer from NO to Fe(III) site through OH^- or/and water. This scheme could explain why we did not observe formation of [Fe^{III}(NO)TMPyP] as an intermediate species in reductive nitrosylation.

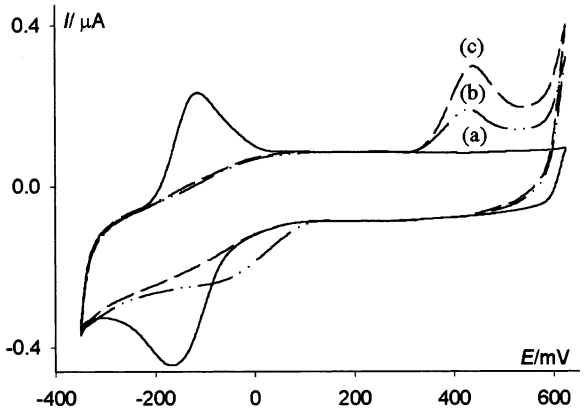


Figure 6. Cyclic voltammograms of 0.1 mM Fe^{III}TMPyP: (a) before and (b) after 5% NO bubbling; (c) [Fe^{II}(NO)TMPyP] prepared by bubbling 5% NO into Fe^{II}TMPyP solution obtained by electrochemical reduction. Scan rate: 10 mV s^{-1}. pH = 7.4.

Electrochemical and Spectroelectrochemical Studies. CV studies on the FeTMPyP/NO system showed that the electrochemical behavior of the porphyrin in the presence of nitric oxide is completely different from that of pure FeTMPyP (Figure 6). Fe^{III}TMPyP shows a reversible electrochemical behavior corresponding to the Fe^{III}/Fe^{II} redox couple. The formal potential was −0.15 V in pH 7.4 solution. Broadening and a positive shift of the reduction peak for iron(III) porphyrin, as well as disappearance of an oxidation peak around −0.15 V on the anodic scan, were observed in the solution containing NO (0.1 mM). It is well-known that nitric oxide has very high affinity toward iron(II) porphyrins comparing with their iron(III) forms.[20,23] Therefore, the positive shift of the reduction peak could be explained by the presence of NO as a ligand with high affinity to the reduced state of iron. Irreversibility of the redox of Fe^{III}TMPyP comes from the complexation of nitric oxide to iron(II) porphyrin formed during cathodic scan.

On the other hand, a new oxidation peak appeared around +0.45 V on the second and following scans. This seems to

(23) (a) Barley, M. H.; Takeuchi, K. J.; Meyer, T. J. *J. Am. Chem. Soc.* **1986**, *108*, 5876−5885. (b) Barley, M. H.; Rhodes, M. R.; Meyer, T. J. *Inorg. Chem.* **1987**, *26*, 1746−1750. (c) Fan, C.; Chen, X.; Li, G.; Zhu, J.; Zhu, D.; Scheer, H. *Phys. Chem. Chem. Phys.* **2000**, *2*, 4409−4413.

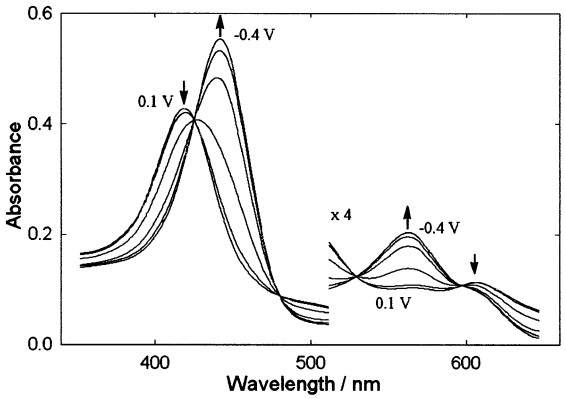


Figure 7. Electronic absorption spectral changes obtained in 0.1 mM Fe^{III}TMPyP solution with pH 7.4 at 0.5 mV s^{-1} CV in a potential range from 0.1 to −0.4 V (every 0.1 V).

be oxidation of [Fe^{II}(NO)TMPyP] formed at the cathodic scan. If the negative limit for CV does not reach the potential of iron(III) reduction, no peak was observed in that potential region, because the formation of Fe^{II}TMPyP and, as a consequence, formation of [Fe^{II}(NO)TMPyP] become impossible. The same peak was observed in a solution of [Fe^{II}(NO)TMPyP] generated by the reductive nitrosylation or prepared from an electrochemically reduced porphyrin. Therefore, the reaction that takes place at +0.45 V is assigned to electrooxidation of [Fe^{II}(NO)TMPyP].

To confirm reactions corresponding to each peak, spectroelectrochemical experiments were carried out in thin layer cell with Pt mesh as the test electrode. A solution containing 0.1 mM Fe^{III}TMPyP was introduced into the spectroelectrochemical cell, and then cyclic voltammetry at a slow scan rate (0.5 mV s^{-1}) was applied to a potential range between 0.1 and −0.4 V. Reversible spectral changes corresponding to the Fe^{III}/Fe^{II} redox couple were observed (Figure 7), which corresponded well with CV data in Figure 6. When the solution containing 0.1 mM Fe^{III}TMPyP and 0.1 mM NO (5% NO bubbling for 15 min) was examined under the same conditions, spectral changes indicating the formation of new species were observed from about 0 V and followed by their transformation into [Fe^{II}(NO)TMPyP]. The CV time scale was, however, too short to complete spectral changes within a single negative scan despite the lowest possible scan rate. After the reaction was completed, the spectrum was stable during further cycling, indicating that [Fe^{II}(NO)TMPyP] was stable in the studied potential range and was not electrooxidized at an anodic scan. The CV mode was then replaced by potentiostatic electrolysis. When a potential of −0.1 V was applied to the spectroelectrochemical cell containing both NO and Fe^{III}TMPyP, spectral changes indicating an accumulation of the same species as an intermediate in the reductive nitrosylation were observed during first 15 min (Figure 8a). The transient spectrum continuously changed until that of [Fe^{II}(NO)TMPyP] has been obtained (Figure 8b). The observed phenomenon could be explained by the formation of dimeric porphyrin nitrosyl complexes as an intermediate (Scheme 3).

When the potential was scanned to positive in the solution containing [Fe^{II}(NO)TMPyP], an electrochemical reaction

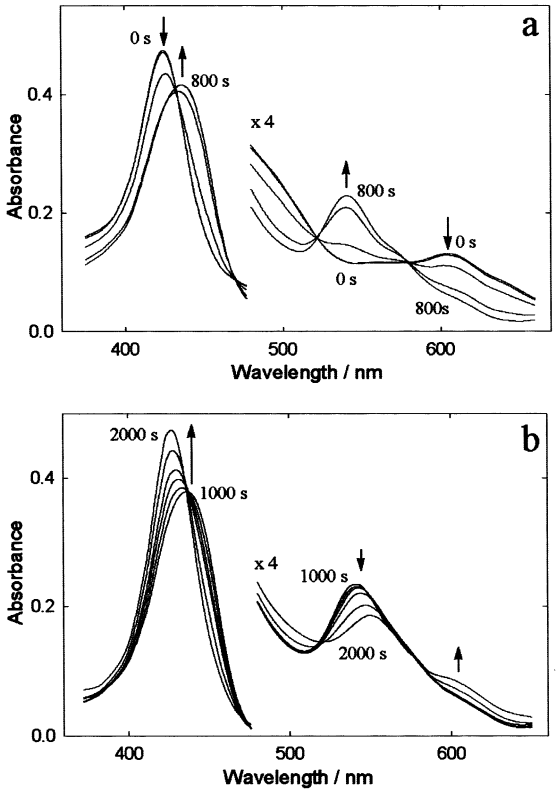
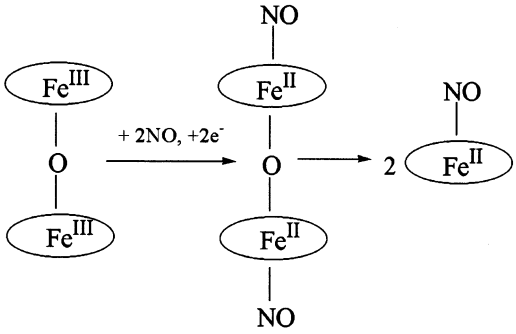


Figure 8. Electronic absorption spectral changes obtained in 0.1 mM Fe^{III}TMPyP solution with pH 7.4 containing 0.1 mM NO during electrolysis at −0.1 V for the following times (s): (a) 0, 200, 400, 600, 800; (b) 1000, 1200, 1400, 1600, 1800, 2000.

Scheme 3. Electroreduction of μ-Oxo Dimeric Species in the Presence of Nitric Oxide



was detected in the potential range between 0.35 and 0.50 V. Spectral change in Figure 9 indicated that the oxidized product was assigned to NO-free iron(III) porphyrin. However, [Fe^{III}(NO)TMPyP] is considered to be the first product but readily releases NO just after the formation (eq 5).

$$[Fe^{II}(NO)TMPyP]^{4+} - e^- \rightarrow \{[Fe^{III}(NO)TMPyP]^{5+}\} \rightarrow Fe^{III}TMPyP^{5+} + NO \quad (5)$$

Discussion

The present research demonstrated that Fe^{III}TMPyP undergoes reductive nitrosylation in aqueous solutions in a wide pH range. From this point of view, the behavior of Fe^{III}TMPyP is somewhat similar to that of natural hemoproteins.[7,12b,14] On the other hand, it was different from that of iron *meso*-tetrakis(4-sulfonatophenyl)porphyrin (FeTPPS)—

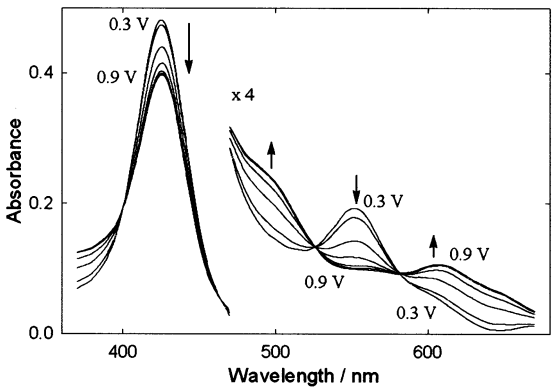


Figure 9. Electronic absorption spectral changes obtained in pH 7.4 PBS containing [Fe^{II}(NO)TMPyP] by applying 0.5 mV s^{-1} CV in potential range from 0.3 to 0.9 V (every 0.1 V).

a synthetic water-soluble porphyrin often used in model studies—which just binds NO reversibly without reductive nitrosylation.[7,10] Two different results demonstrate that binding properties of nitric oxide toward iron porphyrins are strongly dependent on the charges of porphyrin peripheries even when rather simple porphyrins are used. Therefore, the result obtained for a certain porphyrin should be used very carefully when applied to another one to not make hasty conclusions. This could be especially misleading when simplified model porphyrins are exploited instead of natural species.

Charged side groups in the porphyrin structure seem to have a prevalent effect on the nitrosylation reaction. Before demonstration of the possible nature of this effect, it should be noted that expressing the NO ligand in nitrosyl complexes as an uncharged group is done in a formal sense. As has been reported, electron transfer between the iron cation and nitrosyl group is strongly pronounced in iron porphyrin nitrosyl complexes.[1,23] Thus, iron(III) nitrosyl porphyrins could be described by a $Fe^{II}(NO^{+})$P structure[1,23] (P denotes porphyrin), while iron(II) forms have a $Fe^{III}(NO^{-})$P structure to some extent.[23] In a comparison of the nitrosyl complexes of FeTMPyP and FeTPPS, it is expected that positively charged peripheries of the former stabilize the nitroxyl anion and iron(II) nitrosyl complex, consequently, but negative charges on FeTPPS have an opposite effect. This is one of the probable reasons reductive nitrosylation was not observed in the case of FeTPPS.[7,10]

On the other hand, electrophilic properties of nitric oxide coordinated to iron(III) porphyrin should play an important role in reductive nitrosylation, as could be seen from eq 3. Quarternized pyridyl moieties with high electron-withdrawing ability make nitrosonium ion in $[Fe^{II}(NO^{+})TMPyP]^{5+}$ extremely electrophilic and reaction with nucleophiles such as water or hydroxyl anion easy. Negatively charged sulfonato groups in $[Fe^{II}(NO^{+})TPPS]^{3-}$, in contrast, might protect nitrosyl from nucleophilic attack and reductive nitrosylation, therefore. In fact, our recent result on nitric oxide reaction with Fe^{III}TPPS showed that Fe^{III}TPPS also undergoes a reductive nitrosylation in aqueous solutions. Formation of iron(III) nitrosyl complex was detected during the first several minutes of 100% NO bubbling into solution containing Fe^{III}TPPS, and then slow (about 1.5 h) transformation of the spectrum into that of Fe^{II}(NO)TPPS occurs. This result clearly illustrates the stabilizing effect of the peripheral negative charge on the iron(III) nitrosyl complex of FeTPPS, compared with the positive charge on $[Fe^{III}(NO)$-TMPyP$]^{5+}$. The stability of iron(II) nitrosyls shows an opposite tendency for two porphyrins. Fe^{II}(NO)TMPyP was stable against argon bubbling while Fe^{II}(NO)TPPS formed NO-free Fe^{III}TPPS. The possible explanation of the observed effect is given above.

In a summary of our results, an assumption could be done that reductive nitrosylation is a general feature for natural and synthetic iron(III) porphyrins in aqueous solutions. The ability of a certain porphyrin to react in this way, however, depends on its structure, namely the electronic properties of the porphyrin side groups. Furthermore, formation of dimeric intermediate species, which we assume during reductive nitrosylation of FeTMPyP around neutral pH, could be an interesting illustration of the effect of the axial ligand if we imagine one of the oxo-bridged porphyrin moieties as a kind of ligand to another one moiety.

Acknowledgment. The authors thank Professor T. Sakurai for his helpful assistance with ESR measurements.

IC025830N