Substituted Deuteroporphyrins. IV. On the Kinetics and Mechanism of Reactions of Iron(II) Porphyrins with Oxygen*

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ABSTRACT: The kinetics of the autoxidations of dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II) and of dipyridine protoporphyrin IX dimethyl ester iron(II) were observed in benzene–pyridine and benzene–ethanol–pyridine solutions at 25°. The kinetic order of the oxidation of the diacetyl derivative was found first order in oxygen and inverse second order in pyridine. With the protoheme derivative the kinetic order of the oxidation was found to be dependent on the [oxygen]:[pyridine] ratios and varied from first order in heme when the ratio was high to second order in heme when the ratio was low; the rate at high pyridine concentration was first order in oxygen and inverse second order in pyridine while the rate at high oxygen concentration approached a limit independent of oxygen and pyridine. The mechanism presented, which is consistent with all the observed kinetic data, involves first the dissociation of a pyridine molecule from the initial dipyridine complex to yield a five-coordinate monomer. This monomer is attacked by an oxygen molecule and then by a second five-coordinate monomer to yield an oxygen-bridged dimer. Rapid decomposition of the dimer yields products. The results lead to the conclusion that the efficient reduction of oxygen by heme systems in nonaqueous media requires at least two reducing equivalents of heme per oxygen.

The participation of superoxide ion was not indicated. These results are compared with the autoxidizability of some heme proteins.

The reaction of the iron(II) porphyrin or heme moiety with oxygen constitutes the biological function of many heme proteins (*cf.* Caughey, 1967). Elucidation of mechanisms for oxygen reduction by iron(II) porphyrins is also directly relevant to the manner by which protein structure permits oxygen to bind reversibly, without reduction, in hemoglobins and myoglobins. Kao and Wang (1965) investigated the kinetics of autoxidation of protoheme reduced *in situ* in aqueous pyridine and also, to a lesser extent, the autoxidation of the corresponding heme dimethyl ester in ethanol–benzene–pyridine solutions. In aqueous pyridine they found a rate law of the form of eq 1, where py is pyridine and k_a and k_b are first-order rate constants; they

$$-\mathrm{d[heme]}/\mathrm{d}t = 4(k_a/[\mathrm{py}] + k_b)/[\mathrm{O_2}][\mathrm{heme}] \quad (1)$$

proposed a mechanism in which one-electron transfers occured by both an inner and an outer sphere path to yield iron(III) and superoxide ion (which in turn rapidly oxidized three more hemes). Although, in extending the work to ethanol–benzene solutions, effects upon the reaction rate of changes in oxygen or pyridine concentrations were not reported, they assumed that the rate law operative in ethanol–benzene was at least in part identical with the rate law found in the aqueous system. We have now examined the rates of autoxidation of dipyridine 2,4-diacetyldeuteroheme dimethyl ester in benzene solutions under a variety of experimental conditions. The data obtained cannot be accounted for by the rate law assumed by Kao and Wang for their systems. The simplest mechanism which does comply with the observed rate law, in fact, differs markedly in its essential features from that presented by Kao and Wang. We have, therefore, also reexamined the autoxidation of dipyridineprotoheme dimethyl ester in benzene and benzene–ethanol. A portion of this work has been presented in preliminary form (Cohen and Caughey, 1966a,b).

Experimental Section

Materials. The preparation and characterization of the hemes were described elsewhere (Alben *et al.*, 1968).[1] Benzene (reagent grade) was washed with sulfuric acid and then with water, and distilled from calcium oxide. Pyridine (reagent grade) was redistilled. Oxygen, air, and prepurified nitrogen, each supplied by Matheson Co., were used as received.

Stoichiometric Measurements. The concentration of

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[1] The protoheme derivative used was prepared from chloroprotohemin dimethyl ester.

free oxygen in benzene–glacial acetic acid (9:1, v/v) was monitored with a Beckman oxygen electrode with a Teflon membrane standardized against air-saturated benzene using published solubility data (Morgan and Pyne, 1930). Typically addition of 2.0 mg (2.39×10^{-6} mole) of solid dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II) to 1.8 ml of benzene–acetic acid in a closed system with no air space resulted in the consumption of 22.7% (*i.e.*, 0.605×10^{-6} mole) of the oxygen initially present. Each mole of heme thus consumed 0.25 mole of oxygen in accord with the following

$$4\overset{\vee}{\underset{\wedge}{\text{PyFePy}}} + O_2 + 4\text{HOAc} \longrightarrow 4\overset{\vee}{\underset{\wedge}{\text{FeOAc}}} + 2H_2O \quad (2)$$

Kinetic Measurements. Reaction rates were studied by observing the decay of the hemochrome spectrum with a Cary Model 11 spectrophotometer equipped with a thermostatted cell chamber maintained at 25°. When an oxygen concentration which corresponded to air saturation was desired, the sample was contained in a glass-stoppered cell in the normal fashion. For other oxygen concentrations a 1-cm cell was capped with a rubber top which contained a vent; the rubber cap held a stainless-steel needle which extended nearly to the bottom of the cell for use as a gas bubbler. Oxygen and nitrogen were mixed, flowed past an oxygen electrode to determine the true fraction of oxygen present, bubbled through a tube containing solvent, and finally bubbled through the reaction cell. To maintain a nearly constant flow rate while spectrophotometer readings were being taken the gas flow was diverted around the reaction cell through a dummy cell.

The reactions were initiated upon addition of approximately 0.3 mg of heme to 3 ml of benzene–pyridine solution exposed to air. The solution was shaken for 15 sec, transferred to a syringe, and injected into the cell through a 0.45-μ pore filter (Millipore Corp.) to remove undissolved particles. The first spectrophotometer reading was obtained ~1 min after mixing.

Treatment of the Data. The oxygen and pyridine concentrations were set high enough so that the heme concentration was the only variable in any single kinetic run. Whenever possible the data were treated in the conventional manner for *pseudo*-first- or second-order reactions and the results were accepted if the reaction plot maintained linearity for 85% of the reaction. For the many reactions which did not maintain pure apparent first or second order, the data were statistically fitted to the simplest rate law which would describe it. This analytical approach was carried out on an IBM 7094 digital computer[2] which was programed to accept the data in the form of a set of times (t_i) and absorbance minus final absorbance (ΔA_i). The first phase of the routine generated a set of constants (C_0, C_1, $C_2 \ldots C_9$) by a least-squares fit of the data to eq 3. The routine then evaluated the first derivative of eq 3 at each data point and thus generated a set of $(d\Delta A/dt)_i$'s to correspond to the ΔA_i's. One of these terms is a function of the concentration of the heme and the other is equal to the instantaneous rate of the reaction; thus the rate law must be of the form of eq 4. Various types of functions of heme concentration were substituted for $f(\Delta A_i)$ to find that which gave the best consistent fit of the data in the least-squares sense. The program also included a plot of the data as applied to eq 4 to indicate whether the reaction curve was smooth and whether the function did fit the data for the entire course of the reaction. If 35 data points were provided, the statistics could easily satisfy both conditions to within experimental error.

$$\Delta A_i = C_0 + C_1 t_i + C_2 t_i^2 + C_3 t_i^3 + C_4 t_i^4 + \ldots\ldots + C_9 t_i^9 \quad (3)$$

$$-(d\Delta A/dt)_i = f(\Delta A_i) \quad (4)$$

Results

Reactions of Dipyridine-2,4-diacetyldeuteroheme Dimethyl Ester in Benzene. The spectral changes accompanying a typical autoxidation reaction are shown in Figure 1. The change in absorbance was followed at 570 mμ, near the wavelength of maximum absorption for the α band (568 mμ). The total heme concentration was varied from one run to another over a factor of ten and was determined spectrophotometrically for each experiment. The product spectrum was found to obey Beer's law over the concentration ranges used in these experiments. The spectrum of the initial material could not be checked for accordance with Beer's law directly, due to its reactivity, but the maintenance of an isosbestic point during the rate studies indicated the absence of deviations from Beer's law.

The kinetic order in unreacted dipyridineheme observed for the autoxidation was found to be variable and dependent upon experimental conditions even when concentrations of both oxygen and pyridine were more than tenfold greater than the initial heme concentration. Orders in heme of between one and two were found and the order was often varied during a given run. However, when reactions were run in identical duplicates using a stock solution in pyridine as a source of heme (thereby assuring the same heme concentration for each duplicate run), the reaction plots for the pair of kinetic runs were completely superimposable.

Several attempts to suppress the variability of the

[2] These computations were carried out in the Computing Center of The Johns Hopkins Medical Institutions, which is supported by Research Grant FR-00004 from the National Institutes of Health and by Educational Contributions from the International Business Machines Corp. Two library subroutines were used: least squares by orthogonal polynomials (J. Monteabaro) and a multifunction plot routine (D. Frederick).

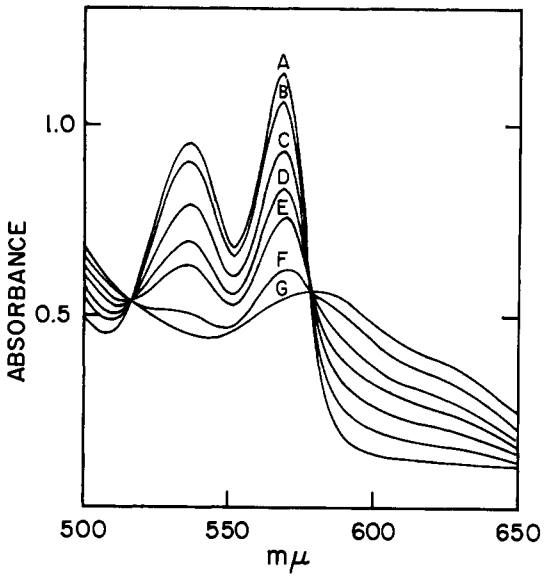

FIGURE 1: The spectral changes accompanying the autoxidation of dipyridine 2,4-diacetyldeuteroprophyrin IX dimethyl ester iron(II) in benzene at 25°. $[O_2]$ = 1.48 mM; [pyridine] = 6.0 mM; A, 2 min; B, 20 min; C, 50 min; D, 96 min; E, 147 min; F, 383 min; and G, 12 hr.

reaction order exhibited by the system were in each case unsuccessful. For example, none of the following had significant effects upon the observed kinetics: addition of 1 drop of water directly to the reaction cell; absence or presence of light; presence of a tenfold excess of product; pretreatment of the heme with pyridine; and reduction and precipitation of the dipyridineheme immediately prior to the kinetic run. These observations in addition to the reproducibility of reactions in which heme concentrations were carefully duplicated lead to the conclusion that the variability of the order of the reaction was indicative of a complex rate law rather than the result of an irreproducible artifact. (The addition under vacuum of the dipyridineheme to a benzene solution saturated with 30% aqueous hydrogen peroxide resulted in production of the spectrum of oxidation product at a rate too fast to measure by the methods employed here.)

When various functions of ΔA were substituted into eq 4, the simplest function that generated a smooth reaction plot which fit the data over the entire course of the reaction was that function shown in eq 5, where k_1' and k_2' are *pseudo*-first- and second-order rate constants, respectively. The actual function used also

$$-(d\Delta A/dt)_i = k_1'(\Delta A_i) + k_2'(\Delta A_i)^2 \qquad (5)$$

had a zero-order term which was essentially zero for every experiment and could be disregarded.

The resultant first- and second-order rate constants found are listed in Table I. The total heme concentration was determined for each run but these data are not included in Table I because no systematic relationship between the observed rates and the total heme concentration was ever found. A value of k_1' of zero was assigned to those experiments where the reaction run maintained clean second-order character for 85% of the reaction. Those results are included in the same table but are averaged separately from the reactions which were not simply second order in heme. The second-order rate constants are given in reciprocal absorbance units which better indicate the relative magnitudes of the k_1' and k_2' found for each experiment (these units can be converted into molar units using the value of 9200 as the difference between the molar extinction coefficients of the reactant and product at 570 mμ).

TABLE I: Rates of Autoxidation of Dipyridine 2,4-Diacetyldeuteroporphyrin IX Dimethyl Ester Iron(II) in Benzene at 25°.[a]

[Pyridine] (mM)	$[O_2]$ (mM)	$10^3 k_2'$ (A^{-1} sec^{-1})[b]	$10^3 k_1'$ (sec^{-1})
1.0	1.48	13.2 ± 20% (5)[c]	1.6
1.0	1.48	14.9 ± 5% (3)	0
1.5	1.48	5.69 ± 20% (12)	1.1
1.5	1.48	5.65 ± 5% (5)	0
3.0	1.48	1.21 ± 10% (7)	0.12
3.0	3.53	2.87 ± 10% (3)	0.26
3.0	5.30	3.98 ● 20% (3)	1.3
20.8	1.48	0.034 ± 25% (2)	0

[a] Total heme between 10^{-4} and 10^{-5} M. [b] A = absorbance units at 570 mμ. [c] Number of kinetic runs is included in parentheses.

The values of k_1' found for the experiments reported in Table I varied markedly, occasionally over a factor of ten. However the average values of k_1' were comparable to the mean deviations found for k_2'. Thus it would not be reasonable to interpret any quantitative relationships or trends regarding the first-order rate component. The second-order term is independent of the scatter of k_1' and, being the major portion of the reaction rate, is quite amenable to interpretation. At constant oxygen concentration, the value of k_2' over a range of 400 was strictly proportional to the inverse of the square of the pyridine concentration. At constant pyridine, the k_2' was directly proportional to the oxygen concentration. No portion of the second-order rate was independent of either oxygen or pyridine. The data in Table I can be summarized by eq 6.

$$k_2' = 7.6 \times 10^{-2} [O_2]/[\text{pyridine}]^2 \ M^{-1} \ sec^{-1} \qquad (6)$$

Reactions of Dipyridineprotoheme Dimethyl Ester in Benzene. The pyridines of dipyridineprotoheme dimethyl ester were more labile than those of the

dipyridinediacetyl derivative. Solid samples of the dipyridineprotoheme ester often lose pyridine on standing; elemental analyses of samples of this compound often indicated the presence of less than two pyridines per heme and the uptake of oxygen (Alben *et al.*, 1968). However, this does not affect the present kinetic study because any oxygen in the solid must be due to either oxidation or oxygenation of the heme. The presence of oxidation products was shown to affect neither the rate nor the order of the reaction. And, dissolving an oxygenated heme in pyridine solutions should, depending upon the pyridine concentration, either allow the rapid production of the oxidized product or displacement of the oxygen by pyridine to yield the dipyridineheme. Thus the initial spectrum was always indicative of the presence of only the dipyridineheme and the final product. The rates of autoxidation were determined by changes in the absorbance at 560 mμ, near the maximum of the α band (555 mμ). The maintenance of isosbestic points provided further evidence for the presence of only initial dipyridineheme and oxidation product during the entire course of the reaction.

For this substrate the results at low oxygen concentration are analogous to those found for the diacetyldeuteroheme and are summarized in Table II. Because of the larger values of k_1' found for this substrate, the mean deviations of k_2' in Table II are relatively

TABLE II: Rates of Autoxidation of Dipyridine Protoporphyrin IX Dimethyl Ester Iron(II) in Benzene at 25° and $[O_2]$ = 1.48 mM.[a]

[Pyridine] (mM)	$10^3\ k_2'$ (A^{-1} sec^{-1})[b]	$10^3\ k_1'$ (sec^{-1})
1.5	22 ± 50% (5)[c]	8.9
3.0	5.9 ± 35% (7)	3.5
4.5	2.8 ± 25% (4)	1.0
20.8	0.11 ± 10% (3)	0.09

[a] Total heme between 10^{-4} and 10^{-5} M. [b] A = absorbance units at 560 mμ. [c] Number of kinetic runs is included in parentheses.

large; nevertheless the average values of k_2' were, over a range of 200, again found to be proportional to the square of the inverse of the pyridine concentration. By analogy with eq 6, the data in Table II can be summarized by eq 7 (using a value of 8700

$$k_2' = 29 \times 10^{-2}\ [O_2]/[\text{pyridine}]^2\ \text{M}^{-1}\ \text{sec}^{-1} \qquad (7)$$

as the difference in the extinction coefficients of the reactant and product for the protoporphyrin derivative at 560 mμ).

The effect of high oxygen concentration upon the rate of oxidation of this substrate is important and unusual in that it caused a change in the apparent order of the reaction in heme. This is shown in Table III. The reaction was predominantly second order in heme below 3.6 mM O_2 but at higher oxygen levels, it became mainly first order. It is also apparent that the first-order constant (k_1') is not related to any simple function of either the oxygen or pyridine concentrations. As the reaction rate increased it became less sensitive to changes in the oxygen or pyridine levels.

TABLE III: Rates of Autoxidation of Dipyridine Protoporphyrin Dimethyl Ester Iron(II) in Benzene at 25°.[a]

[Pyridine] (mM)	$[O_2]$ (mM)	$10^3\ k_2'$ (A^{-1} sec^{-1})[b]	$10^3\ k_1'$ (sec^{-1})
3.0	0.92	4.2	2.4 ± 25% (3)[c]
3.0	1.48	5.9	3.5 ± 10% (7)
3.0	3.65		7.6 ± 25% (5)
3.0	5.30		9.4 ± 30% (6)
3.0	7.05		11.5 ± 10% (5)
2.0	7.05		16.9 ± 6% (3)
4.5	7.05		7.6 ± 20% (6)
6.0	7.05		2.6 ± 25% (5)

[a] Total heme between 10^{-4} and 10^{-5} M. [b] A = absorbance units at 560 mμ. [c] Number of kinetic runs is included in parentheses.

Reaction of Dipyridineprotoheme Dimethyl Ester in Ethanol–Benzene. Because the results found for the reaction in benzene were considerably more complex than those reported by Kao and Wang (1965) we also examined reactions carried out in benzene–ethanol, a solvent they had used.

Results obtained are presented in Table IV. The first entry in Table IV represents a duplication of the conditions reported by Kao and Wang for which they observed a first-order reaction with $k_1' = 8.4 \times 10^{-3}$ sec^{-1}. We found the average result of four runs to be $k_1' = (9.2 \pm 10\%) \times 10^{-3}$ sec^{-1} but the reactions we observed maintained linear first-order character for only 2–3 half-lives. Increasing the oxygen level to 1 atm extended the linearity, with eight runs maintaining first-order character for 3–4 half-lives. On the other hand, decreasing the oxygen pressure to 0.2 atm revealed that in benzene–ethanol, as in benzene, at low oxygen concentrations the reaction became second order in heme. The apparent order of the reaction was likewise affected by the pyridine concentration. As shown in Table IV, even at the highest oxygen concentration an increase in the pyridine concentration to 0.65 M brought out considerable second-order character in the reaction rate. It is apparent that the range of these experiments, as well as those of Kao and Wang, in ethanol–benzene is too limited to provide data of adequate scope to clearly support any mechanistic model.

TABLE IV: Rates of Autoxidation of Dipyridine Protoporphyrin IX Dimethyl Ester Iron(II) in Ethanol–Benzene (1:1, v/v) at 25°.[a]

pO_2 (atm)[b]	[Pyridine] (M)	$10^3 k_1'$ (sec^{-1})	$10^3 k_2'$ ($A^{-1}sec^{-1}$)[c]
0.50	0.5	9.2 ± 10% (4)[e]	0
1.0	0.5	12.4 ± 15% (8)	0
0.20	0.5	2.0 ± 30% (6)	4
1.0	0.29	18.3 ± 15% (3)	0
1.0[d]	0.65	6.6 ± 20% (3)	0
1.0[d]	0.65	5.5 ± 15% (3)	4

[a] Total heme between 10^{-4} and 10^{-5} M. [b] Oxygen pressure in equilibrium with the reaction solution with a pO_2 of 0.5 corresponding to $[O_2]$ = 4.3 mM (Kao and Wang, 1965). [c] A = absorbance units at 560 mμ. [d] Half of six runs under these conditions showed no first-order character while the other half exhibited both rate components. The two sets of runs are shown separately in the table. [e] Number of kinetic runs is included in parentheses.

Discussion

The autoxidations studied were characterized by a variability in the kinetic order of the reactions in heme. Because of this variability the observed rate constants were found to be associated with sufficiently large experimental deviations to necessitate multiple kinetic runs. The magnitude of these deviations restrains us from overemphasizing the absolute values of the rate constants. However, the range of experimental conditions used was clearly large enough to justify consideration of the trends of the results and their mechanistic implications.

The rates for the diacetylheme were mainly second order in heme but deviated toward first-order behavior. The data indicated that the term in eq 5 which was second order in heme was also first order in oxygen and inverse second order in pyridine.

The protoheme dimethyl ester in benzene–pyridine solutions underwent autoxidation with a rate which was first order in heme at both high oxygen and low pyridine concentrations and second order in heme at both low oxygen and high pyridine concentrations. In benzene–ethanol (1:1, v/v) the rate was first order in heme at 0.5 M pyridine and 4.3 mM oxygen but at higher pyridine or lower oxygen concentrations significant second-order character was observed.

A mechanism which is consistent with all the observed results is shown in eq 8–10 (where py is pyridine and N_4Fe is the in-plane iron porphyrin). The peroxide

$$\text{py–N}_4\text{Fe(II)–py} \underset{k_{II}}{\overset{k_I}{\rightleftharpoons}} [\text{py–N}_4\text{Fe}] + \text{py} \quad (8)$$

$$2[\text{py–N}_4\text{Fe}] + O_2 \xrightarrow{k_{III}} [\text{py–N}_4\text{Fe–O}_2\text{–FeN}_4\text{–py}] \quad (9)$$

$$[\text{py–N}_4\text{Fe–O}_2\text{–FeN}_4\text{–py}] + 2H_2O \xrightarrow{\text{fast}} 2\text{N}_4\text{Fe(III)OH} + H_2O_2 + \text{py} \quad (10)$$

formed in eq 10 presumably oxidizes rapidly two more hemes in accordance with an over-all stoichiometry of four hemes oxidized by each oxygen molecule. This requirement was checked experimentally (*vide supra*) and would appear satisfied in regard to both the over-all stoichiometry and the rapidity of the oxidation by hydrogen peroxide. The water participating in eq 10 could arise from water which was always present in sufficient amount in the reaction mixture.

The fast terminal reactions could also lead to a product with a single bridging oxygen such as $N_4Fe–O–FeN_4$. Formation of such a product would not require utilization of water nor the formation of peroxide and would conform to a stoichiometry of four hemes to one O_2. A product consistent with such a structure has been isolated from addition of the dipyridine diacetyldeuteroheme to aerated benzene (Alben *et al.*, 1968).[3] Furthermore, a product isolated from the autoxidation of dipyridinephthalocyaninemanganese(II) contained such an oxygen atom bridge between two manganese atoms (Vogt *et al.*, 1966).

Because the dipyridineheme contains low-spin iron(II) we cannot assume that reaction 8 is rapid but can use a steady-state approach as in eq 11. If reaction 10 is

$$0 = k_I[\text{py–N}_4\text{Fe–py}] - k_{II}[\text{py–N}_4\text{Fe}][\text{py}] - k_{III}[\text{py–N}_4\text{Fe}]^2[O_2] \quad (11)$$

rapid the over-all rate of the autoxidation is given by eq 12 and upon specifying particular chemical

$$-\text{d[Fe(II)]}/dt = k_{III}[\text{py–N}_4\text{Fe}]^2[O_2] \quad (12)$$

conditions the rate law can be simplified considerably. For example, at high pyridine and low oxygen concentrations, $k_{II}[\text{py}] >> k_{III}[\text{py–N}_4\text{Fe}][O_2]$ and the concentration of the reactive species is simply given by eq 13. This then leads to a rate law as in eq 14. On

$$[\text{py–N}_4\text{Fe}] = k_I[\text{py–N}_4\text{Fe–py}]/k_{II}[\text{py}] \quad (13)$$

$$-\text{d[Fe(II)]}/dt = (k_I^2 k_{III}/k_{II}^2)\,[\text{py–N}_4\text{Fe–py}]^2[O_2]/[\text{py}]^2 \quad (14)$$

the other hand, at low pyridine and high oxygen concentrations, $k_{III}[\text{py–N}_4\text{Fe}][O_2] >> k_{II}[\text{py}]$ and the concentration of the reactive species is as in eq 15. That

$$[\text{py–N}_4\text{Fe}] = (k_I[\text{py–N}_4\text{Fe–py}]/k_{III}[O_2])^{1/2} \quad (15)$$

[3] That neither of the hemes reacted with oxygen in a manner to modify peripheral substituent groups on the porphyrin in the course of the autoxidation reaction was supported by conversion of the autoxidation products by reduction in aqueous pyridine with dithionite to species with absorption spectra characteristic for the initial hemes.

factor leads to a rate law as given in eq 16.

$$-\mathrm{d[Fe(II)]}/\mathrm{d}t = k_1[\mathrm{py\text{-}N_4Fe\text{-}py}] \qquad (16)$$

Because the reaction presented in eq 8–10 involves the slow first-order formation of an intermediate and its slow second-order decay to product, the one mechanism is consistent with both the observed first- and second-order rate laws and explains the dependence of the reaction order upon the pyridine and oxygen concentrations.

The reaction mechanism envisioned as applicable to both the dipyridinehemes in benzene and benzene–ethanol involves two factors quite different from those found in water by Kao and Wang. First, it is implied that oxygen occupies a coordination position during the reaction with the oxidation totally an inner sphere electron-transfer process. Second, one oxygen molecule associates with two hemes during the reaction, which implies that the oxidation proceeds directly to products with no formation of higher oxidation states of iron[4] or the superoxide ion. Both these features have also been observed in autoxidations in aqueous systems. Haim and Wilmarth (1961) found that the oxidation of the pentacyanocobaltate ion by oxygen yielded decacyano-μ-peroxodicobalt(III) ion and was thus an inner sphere process. Joyner and Wilmarth (1961) also proposed the formation of decaammine-μ-peroxodichromium(III) during the reaction between oxygen and the hexaamminechromium(II) ion. These O_2-bridged dinuclear complexes are analogous to the product of reaction 9. The reaction between oxygen and ferrous perchlorate was found by George (1954) to be kinetically second order in iron and first order in oxygen, thus indicating a similarity between the reaction path in that system and that found in this work. It was also observed by Huffman and Davidson (1956) that the presence of anions which coordinate with iron(II) can greatly influence the aqueous autoxidation and in fact cause the reaction to proceed with a rate law first order in iron. This too is analogous to the effect of high pyridine:oxygen ratios found with the protoheme. We therefore believe that the possibility remains that aqueous autoxidations of hemes also proceed by a mechanism similar to that presented here. Consistent with all the above is an alternative explanation for Kao and Wang's observations in water based on the first-order limit of this mechanism. It is, however, difficult to evaluate this possibility at present due to the lack of information regarding the kinetic effect of oxygen concentration and of the heme–heme, heme–pyridine, and heme–buffer interactions in the aqueous system used by Kao and Wang.

Our results clearly indicate that in benzene solutions at least two reducing equivalents must be simultaneously provided for the efficient reduction of oxygen by a heme system. It is thus interesting to note that myoglobins and hemoglobins, which autoxidize very slowly, contain but one heme per molecule, whereas oxidases contain two or more reducing equivalents at the same site.

[4] It should be noted that an intermediate formulated as $\mathrm{py}\overset{\vee}{\underset{\wedge}{\mathrm{FeO}}}$, proposed by Alben *et al.* (1968) as likely to participate in the formation of $\mathrm{Fe}\overset{\vee}{\underset{\wedge}{\mathrm{O}}}\mathrm{Fe}$ as an autoxidation product, is formally equivalent to a ferryl iron and an iron(IV) oxidation state.

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