Oxidation of Iron(II) Porphyrins and Hemoproteins by Nitro Aromatics

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Abstract: Nitro aromatics oxidize high-spin iron(II) porphyrins in 1:1 v/v *N*-methylpyrrolidone–acetic acid at room temperature. The corresponding aniline and iron(III) porphyrin are obtained. The overall stoichiometry and kinetics of the reactions require a multistep pathway that entails the arylnitroso compound and arylhydroxylamine as intermediates. The rate-limiting reduction of arylnitro to arylnitroso compound proceeds from either 1:1 or 2:1 iron(II) porphyrin–nitro aromatic adduct depending upon substrate. The reaction is inhibited by good axial ligands of iron(II) and neither hexa- nor pentacoordinate low-spin hemes are oxidized by nitro aromatics. Two related but distinct axial inner sphere mechanisms for the oxidation of hemes by nitro aromatics are apparent and both are acid dependent. The reactivity of iron(II) myoglobin and iron(II) cytochrome *c* toward selective substrates of either mechanistic class accords with simple theory.

Nitro aromatics represent one of the classes of organic molecules capable of rapidly oxidizing high-spin iron(II) porphyrins (Fe^{II} Porp) in homogeneous solution.[1] Moreover, compounds of this kind can induce methemoglobinemia in mammals,[2] and the substance 2,4-dinitrophenol is well known as an effective "uncoupler" of oxidative phosphorylation from mitochondrial electron transport.[3] As part of our effort to delineate the chemistry of these biological processes, we describe here the scope and mechanism of the oxidation of iron porphyrins by nitro compounds. Selected mechanistically defined substrates have been applied to deoxymyoglobin and iron(II) cytochrome *c* to further test the validity of simple theory.[4]

Results

Stoichiometry. Products of the reduction of a series of nitro compounds by iron(II) deuteroporphyrin IX in 1:1 v/v *N*-methylpyrrolidone–acetic acid are given in Table I. In all cases, the reported yields reflect the adequacy of the workup rather than the efficiency of the reaction (cf. Experimental Section). No other products were detected. The quantitative transformations accord with the overall stoichiometry in eq 1.

$$6Fe^{II} + ArNO_2 + 6H^+ \rightarrow 6Fe^{III} + ArNH_2 + 2H_2O \quad (1)$$

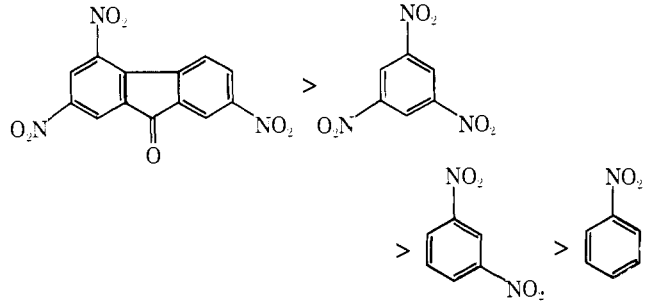
No intermediates could be detected in these reactions, but the kinetics demand their presence. The quantitative yields of aniline from nitrosobenzene and phenylhydroxylamine reflect the stoichiometry of eq 2 and 3.

$$4Fe^{II} + ArNO + 4H^+ \rightarrow 4Fe^{III} + ArNH_2 + H_2O \quad (2)$$

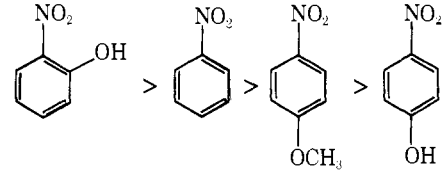
$$2Fe^{II} + ArNHOH + 2H^+ \rightarrow 2Fe^{III} + ArNH_2 + H_2O \quad (3)$$

Reactivity, Kinetics, and Pathway. (a) Reactivity. Nitro aromatic substrates are listed in Table II in a decreasing order of reactivity. Relative rates are calculated with nitrobenzene as the reference. A minimal assessment of the speed of oxidation of the heme by the likely intermediates, nitrosobenzene and phenylhydroxylamine, is given at the bottom of the table along with the rate constant for phenylhydrazine.

In addition to the substances in the table, 3,5-dinitroaniline and *m*-trifluoromethylnitrobenzene[5] were reactive. Nitromethane also oxidizes the heme very slowly. In general, structural features that enhance the delocalization of the π system and are electron withdrawing in character increase the rate. The series:



is illustrative. The relative reactivity of the dinitro compounds reflects the beneficial conjugative influence of one nitro grouping upon another. An ortho hydroxy substituent markedly increases the rate of reaction but, in general, electron-releasing substituents slow the process considerably. The series:



is exemplary. For the most part, neglecting the activating influence of an ortho hydroxy group, the reactivity toward reduction by deuteroheme very roughly follows the electron affinity of the nitro compound (cf. Figure 1) and what might be expected for the stability of a coordinated nitro aromatic radical, but there is no simple correlation.[6] The rates do not parallel the thermodynamics. It should be emphasized here that the reduced congeners of nitrobenzene, nitrosobenzene and phenylhydroxylamine, are two of the most reactive substrates encountered.

The relatively mild influence of the two 2 and 4-substituents of deuteroheme upon the rate of oxidation by nitrobenzene stands in the series $CH_3CH_2 > H > CH{=}CH_2$, while the corresponding iron(II) complexes of mesoporphyrin IX, deuteroporphyrin, and protoporphyrin react at about the same rate with *m*-dinitrobenzene. The data are presented in Table III.

(b) Kinetics. Two different rate laws are obtained in 1:1 v/v *N*-methylpyrrolidone–acetic acid, and the kinetics are well behaved throughout the reaction. Generally, the more rapidly reacting substrates reduce by a process first order in heme, eq 4.

Table I. Products of the Reduction of Nitro Aromatics and Reduced Congeners by Deuteroheme in 1:1 v/v *N*-Methylpyrrolidone-Acetic Acid at 25 °C

Substrate	Product	Yield, %	Eq
sym-Trinitrobenzene	3,5-Dinitroaniline	65[a]	1
2,4-Dinitrophenol	2-Amino-4-nitrophenol	28[b]	1
p-Methoxynitrobenzene	*p*-Methoxyaniline	78[a]	1
Nitrobenzene	Aniline	100[a,c]	1
Nitrosobenzene	Aniline	100[c]	2
Phenylhydroxylamine	Aniline	100[c]	3

[a] Calcd from the weight of isolated acetanilide. [b] As determined by thin-layer chromatography and visible spectra. [c] By gas chromatography; the solid acetanilide was isolated.

Table II. Rates of Oxidation of Iron(II) Deuteroporphyrin IX by Nitro Aromatics and Partially Reduced Derivatives in 1:1 v/v *N*-Methylpyrrolidone-Acetic Acid at 25 °C

$ArNO_2$	Rel rate[d]	k_2, L mol^{-1} s^{-1} [c]	k_3, L^2 mol^{-2} s^{-1} [c]
2,4,7-Trinitrophlorenone	>34 000	>8 × 10^3 [a]	
sym-Trinitrobenzene	3 300	7.0 × 10^2	
p-Dinitrobenzene	850	2.0 × 10^2	
2,6-Dinitrophenol	810	1.9 × 10^2	
2,4-Dinitrophenol	680		58 × 10^4
o-Dinitrobenzene	42		4.0 × 10^4
m-Dinitrobenzene	45	9.9	
o-Nitrophenol	3.3		2.8 × 10^3
Nitrobenzene	1.0		8.5 × 10^2
p-Methoxynitrobenzene	0.14		1.2 × 10^2
p-Hydroxynitrobenzene	0.039		33
Nitromethane	0.031		27[b]
1,3,5-Tri-*tert*-butylnitrobenzene	0.0047		4[b]
Reduced derivatives			
Nitrosobenzene	>34 000	>8 × 10^3 [a]	
Phenylhydroxylamine	>34 000	>8 × 10^3 [a]	
Phenylhydrazine	4.8		4.1 × 10^3

[a] The limits of our methodology; the rate law is assumed. [b] From initial slopes. [c] Reproducibility ±7%. [d] With $[Fe^{II}\ Porp]_0 = 2.75 \times 10^{-4}$ M.

$$\text{rate} = \frac{d(Fe^{III}\ Porp)}{dt} = k(Fe^{II}\ Porp)(ArNO_2) \qquad (4)$$

In contrast, the slower reacting substrates (with the exception of *m*-dinitrobenzene) undergo a two-electron reduction in the primary step, eq 5.

$$\text{rate} = \frac{d(Fe^{III}\ Porp)}{dt} = k(Fe^{II}\ Porp)^2(ArNO_2) \qquad (5)$$

Typical pseudo-order plots of the data for *m*-dinitrobenzene and *sym*-trinitrobenzene corresponding to eq 4 and nitrobenzene corresponding to eq 5 are presented in Figures 2 and 3. In addition to the good linearity of the plots, the same rate law was obtained from an initial slope treatment, and the same rate constant for any run could be obtained from the slope at any time. This multislope treatment was employed to demonstrate that ferrous acetate had *no* influence upon the rate.[7] Thus, ferrous acetate does not reduce nitrobenzene under reaction conditions and, more significantly, the rate constant for

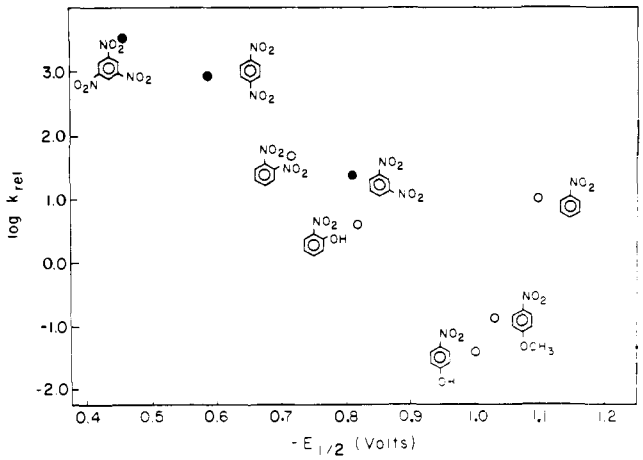
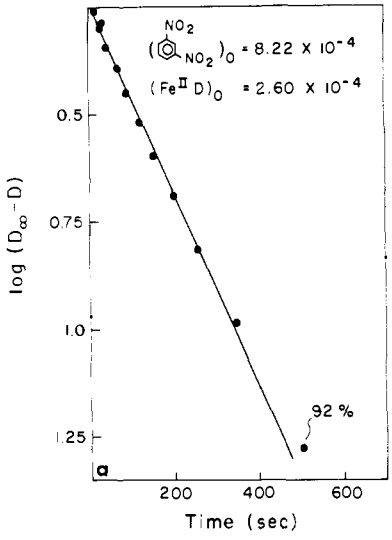


Figure 1. Plot of the relative rate of oxidation of iron(II) deuteroheme by various nitro aromatics vs. their reduction potential in DMF.



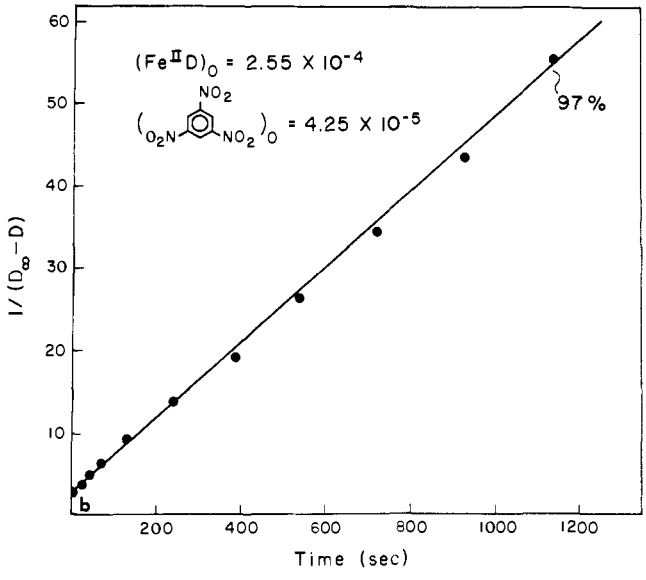


Figure 2. (a) Pseudo-first-order plot for the oxidation of deuteroheme by *m*-dinitrobenzene in 1:1 v/v *N*-methylpyrrolidone-acetic acid at 25 °C. (b) General second-order plot with stoichiometric ratios of reactants for the oxidation of deuteroheme by *sym*-trinitrobenzene in 1:1 v/v *N*-methylpyrrolidone-acetic acid at 25 °C.

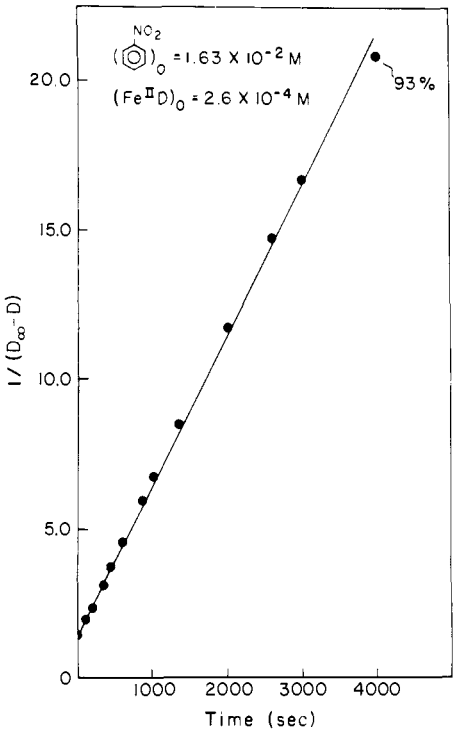
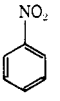
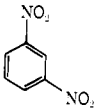


Figure 3. Pseudo-second-order plot for the oxidation of deuteroheme by nitrobenzene in *N*-methylpyrrolidone–acetic acid at 25 °C.

Table III. Comparative Rates of Oxidation of Iron(II) Porphyrins by Nitro Aromatics in 1:1 v/v *N*-Methylpyrrolidone–Acetic Acid at 25 °C

Iron(II) porphyrin	 k_3, L^2 mol^{-2} s^{-1}[a]	 k_2, L mol^{-1} s^{-1}[b]
Mesoporphyrin IX dimethyl ester	16×10^2	10
Deuteroporphyrin IX	8.5×10^2	9.9
Protoporphyrin IX	4.8×10^2	9.2

[a] Rate is second order in iron. [b] Rate is first order in iron.

Table IV. Activation Parameters for the Oxidation of Deuteroheme by Nitro Aromatics in 1:1 v/v *N*-Methylpyrrolidone–Acetic Acid at 25 °C

Substrate	kcal/mol $\Delta H^{\ddagger}$	kcal/mol $\Delta F^{\ddagger}$	$\Delta S^{\ddagger}$, eu
Nitrobenzene[a]	14	13.4	2
m-Dinitrobenzene[b]	10	16	−20

[a] Rates second order in heme. [b] Rates first order in heme.

the oxidation of nitrobenzene was not altered by cycling the process with iron powder repetitively, eq 6.

$$PhNH_2 + Fe^{III}\ Porp \xrightarrow{Fe^0} Fe^{II}\ Porp + Fe(OAc)_2 \quad (6)$$
$$\uparrow \qquad\qquad PhNO_2 \qquad\qquad \downarrow$$

Both of the mechanisms that are represented by eq 4 and 5 are, in addition, acid dependent. The influence of added *p*-toluenesulfonic acid upon the rates of reduction of two typical substrates, nitrobenzene (process 5) and *m*-dinitrobenzene (process 4) are depicted in Figures 4 and 5, respectively. Thus,

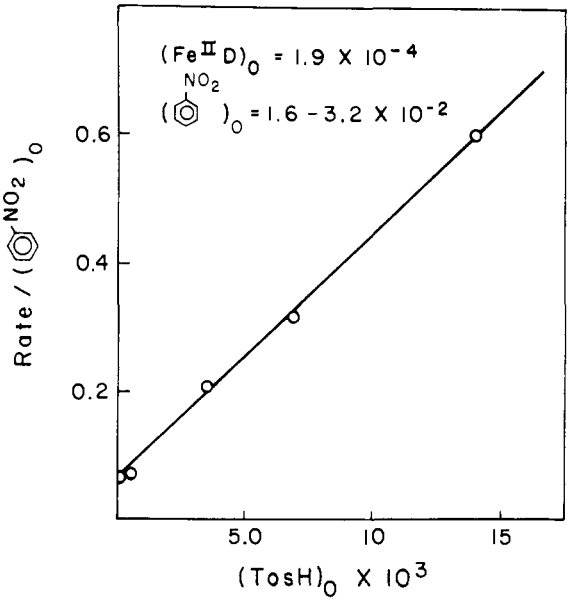


Figure 4. Rate of oxidation of deuteroheme by nitrobenzene as a function of added *p*-toluenesulfonic acid.

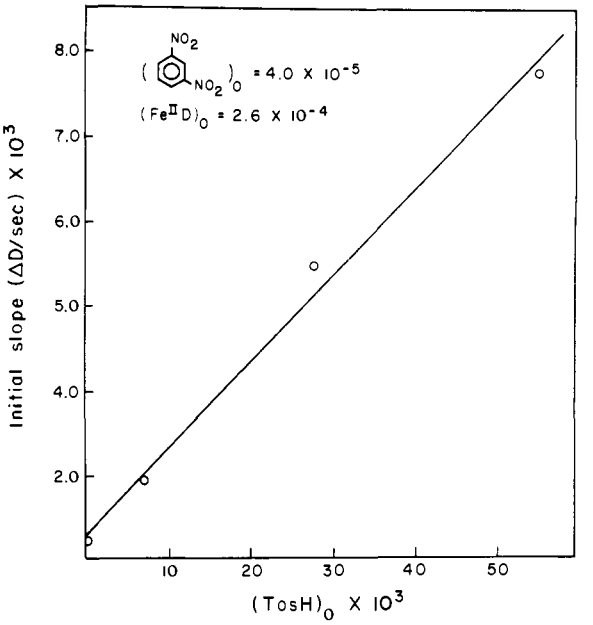


Figure 5. Rate of oxidation of deuteroheme by *m*-dinitrobenzene as a function of added *p*-toluenesulfonic acid.

the overall rate processes are termolecular:[7a]

$$\frac{d(Fe^{III}\ Porp)}{dt} = k(Fe^{II}\ Porp)(ArNO_2)(H^+) \quad (7)$$

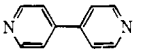
$$\frac{d(Fe^{III}\ Porp)}{dt} = k(Fe^{II}\ Porp)^2(ArNO_2)(H^+) \quad (8)$$

Activation parameters are given in Table IV.

(c) Pathway. The kinetics and the dramatic difference in reactivity of nitrobenzene and its partially reduced congeners demonstrate a multistep path for the overall conversion of arylnitro compounds to aniline. With nitrobenzene, the first step is the slowest (eq 9) and we presume this is true for all substrates whether the initial step entails a one- or two-electron transfer.

$$PhNO_2 \xrightarrow[slow]{k} PhNO \longrightarrow PhNHOH \longrightarrow PhNH_2 \quad (9)$$

Table V. Reactivity of Low-Spin Iron(II) Porphyrin Solutions toward Oxidation by Trinitrobenzene in 1:1 v/v NMP–HOAc[e] at 25 °C

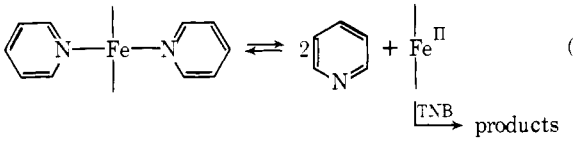
Iron complex	mol/L	Ligand	mol/L	Rate
L —Fe— 1	1.0×10^{-3}	NO	(Satd)	Nil[a]
	2.8×10^{-4}	CO	(Satd)	Very slow[b]
L —Fe— L 2	1.0×10^{-4}		0.08	Slow[c]
	1.4×10^{-4}		Solvent (pyr) + 10^{-4} M pyr H^+Cl^-	Nil
	1.1×10^{-4}		63:35 pyr:HOAc	Nil
	6×10^{-5}		0.06	Nil
	1×10^{-4}		0.01	Nil
	1×10^{-4}			*d*

[a] Nil = no oxidation in 1 day with $\sim 10^{-5}$–6×10^{-4} TNB. [b] With 6.5×10^{-5} M TNB ~50% oxidation occurred in 18 h. [c] The reaction is inverse order in $(pyr)^2$; cf. Figure 6. [d] Imidazole reacts with TNB. [e] NMP is *N*-methylpyrrolidone.

(d) Axial Ligands and Spin State. Both penta- (**1**) and hexacoordinate (**2**) low-spin iron(II) porphyrins were examined for reactivity toward *sym*-trinitrobenzene in *N*-methylpyrrolidone–acetic acid (Table V). In contrast to the results with quinones[8] or oxygen,[9] the reactivity toward nitro aromatics can be wholly suppressed by axial ligation. There is, in fact, no reaction at all of any of the low-spin adducts of Table V with trinitrobenzene (TNB). While an apparent reaction does ensue at lower concentrations of pyridine in *N*-methylpyrrolidone–acetic acid, the rate under these conditions is inverse order in $(pyridine)^2$; that is:

$$\text{rate} = \frac{k(\text{Fe}^{\text{II}}\ \text{Porp})(\text{TNB})}{(\text{pyridine})^2}$$

This is illustrated in Figure 6. The reaction is the result of the presence of some nonligated high-spin Fe^{II} adduct (eq 10)



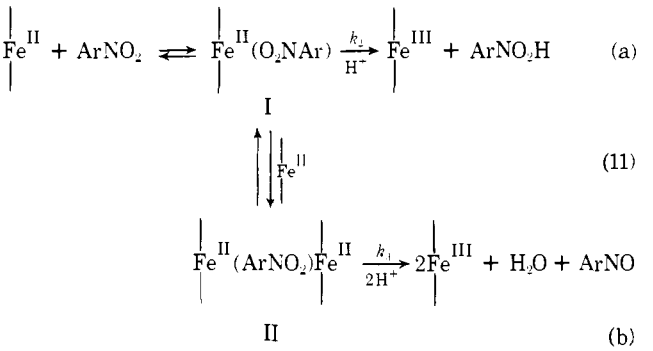
(10)

(Figure 7). However, in neat pyridine, the spectrum of low-spin iron(II) octaethylporphyrin or iron(II) deuteroporphyrin IX dimethyl ester is unaffected by the addition of massive amounts of TNB in the presence or absence of pyridinium chloride. Moreover, a solution of iron(II) deuteroporphyrin IX in 65:35 v/v pyridine–acetic acid is inert to this substrate. Similar results are obtained with nitrobenzene. We conclude that low-spin iron(II) porphyrins *are not* oxidized by nitro aromatics. Clearly, reaction by either mechanism suggested by eq 7 or 8 requires substitution into the inner coordination sphere of iron.

Hemoproteins. Deoxymyoglobin and iron(II) cytochrome *c* were chosen as examples of extreme conformation and spin state. The reactivity of these proteins to trinitrobenzene and 2,4-dinitrophenol is given in Table VI.

Discussion

Taken together, all of our data and, in particular, the inhibition by good axial ligands point to a general mechanism for the oxidation of iron(II) porphyrins by nitro compounds that is an "axial inner sphere" process.[10] In many ways the reaction parallels the reactivity we have encountered with alkyl halides.[5] We formulate the general process in eq 11 with the equilibria to I or II being rapidly established.

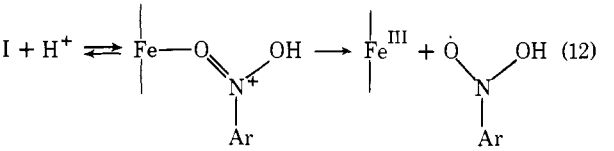


(11)

(a)

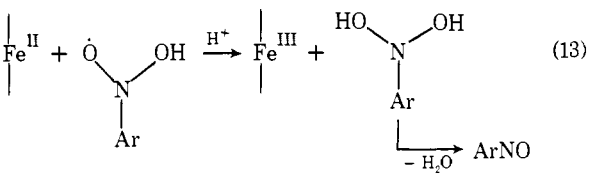
(b)

Thus, depending upon substrate, electron transfer may ensue subsequent to protonation of the 1:1 adduct I or the binuclear species II. In slight amplification, the most reasonable rate-limiting step for eq 11a would seem to be dissociation of the protonated nitro aromatic ligand (eq 12).



(12)

The nitrooxyl radical emanating from eq 12 would, like other oxy radicals,[11] be expected to very rapidly oxidize a second high-spin iron(II) complex in a subsequent step (eq 13).



(13)

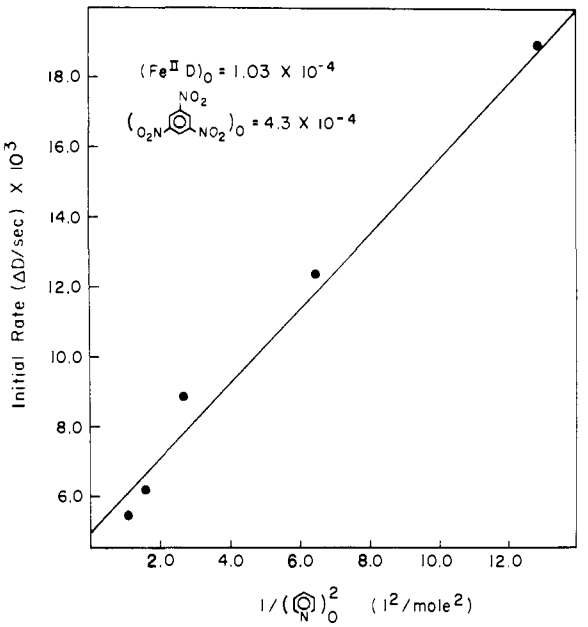


Figure 6. Rate of oxidation of deuteroheme by *sym*-trinitrobenzene as a function of added pyridine.

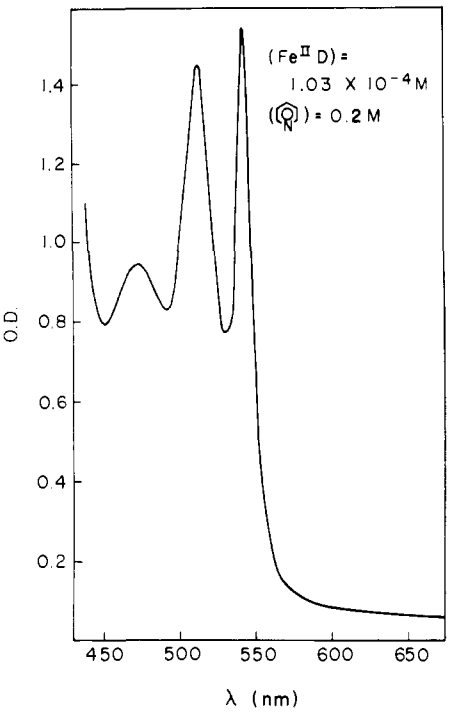


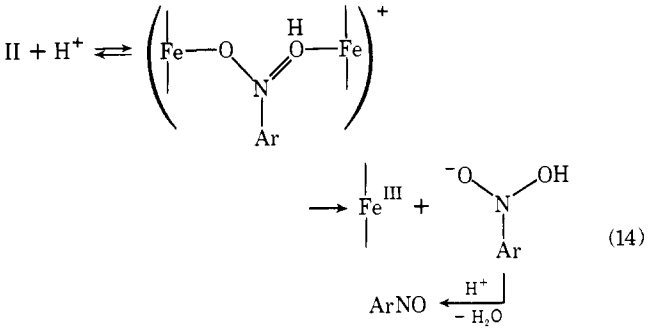
Figure 7. Visible spectrum of deuteroheme in 1:1 v/v *N*-methylpyrrolidone–acetic acid containing 0.2 M pyridine.

Table VI. Reactivity of Hemoproteins to Nitro Aromatics in Aqueous Buffer at 25 °C

Protein	Substrate	Rate, L mol^{-1} s^{-1}
Fe^{II} myoglobin	1,3,5-Trinitrobenzene	0.15[c]
Fe^{II} myoglobin	2,4-Dinitrophenol	NR[a]
Fe^{II} cytochrome *c*	1,3,5-Trinitrobenzene	NR
Fe^{II} cytochrome *c*	2,4-Dinitrophenol	NR

[a] NR = no reaction after 4 h. [b] pH 7.4, 0.01 M phosphate buffer, 0.1 M KCl. [c] k_2 = 2.7 at pH 6.0.

The operation of eq 11a (eq 12 and 13) is favored by the more easily oxidized substrates. The actual electron transfer from the binuclear adduct II can be envisioned most reasonably in similar fashion (eq 14).

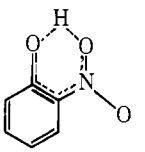


(14)

There are a number of possible formulations for II; thus, each iron may be coordinated to different nitro groups on opposite sides of the ring (*o*-dinitrobenzene) or perhaps to a phenolic and nitro moiety. Indeed, the possibility of a backsided attack on the iron porphyrin of a protonated I also exists. In light of the basicity and incipient radical character of the coordinated ligand,[12] we prefer the formulation given. As written, the process 11b (eq 14) resembles the mechanism of oxidation of high-spin iron(II) porphyrins by molecular oxygen.[13] We ascribe the activating influence of an ortho hydroxy substituent to the increased delocalization embodied in structures like III and, more importantly, to the close juxtaposition of a proton source to the site of reaction.

In sum, as with quinones[8] and oxygen,[9] nitro aromatics oxidize high-spin iron(II) porphyrins much more rapidly than the corresponding low-spin adducts, but in contrast to the former oxidants no reaction occurs with the low-spin hexacoordinate species in this case. We have *no* evidence for an outer sphere pathway with nitro aromatics, nor do we have any evidence for molecular complex formation with these species.

Molecular Complexes. The inability to observe any change in the visible or NMR spectrum upon addition of trinitrobenzene to pyridine solutions of iron(II) porphyrins was surprising in light of the reported existence of such species with analogous iron(III) and Co(II) complexes.[14] However, no solid derivatives of iron(III) could be isolated.[15]



III

Thus, if pyridine enhances metal-porphyrin d-π interaction, a peripheral π complex with trinitrobenzene should be favored. Moreover, an iron(II) adduct should undergo molecular complex formation in such an arrangement more readily than an iron(III) species by virtue of possessing an additional electron. Yet all of our evidence indicates only coordination with iron. In this sense, the loose affiliation solely with the aromatic ring proposed for some iron(III) adducts may be too restrictive.

On the other hand, a very feeble interaction would not be detected by the methods employed herein. We can say only that if an outer sphere adduct of nitro aromatics with low-spin iron(II) porphyrins is formed, an electron-transfer reaction does not ensue in the presence or absence of protons at room temperature.

The Hemoproteins. The response of the hemoproteins accords perfectly with the mechanism delineated herein and the reactivity pattern matches exactly that predicted for axial inner

sphere oxidation with these proteins.[4] Thus, only a high-spin heme in the G conformation could possibly react, and it may only oxidize by a process that is first order in iron. Hence, deoxymyoglobin does oxidize with trinitrobenzene but is inert to an oxidant that requires the attack of a second heme on the 1:1 adduct (2,4-dinitrophenol). The C conformation of the cytochrome *c* precludes reaction by either substrate by blocking the axial positions of iron. This is the identical pattern observed with alkyl halides.[16] Moreover, it is interesting to note that the attenuation of reactivity imparted by the apoprotein of myoglobin to the heme is significant for either class of oxidant. Thus, the relative rates of oxidation of iron(II) porphyrin and myoglobin for the halide α-bromoisobutyronitrile are $\sim 6 \times 10^4$. Under the same conditions, the relative rate with trinitrobenzene is 6×10^3. These ratios are in keeping with the steric constraint imposed by the protein to coordination with the heme. Thus, the rate-limiting step with the globins is the formation of the 1:1 adduct.

Experimental Section

Materials. Nitro Aromatics. Nitromethane (spectrograde, Matheson Coleman and Bell) and 2,4,6-tri-*tert*-butylnitrobenzene (reagent, Frinton Labs) were used without further purification. Phenylhydroxylamine[17] and nitrosobenzene[18] were prepared from nitrobenzene. All other substances were purified before use; their physical constants checked the literature. Pyridine was distilled over benzenesulfonyl chloride (~0.5%) to remove traces of amine reductant and redistilled after being dried over a 4-Å molecular sieve.

Porphyrins. All porphyrins were obtained and purified as previously described.[5,19]

Product Isolation and Characterization. The reaction with nitrobenzene is illustrative.

A solution of iron(II) deuteroporphyrin IX (3.75×10^{-3} M) in 1:1 v/v *N*-methylpyrrolidone-acetic acid (1.5 L) was prepared and reacted with 0.24 g (1.96×10^{-3} M) of nitrobenzene under argon. After 20 h the reaction was opened to air and the whole was treated with a large excess of acetic anhydride (25 mL). The solution was heated in a water bath for 2–3 h. The reaction was then concentrated to almost dryness by means of a rotary evaporator (94 °C (0.5 mm)). The concentrate was extracted several times with acetone and passed through a Fisher A540 alumina column to separate most of the coextracted hemin. The eluate was concentrated and the product was isolated by preparative gas chromatography (1 ft × 0.25 in. 10% SE Gum-30 on Chromosorb W treated with acetic anhydride, ~140 °C).

Acetanilide and *p*-methoxyacetanilide isolated in this fashion had mp and mmp of 113–114 and 126.5–127.5 °C, respectively. The infrared spectra were identical with authentic samples. Quantitation was effected by direct analysis of the concentrated eluate by flame ionization gas chromatography. A 7 ft × 0.125 in. column of 5% SE Gum-30 on Chromosorb W (125 °C) was used. An internal standard was employed.

Quantitation of 3,5-dinitroacetanilide after chromatography was accomplished by sublimation and crystallization from 95% ethanol (mp and mmp 190.5 °C). The 2-amino-4-nitrophenol was not amenable to isolation by the above methods or by far more elaborate separation procedures. An ether extract of the concentrated reaction mixture was gassed with HCl to precipitate the hydrochloride. The latter was taken up in methanol and chromatographed on thin-layer plates (silica gel G). The TLC plate was eluted with 5% ethanol in benzene. The product cochromatographed with an authentic sample (R_f = 0.27). It was scraped from the plate and determined by the absorbance at 380, 308, and 260 nm as compared to that of an authentic sample that had undergone chromatography. It should be emphasized that the para isomer was resolved on TLC and detectable by these means. None was apparent in the product mixture.

Kinetics. Kinetics were monitored at 623 nm in the manner previously described.[5a] The solvent *N*-methylpyrrolidone-acetic acid (1:1 by volume) was employed throughout except where indicated. Heme solutions ($0.4–2.8 \times 10^{-4}$ M) were prepared in 3-mL spectrophotometric cells in a manner similar to that previously employed.[5a] After four cycles of evacuation and argon flushing, the stock iron(III) solution was transferred under argon into an argon-flushed cell that contained ~0.4 mg of iron powder via stainless steel hypodermic tubing. The solution in the serum capped cell was completely reduced after 20 min of stirring. Chromous perchlorate scrubbed 99.998% argon was employed throughout.

Acknowledgment. This research was supported in part by Research Grant No. AM 17936 from the National Institute of Arthritis, Metabolism and Digestive Diseases. We thank Eleanor W. Bartnicki for experimental assistance.

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