Ligand-Promoted Rapid Nitric Oxide Dissociation from Ferrous Porphyrin Nitrosyls†

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Ferrous complexes of both natural and synthetic porphyrins generally have extremely high affinities for nitric oxide.[1] For example, in nitrosyl hemoglobin (HbNO), the most intensively studied system, the equilibrium constant for nitric oxide binding to deoxyhemoglobin is estimated by partition experiments with carbon monoxide to be at least 2×10^{11} M^{-1}, some 1500-fold greater than that for carbon monoxide binding to deoxyhemoglobin.[2,3] These high affinities are in part due to the extremely rapid rates of geminate recombination, *cf.* $k_{-2} = 1.1(1) \times 10^{11}$ M^{-1} s^{-1} for elephant myoglobin and $k_{-2} = 1.6 \times 10^{10}$ M^{-1} s^{-1} for Fe(TPP)(pyridine),[4] with the net result being a very low rate of nitric oxide dissociation. Among the factors which can potentially modulate nitric oxide dissociation kinetics from heme proteins are iron oxidation state,[5,6] distal pocket geometry, proximal ligand conformation and identity,[5,7,8] and porphyrin conformation.[9] Moreover, a recent report describing the reversible binding of nitric oxide to the ferrous form of a heme enzyme from *Bacillus halodenitrificans* suggests that a variety of as yet unrecognized factors may influence ligand binding.[10] In connection with our studies of the fundamental chemistry of heme NO adducts, we have recently discovered a class of ferrous nitrosyl complexes with synthetic tetraarylporphyrins, **1a−f**, where ligand-promoted denitrosylation is extremely facile at room temperature. Herein we describe (1) the synthesis of a series of ferrous nitrosyls with substituted tetraarylporphyrins and their characterization by ESR, electrochemistry, elemental analysis, and IR; (2) the structure of one of these, Fe(OBTPP)(NO), (**1f**), which contains a typical Fe(NO) fragment in a severely distorted porphyrin; and (3) the spectrophotometric titration and facile dissociation kinetics of nitric oxide from some of these complexes in the presence of pyridine and *N*-methylimidazoles. Together these results suggest that a variety of factors can lead to a dramatic variation in the rate of NO loss from Fe(porphyrin)(NO) by at least by 6 orders of magnitude.

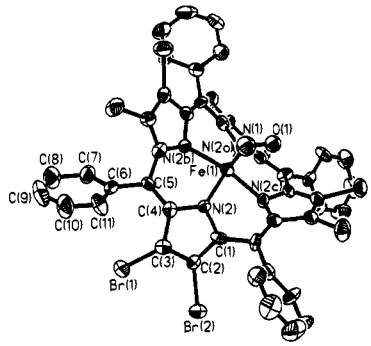

Figure 1. ORTEP view of the single crystal structure of **1f** showing one position for the crystallographically disordered FeNO. Significant metric parameters: Fe(1)−N(1) 1.75(6) Å, Fe(1)−N(2) 2.00(1) Å, Fe(1)−N(2)C 1.97(1) Å; Fe(1)−N(1)−O(1) 146(2)°.

The new Fe(porphyrin)(NO) complexes **1a−f** are readily prepared by reductive nitrosylation of the corresponding Fe(porphyrin)Cl complexes by treating them with nitric oxide in the presence of excess methanol and 2,6-lutidine.[11] The characteristic data collected in Table 1 are typical of paramagnetic ferrous nitrosyl adducts, in which the nitric oxide adopts a bent geometry and the iron is five coordinate.[12] The only significantly variable type of data for **1a−f** in Table 1 is the potential of the Fe(II)/Fe(III) couple, which increases by ~500 mV upon incorporation of more electron-withdrawing substituents on the aryl ring or the porphyrin ring. Over this same series, there is little variation in either the ν(NO) or the isotropic g and hyperfine a_N values from the ESR spectra, thus suggesting that the unpaired electron is in a $d_{z^2} - \sigma_N$ orbital.[11,13]

$$\mathrm{Fe(por)\{NO\} + L} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Fe(por)\{NO\}L} \qquad K_1 \qquad (1)$$

$$\mathrm{Fe(por)\{NO\}L} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{Fe(por)L + NO} \qquad K_2 \qquad (2)$$

$$\mathrm{Fe(por)L + L} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{Fe(por)L_2} \qquad K_3 \qquad (3)$$

Three of the five-coordinate nitrosyl adducts, **1a**,**d**,**f**, have been structurally characterized by single crystal X-ray diffraction.[14] A view of **1f**, shown in Figure 1 for one of the four nitrosyl orientations that result from crystallographically imposed disorder, illustrates the distorted geometry of the porphyrin.[15] Surprisingly, this severe saddle-shaped distortion does not significantly alter the geometry of the iron−nitrosyl moiety from that found in **1a**,**d** or the two comparable structures that have been reported for five-coordinate ferrous porphyrin nitrosyl compounds.[16]

† The tetraarylporphyrins employed in this study have the following substituents: **a**, *p*-tolyl; **b**, 2,6-difluorophenyl; **c**, mesityl; **d**, 2,6-dichlorophenyl; and **e**, pentafluorophenyl. The compound **1f** is octabromotetraphenylporphyrin.

(1) (a) Romberg, R. W.; Kassner, R. J. *Biochemistry* **1979**, *18*, 5387−5392. (b) Shimizu, M.; Basolo, F.; Vallejo, M. N.; Baldwin, J. E. *Inorg. Chim. Acta* **1984**, *91*, 251−255. (c) Stynes, D. V.; Stynes, H. C.; James, B. R.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 4087−4089.

(2) (a) Gibson, Q. H.; Roughton, F. J. W. *Proc. R. Soc. London B* **1965**, *163*, 197−205; (b) Gibson, Q. H.; Roughton, F. J. W. *Proc. R. Soc. London B* **1957**, *147*, 44−56.

(3) Gibson, Q. H.; Roughton, F. J. W. *J. Physiol.* **1957**, *136*, 507−526.

(4) (a) Jongeward, K. A.; Marsters, J. C.; Mitchell, M. J.; Magde, D.; Sharma, V. S. *Biochem. Biophys. Res. Commun.* **1986**, *140*, 962−966. (b) Hoshino, M.; Kogure, M. *J. Phys. Chem.* **1989**, *93*, 5478−5484.

(5) Hoshino, M.; Ozawa, K.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc.* **1993**, *115*, 9568−9575.

(6) Antonini, E.; Brunori, M.; *Hemoglobin and Myoglobin in Their Reactions with Ligands*; North-Holland Publishing Co.: Amsterdam, 1971.

(7) Variation in axial ligand binding to a sperm whale myoglobin mutant H93G has recently been described: DePhillis, G. D.; Decatur, S. M.; Barrick, D.; Boxer, S. G. *J. Am. Chem. Soc.* **1994**, *116*, 6981−6982.

(8) Duprat, A. F.; Traylor, T. G.; Wu, G.-Z.; Coletta, M.; Sharma, V. S.; Walda, K. N.; Magde, D. *Biochemistry* **1995**, *34*, 2634−2644.

(9) For a recent discussion of the effects ruffled porphyrin conformations have on their chemistry, see: Munro, O. Q.; Marques, H. M.; Debrunner, P. G.; Mohanrao, K.; Scheidt, W. R. *J. Am. Chem. Soc.* **1995**, *117*, 935−954 and references therein.

(10) (a) Denariaz, G.; Ketchum, P. A.; Payne, W. J.; Liu, M.-Y.; LeGall, J.; Moura, I.; Moura, J. J. *Arch. Microbiol.* **1994**, *162*, 316−322. (b) Ribeiro, J. M. C.; Hazzard, J. M. H.; Nussenzveig, R. H.; Champagne, D. E.; Walker, F. A. *Science* **1993**, *260*, 539−541.

(11) Wayland, B. B.; Olson, L. W. *J. Am. Chem. Soc.* **1974**, *96*, 6037−6041.

(12) An extensive collection of physical data for Fe(porphyrin)(NO) complexes can be found: *Physical Bioinorganic Chemistry Series: The Iron Porphyrins, Vols 1−3*; Lever, A. B. P., Gray, H. B., Eds.; VCH: New York, 1989.

(13) Loew, G. H.; Kirchner, R. F. *Int. J. Quantum. Chem., Quantum. Biol. Symp.* **1978**, *5*, 403−415.

(14) Crystallographic data for **1f**: A green crystal with dimensions 0.6 × 0.3 × 0.5 mm^3 was grown from toluene/hexane and then sealed in a capillary tube under nitrogen. Crystallographic information: $C_{44}H_{20}Br_8FeN_5O$, M = 1329.8, tetragonal space group $I4_1/a$, a = 20.750(3) Å, c = 10.554(2) Å^3, V = 4544.2(13) Å^3, Z = 4, d_c = 1.944 g cm^{-3}, μ(Mo K_α) = 7.406 mm^{-1}, F(000) = 2532, T = 295 K. The structure was solved by Patterson methods and refined using difference Fourier syntheses. Crystallographically imposed disorder of the iron and nitrosyl nitrogen was modeled by exact one-half fractional vacancy at both positions, while the nitrosyl oxygen was disordered over four sites. Anisotropic refinement for the iron and porphyrinic non-hydrogen atoms (hydrogens fixed; 138 variables) using 646 reflections with $F > 6\sigma(F)$ from 2114 unique data collected on a Siemens P4 diffractometer by the 2θ scan method ($4.0 \le 2\theta \le 50.0$), gave R = 0.052, R_w = 0.068.

(15) Ochsenbein, P.; Mandon, D.; Fischer, J.; Weiss, R.; Austin, R.; Jayaraj, K.; Gold, A.; Terner, J.; Bill, E.; Müther, M.; Trautwein, A. X. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1437−1439.

0002-7863/95/1517-9584$09.00/0

Table 1. Summary of Physical and Kinetic Data for the New Nitrosyl Heme Complexes **1a−f**

compound	ν(NO) (cm^{-1})[a]	$E_{1/2}$ (V vs Ag/AgCl)[b]	$g(a(^{14}N))$ (a_N Gauss)[c]	∠Fe−N−O (deg)	ligand (concn, M)	k_{obs} (s^{-1})[d]
Fe(TTP)NO (**1a**)	1675	0.43	2.053(18)	149.2(6)	pyridine (12.4)	$4.17(1) \times 10^{-5}$
Fe(TDFPP)NO (**1b**)	1687	0.66	2.056(17)		pyridine (12.4)	$2.00(1) \times 10^{-4}$
Fe(TMP)NO (**1c**)	1676	0.39	2.051(17)		pyridine (12.4)	$1.36(4) \times 10^{-2}$
Fe(TDCPP)NO (**1d**)	1688	0.64	2.053(17)	138.8(9)	pyridine (12.4)	$1.60(1) \times 10^{-2}$
					pyridine (0.1)	$2.11(1) \times 10^{-4}$
Fe(TPFPP)NO (**1e**)	1705	0.88	2.057(18)		pyridine (0.1)	0.51(1)
Fe(OBTPP)NO (**1f**)	1685	0.72	2.047(18)	146.4(24)	pyridine (0.1)	2.69(2)
					N-methylimidazole (0.1)	$3.71(5) \times 10^{1}$

[a] KBr Pellet. [b] CH_2Cl_2 solution with 0.1 M $[N(n\text{-butyl})_4][PF_6]$ as backing electrolyte with a platinum button electrode. [c] Measured in toluene at 22 °C and referenced to diphenylpicrylhydrazyl(DPPH). [d] Pseudo-first-order rate constants for nitric oxide substitution in Fe(porphyrin)(NO) by ligand, eqs 1−3, measured in toluene solution at 25 °C, with [Fe(porphyrin)(NO)] = $(1.5 - 0.59) \times 10^{-4}$ M, and determined by stopped-flow or spectrophotometric techniques.

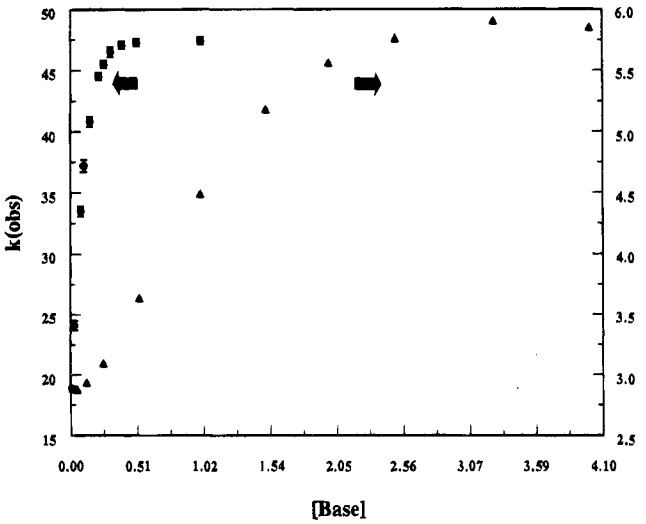

Figure 2. Plot of pseudo-first-order rate constants (k_{obs}) in s^{-1} for the dissociation of nitric oxide from Fe(OBTPP)(NO), **1f**, versus the molar base concentration for pyridine (▲) and *N*-methylimidazole (●) at 25 °C and [**1f**] = 5.9×10^{-5} M.

Given what appears to be similar molecular and electronic structures for the d^7 {FeNO} moieties in **1a−f**, it is surprising to find the large variation in the rates of nitric oxide dissociation when compounds **1a−f** are treated with coordinating ligands. Under pseudo-first-order conditions in the presence of excess pyridine and *N*-methylimidazole, the observed rate constants (k_{obs} in Table 1) for conversion to the corresponding Fe(porphyrin)L_2 complex vary by 6 orders of magnitude, between 10^1 and 10^{-5} s^{-1}. For the complex with the fastest denitrosylation rates, Fe(OBTPP)(NO), the dependence of rate on ligand concentration are shown in Figure 2. Both *N*-methylimidazole- and pyridine-promoted dissociation show saturation behavior, with the lower concentrations of the former being required to attain the maximum rate of 47.1 s^{-1} at 0.4 M, while for pyridine this limiting rate is only 5.9 s^{-1} in ~2 M pyridine. Spectrophotometric titrations of **1f** with *N*-methylimidazole and pyridine exhibit isosbestic behavior and are interpreted in terms of two equilibria, with constants K_a = 84.2(2) M^{-1} and K_b = 176.5(33) M. For pyridine the corresponding values are markedly lower, K_a = 6.3(4) M^{-1} and K_b = 2.3(5) M. In terms of eqs 1−3, these data are best reconciled with an associative substitution mechanism, where the rapidly established prequilibrium ($K_1 = K_a$) precedes the rate-limiting denitrosylation step. At high base concentrations, the rate law is zero-order in base, and therefore $k_2 = k_{obs}$; thus, for this the rate-determining step, k_2 = 47.5 s^{-1} for *N*-methylimidazole and 5.9 s^{-1} for pyridine. The second equilibrium constant, K_b, from the spectrophotometric titrations is thus the product of K_2 and K_3. For the remaining complexes **1a−e**, nitrosyl dissociation is slower, and even under high concentrations of base there is no evidence for a saturation behavior similar to that shown in Figure 2.

The emerging biochemical roles of nitric oxide are almost as diverse as the physiological tissues in which it is generated. Possibly the best understood receptor for intercellular nitric oxide-mediated signal transduction is heme in soluble guanylyl cyclase (sGC).[17] Although it is now well understood that the coordination of nitric oxide to the ferrous heme moiety in sGC is the physiologically significant activating step in the up-regulation of this enzyme,[18] and that in the activated state the iron has an ESR signal consistent with a five-coordinate iron {FeNO} d^7 configuration with a bent nitrosyl, it is not known what the down-regulatory mechanism is for the return of sGC to its rest state.[17] The half-life for the reversible deactivation of isolated sGC can be estimated to be 20 min at 37 °C,[19] but organ studies suggest that it is as little as several minutes.[20] Based primarily on the very slow rates of denitrosylation of hemoglobin and myoglobin nitrosyls, it is often assumed that the direct loss of nitric oxide from sGC is very slow, and thus this is not a possible mechanism for denitrosylative down-regulation.[8,17b] The results presented in this study suggest that this mechanism should not be discounted until we have a better understanding of all aspects of both sGC biochemistry and the nature of the electronic structure for the heme−nitric oxide ensemble.

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Supporting Information Available: Tables of kinetic and spectrophotometric data as well as complete crystallographic data for **1f** including metric parameters, atomic coordinates, and thermal parameters (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current mathead page for ordering information and Internet access instructions.

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(16) (a) Piciulo, P. L.; Rupprecht, G.; Scheidt, W. R. *J. Am. Chem. Soc.* **1974**, *96*, 5293−5295. (b) Scheidt, W. R.; Frisse, M. E. *J. Am. Chem. Soc.* **1975**, *97*, 17−21. (c) Nasri, H.; Haller, K. J.; Wang, Y.; Huynh, B. H.; Scheidt, W. R.; *Inorg. Chem.* **1992**, *31*, 3459−3467.

(17) (a) Ignarro, L. J. *Biochem. Soc. Trans.* **1992**,*20*,465−469; (b) Stone, J. R.; Marletta, M. A. *Biochemistry* **1994**, *33*, 5636−5640.

(18) Yu, A. E.; Hu, S.; Spiro, T. G.; Burstyn, J. N.; *J. Am. Chem. Soc.* **1994**, *116*, 4117−4118.

(19) Estimated from reported $t_{1/2}$ of 3−4 h at 4 °C. Arnold, W. P.; Mittal, C. K.; Katsuki, S.; Murad, F. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3203−3207. See also: Wu, X.-B.; Brüne, B.; von Appen, F.; Ullrich, V. *Arch. Biochem. Biophys.* **1992**, *294*, 75−82. Braughler, J. M. *Biochem. Pharmacol.* **1983**, *32*, 811−818.

(20) Palmer, R. M. J.; Ferrige, A. G.; Moncada, S.; *Nature* **1987**, *327*, 524−526.