Oxidation of Low-Spin Iron(II) Porphyrins by Molecular Oxygen. An Outer Sphere Mechanism†

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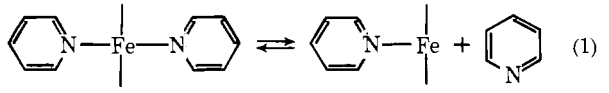
ABSTRACT: Hexacoordinate low-spin iron(II) porphyrins are oxidized by molecular oxygen in amine solvents at room temperature by a process that is acid dependent. The visible and NMR spectra of solutions of the iron complexes and the influence of axial ligands upon the rate of oxidation are consistent with an outer sphere mechanism that entails the dissociation of a protonated 1:1 iron porphyrin–oxygen π complex as the rate-limiting step.

The response of hemoproteins to oxygen varies widely. Indeed, this reactivity typifies the modulating influence that a protein can bring to bear upon its highly defined iron porphyrin active site. A simple theory (Castro, 1971) has been advanced that will accommodate the reactivity patterns exhibited by hemoproteins, but the underlying basis for it, a knowledge of mechanistic iron porphyrin redox chemistry, remains in large measure to be developed. A necessary premise of theory was that an outer sphere mechanism for the oxidation of iron(II) porphyrins by oxygen can occur under certain conditions. This was essential to explain the unusual reactivity of some of the cytochromes, like cytochrome b_5, that are inhibited by neither cyanide nor carbon monoxide and yet are autoxidizable.

Early studies of the kinetics of the oxidation of iron(II) protoporphyrin IX dimethyl ester by oxygen in pyridine–water and pyridine–ethanol–benzene solvents found the apparent rate of the reaction to be first order in heme and inverse order in pyridine (Kao and Wang, 1965). A very small component of the rate was assigned to an "outer sphere" mechanism, but the major path of reaction was thought to be an "inner sphere" mechanism that entailed an iron bonded oxygen intermediate. Subsequent studies of the same and closely related systems (Cohen and Caughey, 1968) seemed to dispute these findings. Thus, an examination of the kinetics of the autoxidation of the above porphyrin and the 2,4-diacetyldeutero-IX-iron(II) derivative in pyridine–benzene and pyridine–benzene–ethanol demonstrated the order in heme varied from first to second, depending upon the reaction conditions. Moreover, even under carefully controlled conditions, the variations in rates were wide and difficult to control experimentally. Nevertheless, the trends in the data indicated the rate law to be mainly:

$$\text{rate} = \frac{k(\text{heme})^2(O_2)}{(\text{pyridine})^2}$$

A first-order component in heme was indicated at high oxygen and low pyridine concentrations. The mechanism inferred from this study was as follows:



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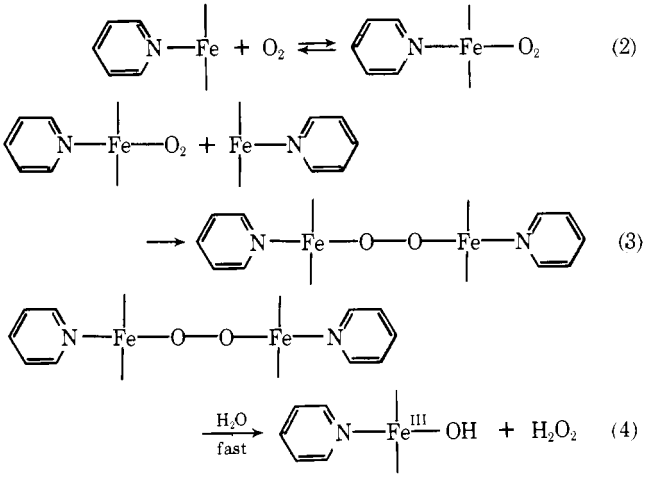

The attack of heme on the 1:1 oxygen adduct step (eq 3) was considered as rate limiting. Moreover, it was observed that the earlier results could be a special limiting case of this mechanism. While not kinetic in nature, there is now a wealth of information that supports the formulation (1)–(4), particularly steps (2) and (3), for the reaction of high spin iron(II) porphyrins. It has recently been summarized (Castro, 1977; Castro et al., 1978). A recent NMR study (Chin et al, 1977) also accords with this formulation.

We present here evidence for an outer sphere mechanism for the oxidation of low spin iron(II) porphyrins by molecular oxygen. The path is analogous to one of those originally suggested by Kao and Wang (1965). The apparent discrepancies between the above works (Kao and Wang, 1965; Cohen and Caughey, 1968) and the variability of some of the data can be explained by our observations. Our basic point is that the outer sphere mechanism is acid dependent. The reactivity encountered here with oxygen is entirely analogous to that of the reaction of iron(II) porphyrins with quinones (Castro et al., 1977).

Experimental Section

Solvents. All solvents were reagent grade and were freshly distilled through a small Vigreux column under argon immediately before use. In general, heart cuts of pyridine (bp 115 °C), *tert*-butylpyridine (bp 94 °C at 14 mm), *tert*-butylamine (bp 45 °C), and *N*-methylimidazole (bp 197 °C) were stored under argon in the receiver of the distillation apparatus and transferred under argon to the spectrophotometric cells. *tert*-Butylamine was particularly refractory toward oxygen removal. A kinetically steady solvent was obtained only after

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redistillation of a heart cut that was treated with traces of aqueous sodium dithionite and solid potassium hydroxide.

Axial Ligands. Imidazole was recrystallized from water, mp 89 °C, and 4-cyanopyridine was sublimed, mp 78 °C. Carbon monoxide (Matheson) was employed without purification.

The Porphyrins. The porphyrins and their iron(III) complexes were obtained and purified in the manner previously described (Castro, 1974; Castro et al., 1974).

The Iron(II) Porphyrins. These were generated in pyridine solution using hydrazine hydrate or *n*-butylamine as a reductant. In general, ~5 mg of the chloroiron(III) porphyrin was dissolved in 15 mL of pyridine and purged with argon in a rotary evaporating apparatus. After 30 min, a solution of 1 mL of 85% hydrazine hydrate in 15 mL of pyridine was added under argon. The contents were stripped to dryness at 30 °C under argon. An additional 15 mL of argon-purged pyridine was admitted, and the solvent was removed again. This process was repeated and the final solid was diluted with pyridine and stored under argon in a flask that was fitted with a stopcock protected with a serum cap. The concentration of iron(II) solutions diluted into spectrophotometric cells was ascertained by comparing the spectrum with that of the same cuvette to which 1 μL of hydrazine hydrate was added.

Alternatively, stock solutions in pyridine were prepared by dissolving the bis(piperidyl)iron(II) adducts in pyridine under argon. The reduction of iron(III) porphyrins by piperidine has been noted (Epstein et al., 1967). We find the reduction of iron(III) porphyrins by amines to be a very general reaction, and we shall report upon it separately. The preparation described (Epstein et al., 1967) for iron(II) protoporphyrin IX and mesoporphyrin IX gave poor analyses. It is *not* possible to "recrystallize" these porphyrins from this solvent. The corresponding salts precipitate.

The following procedure is illustrative of the preparation of the solid bis(piperidyl)hemes employed herein. A solution of 100 mg of chloroiron(III) octaethylporphyrin is dissolved in 10 mL of freshly distilled piperidine by gently warming on a hot plate. Upon dissolution, the cooled solution is poured immediately upon a dry 1 × 40 cm A-540 alumina column and eluted with piperidine. The orange-red iron(II) adduct is the first band off the column. This is followed closely by a small green band. A small dark olive band remains at the top. The heme-piperidine solution is filtered and concentrated to *dryness* on a rotary evaporator at 40 °C (0.01 mm). The absolutely solid mass is chipped from the flask, transferred to a vacuum filter, and washed copiously with water. The solid may be air dried. It is best stored under argon; however, it may be kept in air for a month without spectral change. If the preparation is conducted entirely under argon, the chromatographic step can be eliminated. The NMR spectrum of the octaethylporphyrin iron(II) complex in pyridine-d_5 is given in Figure 4. The above preparation worked well with mesoporphyrin dimethyl ester and deuteroporphyrin dimethyl ester, but the corresponding hemes were less stable to air than the bis(piperidyl)octaethylheme.

Pyridinium chloride was prepared from freshly distilled pyridine by gassing a solution with anhydrous HCl. The concentration of the stock solution was determined by dilution with aqueous nitric acid and direct potentiometric analysis for chloride (Castro and Bartnicki, 1965).

Oxygen concentrations in pyridine and *tert*-butylamine were determined polarographically using the Beckman "Fieldlab" unit and an oxygen electrode covered with a polyethylene membrane. A stock solution of pyridine saturated with oxygen was generally employed. The oxygen concentration upon dilution of the stock solution with pyridine was determined by direct measurement.

Spectra. Visible spectra were recorded on a Cary 118C or a Beckman DBG spectrophotometer. The NMR spectra were taken on a Varian T-60 unit.

Kinetics. In general, reactions were monitored by following the disappearance of the α band of the iron(II) complexes at ~547 nm. An initial slopes treatment was employed throughout to establish the rate laws and determine the rate constants. The rate constants were calculated from the slope of plots of the initial rate (change in heme concentration/second) vs. heme oxygen or acid (pyridinium chloride) initial concentrations. The expression

$$\frac{\Delta OD/\text{second}}{\Delta\epsilon_\alpha} = \text{rate} = k(\text{heme})_0(O_2)_0(H^+)_0$$

was valid over a wide range of initial concentration of reactants. The values obtained in this fashion agreed well with those calculated from integrated pseudo-first-order plots. For pyridine and imidazole, $\Delta\epsilon_\alpha = (\epsilon Fe^{II} - \epsilon Fe^{III}) = 2.7 \times 10^4$; for *tert*-butylamine, *tert*-butylpyridine, and *N*-methylimidazole, this value is 1.7×10^4, 2.2×10^4, and 2.2×10^4, respectively. As noted, integrated plots of the data with good linearity can be obtained; however, there are a variety of artifacts that can alter the D_∞ reading.[1] Thus, an integrated analysis can be difficult, and our experience is that an initial slope treatment is the most reliable and reproducible experimental approach. A typical run in pyridine is accomplished as follows. Three milliliters of a stock heme solution in pyridine is transferred under argon via a gas tight syringe (or pressured through a no. 22 stainless steel hypodermic needle) into a spectrophotometric cuvette that has been purged with argon and is under an argon sweep.[2] The solution is purged an additional 2 min and its spectrum recorded. With argon passing above the stopcock, the requisite amount of stock oxygen-pyridine solution (usually 2–100 μL) is added via glass syringe well below the surface of the solution.

In the absence of pyridinium chloride, imidazole, or other proton source, the spectrum of this solution is unchanged. For some runs, the solution was gassed gently with oxygen for 10 min followed by air equilibration for 30 min before addition of pyridinium chloride.[3] Reaction is commenced by addition of pyridinium chloride in pyridine (1–10 μL of a 0.15 M solution). The cell is shaken and placed in the cavity of the spectrophotometer. Zero time is fixed at the time of injection of the hydrochloride. Reactions in the presence of imidazole or with the imidazole bearing porphyrins were initiated by the addition of oxygen. The more rapid oxidations in *tert*-butylamine could not be conducted in this fashion. A cuvette containing a stopcock and glass pouch was employed. Solid bis(piperidyl)heme was mixed with an oxygenated *tert*-butylamine solution to initiate reaction. Our kinetic estimates for this reaction are consequently lower limits.

Results

Spectra and Bonding of the Hemes. All of the hemes em-

[1] One of the more notable artifacts that can occur with these solutions is worthy of special emphasis. *Rubber serum caps will cause the reduction of iron(III) to iron(II) porphyrins in pyridine!* Indeed, this artifact can be a convenient means for preparing bis(pyridyl) iron(II) adducts for spectral observation. We presume the reaction is due to traces of hydroquinone or mercaptan extracted from the rubber septum by pyridine.

[2] High purity argon (99.998%) was employed throughout. The gas was passed through a chromous sulfate scrubber and then through a scrubber containing pyridine over KOH.

[3] An O_2 (or air) saturated solution in the absence of added proton source oxidizes to the extent of ~17% in 4 days.

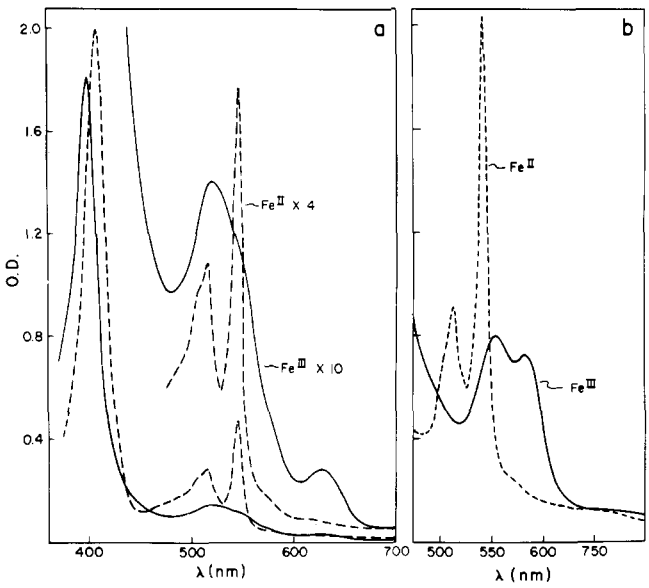


FIGURE 1: (a) Visible spectrum of iron octaethylporphyrin complexes in pyridine [Fe^{II} (- - -) and Fe^{III} (—)], 1.3×10^{-5} M. (b) In *tert*-butylamine, 4.8×10^{-5} M.

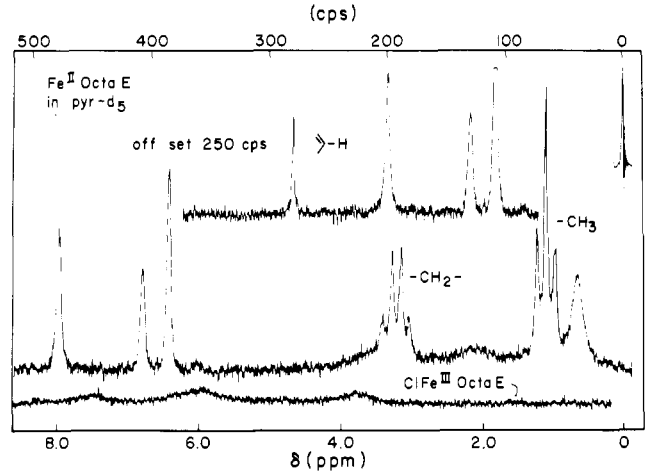


FIGURE 2: The NMR spectra of solid chloroiron(III) octaethylporphyrin and bis(piperidinato)iron(II) octaethylporphyrin dissolved in pyridine-d_5.

ployed in this work are present as hexacoordinate low-spin iron complexes in these solutions. Typical visible spectra of the iron(II) and iron(III) derivatives of octaethylporphyrin in pyridine and *tert*-butylamine are shown in Figure 1.

The corresponding NMR spectrum of the diamagnetic iron(II) complex in pyridine-d_5 is depicted in Figure 2 along with the obliterated iron(III) spectrum in the same solvent. The iron(II) bis(*tert*-butyl)amine adduct in *tert*-butylamine exhibits the same NMR spectrum as the bispyridyl species except that the methyl resonance at δ 1.2 is masked by solvent. The spectrum of iron(II) octaethylmesoporphyrin-d_4 is identical with that of the nondeuterated species except that the meso proton ($\sim\delta$ 9.4) is missing. The addition of pyridine hydrochloride at concentrations (0.088 M) far in excess of that employed in the oxidation reactions did not alter the NMR or visible spectrum of any of the heme solutions. In particular, there was no line broadening and there was no appearance of a meso proton signal in the NMR spectrum of the mesotetradeuteroiron(II) octaethylporphyrin complex. Moreover, reduction of the chloroiron(III) adducts with solid hydrazine hydrochloride or hydrazine sulfate resulted in the same sharp NMR spectra of the iron(II) complexes. We conclude the equilibrium 5

$$\text{Porp Fe}^{II}\text{L}_2 \rightleftarrows \text{Porp Fe}^{II}\text{L} + \text{L} \qquad (5)$$

does not occur in dry pyridine solutions, or it lies exceedingly far to the left, and it is not altered by pyridinium chloride or other amine acid salts.[4] In addition, there is no detectable oxygenation of these solutions in pyridine in the visible spectrum, and no oxidation occurs in the absence of pyridinium chloride during the time of these experiments.[3] The NMR results also demonstrate that the porphyrin *is not* protonated at either the meso or other positions under these conditions.

Stoichiometry. Initial oxygen concentrations in this work usually exceeded those of the starting hemes by ten or more. This is true of all of the kinetics (Table I) except for the runs at highest heme concentration indicated in Figure 4d. Under conditions of excess oxygen, the iron(II) porphyrins are cleanly converted to the iron(III) species. The corresponding spectra given in Figure 1 for the octaethylporphyrin derivatives, for example, are observed. After argon flushing, the iron(III) complex can be quantitatively converted back to the heme with hydrazine. With excess heme, spectrophotometric titration (at the α band) of the moles of heme consumed per mole of oxygen charged sets this ratio at 4.0 ± 0.4. However, the iron(III) product spectrum under these conditions is not simply that of the bis(pyridyl)iron(III) porphyrin but rather it represents a composite of this species and the oxyphlorin and "verdohemochrome" (Figure 3a). The ratio of components is a function of heme/oxygen initial concentrations. When the O_2/heme ratio is less than 5, the verdohemochrome spectrum (λ_{max} 650 nm) becomes detectable. These oxygenated products have been established to result from the reaction of bis(pyridyl)hemes with hydrogen peroxide and oxygen (Bonnet and Dimsdale, 1972). Direct reaction of bis(pyridyl)octaethylheme with hydrogen peroxide in the presence of even traces of oxygen under our conditions (Figure 3b) does produce the "verdohemochrome." The product spectrum matches exactly that reported by Bonnet and Dimsdale for this reaction. The heme is oxidized about three times more rapidly with hydrogen peroxide than it is with oxygen at the same initial concentrations, and this accords with the above observations. Taken together with earlier findings (Bonnet and Dimsdale, 1972; Castro et al., 1974), an overall multistep process for the reaction of low-spin hemes with oxygen is indicated (eq 6 and 7).

$$2\text{Porp Fe}^{II}\text{L}_2 + \text{O}_2 + 2\text{H}^+ \longrightarrow 2\text{Fe}^{III}\text{Porp L}_2 + \text{H}_2\text{O}_2 \qquad (6)$$

$$\text{Porp Fe}^{II}\text{L}_2 + \text{H}_2\text{O}_2 \xrightarrow[a]{} \text{Fe oxyphlorin}$$

$$\xrightarrow[b]{\text{O}_2} \text{verdohemochrome} \qquad (7)$$

Presumably, two hemes are oxidized in the hydrogen peroxide reaction (eq 7a). We have not studied this reaction.

Kinetics. For all porphyrins in pyridine or 4-*tert*-butylpyridine, an overall third order process is observed. The rate law for process 6 is:

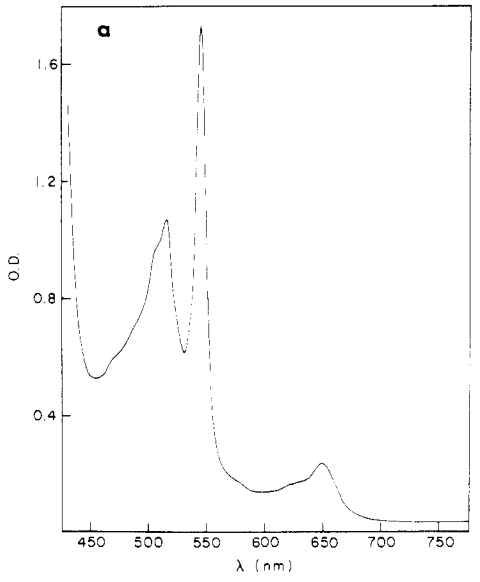
$$\text{rate} = \frac{-\text{d(Fe}^{II})}{dt} = k(\text{Fe}^{II})(\text{O}_2)(\text{H}^+)$$

[4] The presence of even a small amount of high-spin pentacoordinate iron(II) porphyrin should result in rather extensive paramagnetic broadening as it does with the iron(III) complex.

TABLE I: Rate Constants for the Oxidation of Iron(II) Porphyrin Complexes by Oxygen at 30 °C.

Porphyrin	Solvent	Axial ligands	k_3' ($L^2/(mol^2\ s)$)	~Rel rate[a]
Mesoporphyrin IX diimidazole[b]	Pyridine	Imidazole	9.4×10^5	100
Mesoporphyrin IX monoimidazole[c]	Pyridine	Imidazole, pyridine	6.6×10^5	72
Mesoporphyrin IX dimethyl ester + imidazole (0.17 M)	Pyridine	Pyridine	4.6×10^4	5
Mesoporphyrin IX dimethyl ester	Pyridine	Pyridine	9.2×10^3	1
Octaethyl	*N*-Methylimidazole	*N*-Methylimidazole	1.1×10^5	10
Octaethyl	*tert*-Butylamine	*tert*-Butylamine	(85)[d]	(100)[e]
Octaethyl	Pyridine	Pyridine	1.1×10^4	1
Octaethyl + imidazole (0.38 M)	Pyridine	Pyridine	4.9×10^4	5
Octaethyl + 4-cyanopyridine	Pyridine	Pyridine	1.1×10^4	1
Octaethyl + carbon monoxide	Pyridine	Pyridine, CO	0	
Octaethyl	*tert*-Butylpyridine	*tert*-Butylpyridine	5.9×10^3	0.6

[a] Rates relative to bis(pyridinato)iron(II) mesoporphyrin IX dimethyl ester in pyridine. [b] Bis(β-imidazolyl)ethylamide of mesoporphyrin IX (Castro, 1974). [c] Mono(β-imidazolyl)ethylamide of mesoporphyrin IX (Castro, 1974). [d] This is a second-order rate constant. [e] Estimate obtained by dividing k_2 by $(H^+)_0$ used for others. [f] All rate constants ±10% except for t-BuNH_2 (±25%).



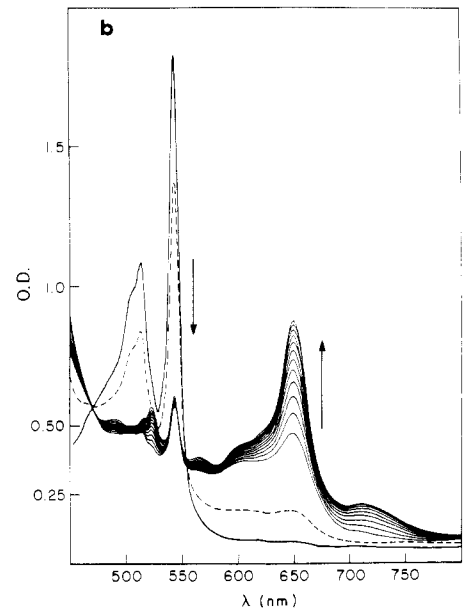


FIGURE 3: (a) Product spectrum from the O_2 oxidation of octaethylheme in pyridine, $(heme)_0/(O_2)_0 = 50$. (b) The oxidation of bis(pyridyloxtaethyl)heme by hydrogen peroxide. Spectral traces are at 1-h intervals except the dashed curve is ~2 min after mixing.

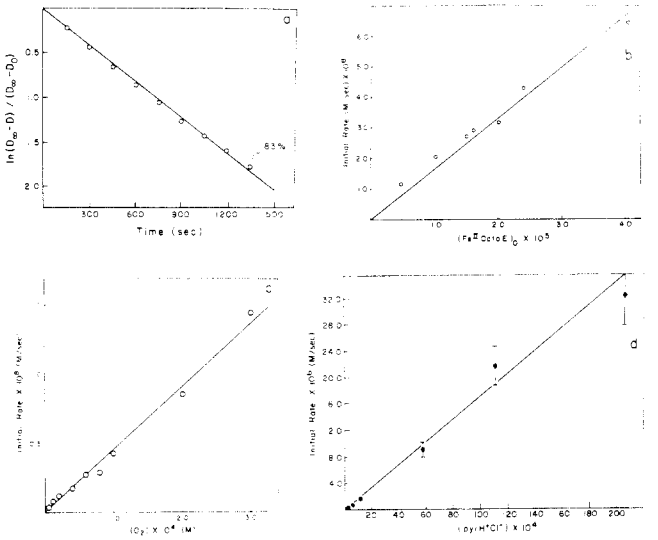


FIGURE 4: (a) Pseudo-first-order plot for the oxidation of iron(II) octaethylporphyrin by oxygen in pyridine containing pyridine hydrochloride, $(OctaE\ Fe^{II})_0 = 2.0 \times 10^{-5}$ M, $(O_2)_0 = 3.0 \times 10^{-4}$ M, $(PyrH^+) = 5 \times 10^{-4}$ M. (b) Initial rate of oxidation of iron(II) octaethylporphyrin in pyridine as a function of heme, $(O_2)_0 = 3.0 \times 10^{-4}$ M, $(pyrH^+Cl^-)_0 = 5.0 \times 10^{-4}$ M. (c) Initial rate of oxidation of iron(II) octaethylporphyrin in pyridine as a function of oxygen, $(Fe^{II}Octa\ E)_0 = 1.5 \times 10^{-5}$ M, $(pyrH^+Cl^-)_0 = 2.5 \times 10^{-4}$ M. (d) The initial rate of oxidation of bis(pyridyl)octaethylheme as a function of pyridinium chloride, $(Fe^{II}Octa\ E)_0 = 7.1 \times 10^{-4}$ M, $(O_2)_0 = 2.4 \times 10^{-4}$ M.

All rates were measured at high oxygen (usually oxygen saturated solvent) concentrations. In runs where the $(O_2)/(Fe^{II})_0$ was low (cf. Figure 4), the initial slope treatment was still representative of reaction 6. Illustrative data are summarized in Figure 4. All of the porphyrins show a linear dependence on pyridinium chloride. Other acids or proton sources may be employed instead of this amine salt. Indeed, imidazole itself can function rather feebly in this capacity. However, the influence of imidazole upon the rate of oxidation in the presence of high pyridinium chloride is negligible. It should be emphasized that nonprotic salts (e.g., sodium chloride, tetramethylammonium chloride) do not influence the rates or initiate reaction. Our data do not allow a distinction between specific (H^+) or general acid dependence.

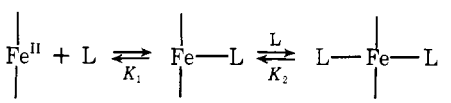
A summary of the third-order rate constants is presented in Table I. It is not possible to compare the rates in *tert*-butylamine with those in pyridine directly, because we could not

significantly alter the proton concentration in this very basic solvent. A comparison in the absence of added proton source would set the bis(*tert*-butyl)amine adduct as our most reactive species. The overall order of reactivity of the iron(II) complexes as a function of axial ligand is: bis(*tert*-butyl)amine ~ bisimidazole > imidazole, pyridine > bis(*N*-methylimidazole) > bispyridine > bis(4-*tert*-butyl)pyridine ≫ pyridine, carbon monoxide.

Activation parameters for the O_2 oxidation of bis(pyridyl)-octaethylheme in pyridine at 30 °C are: $\Delta F^{\ddagger}$, 12 kcal/mol; $\Delta H^{\ddagger}$, 16 kcal/mol; and ΔS, 14 eu.

Discussion

All of the data for the oxygen reaction (eq 6) are consistent with an outer sphere mechanism for the oxidation of low-spin hexacoordinate iron(II) porphyrins by oxygen that is acid dependent. In no case in these anhydrous amine solvents do we have spectral evidence for oxygenation of iron or dissociation of an axial ligand. This evidence, however, is thermodynamic only. On the other hand, both *tert*-butylamine and imidazole as axial ligands markedly enhance the rate of oxidation in pyridine and the diligated species is the most reactive of the imidazole complexes.[5] The hindered *tert*-butylamine is a poorer ligand for the iron(II), but at high concentrations in pyridine the corresponding bis(amine)heme reacts even in the absence of added proton source. Equilibrium constants for the processes 8



(8)

have recently been ascertained in benzene (Broualt and Rougee, 1975) for tetraphenylporphyrin and deuteroporphyrin IX. Assuming relative values are the same in pyridine, the thermodynamic ligand strength for the overall process (K_1K_2) is in the order: imidazole > pyridine > carbon monoxide (only one carbon monoxide binds iron at room temperature). A comparison of K_1 for CO and K_2 for imidazole and pyridine would result in the order: imidazole > carbon monoxide > pyridine for this triad. If an axial ligand were required to dissociate for reaction to ensue, the rate should be inversely proportional to the concentration of the ligand, and both the carbon monoxide and pyridyl adducts should react more rapidly than the imidazole (or *N*-methylimidazole) complexes.

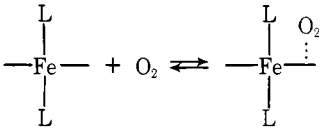
An Outer Sphere Pathway: Acid Dependence. There are four possible mechanistic sequences for the acid dependence. The relative speed of the reactions eliminates the improbable termolecular collision, and we are left with: (a) protonation of the porphyrin followed by hydrogen abstraction of the highly conjugated allylic C–H bond; (b) protonation of oxygen followed by attack on the porphyrin; (c) iron-porphyrin-oxygen affiliation followed by proton attack.

Sequence a is the reverse of the σ-meso addition mechanism for the reduction of iron(III) porphyrins. We eliminate it here by virtue of the fact that the porphyrin is not protonated under reaction conditions at high concentration of pyridinium chloride (cf. Results). Protonation of an axial ligand should result in its dissociation from iron. This is not observed.

Sequence b. The protonation of oxygen is hardly a favorable process in pyridine. However, if it did occur, the interaction of HO_2^+, like the reaction of other electrophiles (Grigg et al., 1972) with iron(II) porphyrins should be expected to result in addition to the meso position. This process with this species may result in oxidation of the metal but a meso hydroperoxy species should ensue. It *does not* under the conditions of high O_2/heme ratios (vide infra).

The remaining sequence, c, is the most reasonable and all of our data are accommodated by it. Two distinct paths for the electron transfer via this sequence can be operative, but both require a prior affiliation of oxygen with the iron–porphyrin complex.

The 1:1 Adduct. As noted above, we have no spectroscopic evidence for any affiliation of oxygen with the iron–porphyrin complex. However, a feeble π interaction akin to the weak molecular complexes noted with metalloporphyrins and nitroaromatics (Gouterman et al., 1962; Hill et al., 1967a,b; Barry et al., 1973; Fulton and LaMar, 1976) would not be expected to be discernible at low concentrations. We formulate the adduct with oxygen as the π acceptor loosely affiliated with the porphyrin ring:



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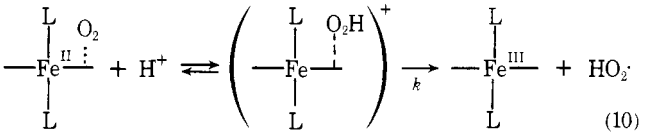
It may also simultaneously or separately interact with the axial ligand.[5]

The Electron Transfer. The most consistent interpretation of our data is that the electron transfer is a "peripheral π-process", and that it proceeds subsequent to the protonation of the 1:1 adduct (eq 10).



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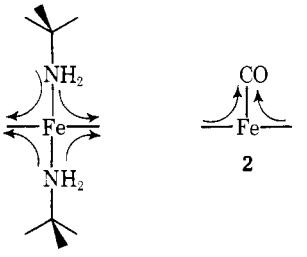
We prefer the protonation of the 1:1 complex at oxygen because this should be the most basic site in the adduct if some degree of charge transfer is manifest in its ground state.

The strong σ-coordinating *tert*-butylamine should enhance metal d porphyrin π interaction by virtue of bringing the metal into the plane of the ring without the capacity to accept metal d electrons, **1.** In contrast, the carbonyl adduct, **2,** decreases

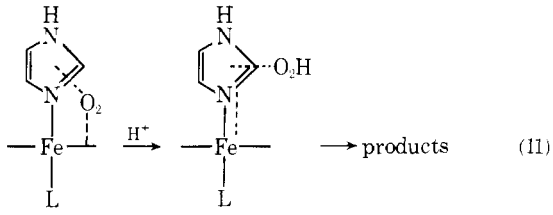


1 2

metal conjugation with the porphyrin by removing it from the plane of the ring and accepting metal electrons. Thus, the electronic and geometric aspects of the bonding of these different low-spin adducts accord exactly with the reactivity found herein and with that predicted for these extreme bond types. However, the inertness of the carbonyl adduct can also be rationalized on a thermodynamic basis. That is, an iron(III) carbonyl does not exist. The lethargy of the carbonyl adduct toward oxidation is consistent with the electrochemistry of related iron porphyrin systems (Brown et al., 1975). The influence of axial ligands upon the rates of oxygen oxidation parallels exactly that observed for the outer sphere oxidation or reduction of these iron complexes by quinone and hydroquinone (Castro et al., 1977). The rate laws for both quinone and oxygen oxidation of these species are identical. For the

[5] Spectral evidence for some degree of π bonding in a 2 heme:2 oxygen adduct has been reported (cf. Fuchsman et al., 1974).

imidazole complexes, we cannot eliminate an "axial through ligand" process in these reactions (eq 11) with the evidence at hand.



(11)

In sum, a π transfer is possible with imidazole or pyridine if "back bonding" from the metal to these heterocycles occurs.[6]

The mechanism described here can account at least in part for the apparent contradiction in the literature (Kao and Wang, 1965; Cohen and Caughey, 1968). The first-order dependency on iron noted by Kao and Wang (1965) in aqueous pyridine and pyridine–ethanol–water could be due in part to an outer sphere path in which water or ethanol functioned as the proton source. In this sense, the apparent rate of the reaction would be expected to increase with "solvent polarity," really ethanol concentration. Our data are in agreement with the observation that oxidation rates in benzene containing pyridine are too slow to be measured. Thus, we find no reaction at all in neat pyridine. It is also true that in the aqueous and ethanol solvents the ratio of low- to high-spin porphyrins represented by the equilibrium 1 can change significantly. The high-spin pentacoordinate species oxidizes at a rate too rapid to follow by means currently at our disposal. This is not to say that the explanation and mechanism advanced by Cohen and Caughey (1968) are incorrect. Quite the contrary (cf. introductory section), their solvent conditions were such that the second order in iron (high-spin) mechanism could well dominate. We note only that the fluctuation in the kinetic order for iron in their measurements could in part be due to the operation of the outer sphere mechanism observed here. In sum, both of the earlier general interpretations of Kao and Wang (1965) and Cohen and Caughey (1968) are correct. An inner and an outer sphere mechanism for the oxidation of iron(II) porphyrins can operate. As demonstrated by Cohen and Caughey (1968), the inner sphere process is second order in iron. We show here that the outer sphere process envisioned by Kao and Wang (1965) can occur but it requires a proton source.

[6] The visible spectra of all amine base iron(II) porphyrin adducts are remarkably similar whether or not the amino moiety is part of a conjugated π system (cf. Figure 1). Indeed, iron(II) octaethylporphyrin exhibits nearly identical spectra in piperidine, *tert*-butylamine, and *N*-methylimidazole.

References

Barry, C. D., Hill, H. A. O., Mann, B. E., Sadler, P. J., and Williams, R. J. P. (1973), *J. Am. Chem. Soc. 95,* 4545.

Bonnet, R., and Dimsdale, M. J. (1972), *J. Chem. Soc. Perkins Trans. 1,* 2540.

Broualt, D., and Rougee, M. (1975), *Biochemistry 14,* 4100.

Brown, G. M., Hopf, F. R., Meyer, T. J., and Whitten, D. G. (1975), *J. Am. Chem. Soc. 97,* 5385.

Castro, C. E. (1971), *J. Theor. Biol. 33,* 475.

Castro, C. E. (1974) *Bioinorg. Chem. 4,* 64.

Castro, C. E. (1977), in The Porphyrins, Vol. V, Dolphin, D., Ed., New York, N.Y., Academic Press, (in press).

Castro, C. E., and Bartnicki, E. W. (1965), *Biochim. Biophys. Acta 100,* 384.

Castro, C. E., Robertson, C., and Davis, H. F. (1974), *Bioorg. Chem. 3,* 343.

Castro, C. E., Hathaway, G. M., and Havlin, R., (1977), *J. Am. Chem. Soc. 99,* 8032.

Castro, C. E., Wade, R. S., and Belser, N. O. (1978), *Biochemistry* (in press).

Chin, D. H., Del Gaudio, J., LaMar, G. N., and Balch, A. L. (1977), *J. Am. Chem. Soc. 99,* 5488.

Cohen, I. A., and Caughey, W. S. (1968), *Biochemistry 7,* 636.

Epstein, C. J., Straub, D. K., and Maricondi, C. (1967), *Inorg. Chem. 6,* 1720.

Fuchsman, W. H., Barlow, C. H., Wallace, W. J., and Caughey, W. S. (1974), *Biochem. Biophys. Res. Commun. 61,* 635.

Fulton, G. P., and La Mar, G. N. (1976), *J. Am. Chem. Soc. 98,* 2119, 2124, and references therein.

Gouterman, M., Stevenson, P. E., and Stevenson, J. (1962), *J. Chem. Soc. 37,* 2266.

Grigg, R., Shelton, G., Sweeney, A., and Johnson, A. W. (1972), *J. Chem. Soc. Perkins Trans. 1,* 1789.

Hill, H. A. O., MacFarlane, A. J., and Williams, R. J. P. (1967a), *Chem. Commun.,* 905.

Hill, H. A. O., Mann, B. E., and Williams, R. J. P. (1967b), *Chem. Commun.,* 906.

Kao, O. H. W., and Wang, J. H. (1965), *Biochemistry 4,* 342.