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Oxidation and Reduction of Iron Porphyrins and Hemoproteins by Quinones and Hydroquinones

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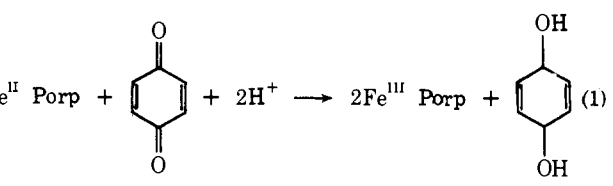
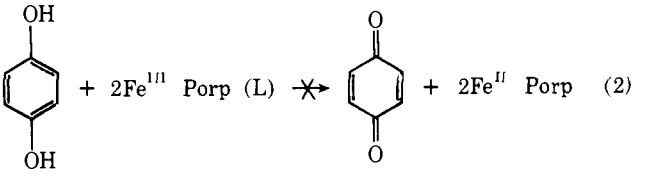
Abstract: High-spin iron(II) porphyrins in *N*-methylpyrrolidone–acetic acid, methanol, or benzene are very rapidly oxidized by quinones at room temperature. The corresponding iron(III) porphyrins and hydroquinones are the only products. The reaction is *not* reversible. The oxidation is inhibited by amine ligands that impart a low-spin state. The corresponding low-spin adducts are inert to benzoquinone in the absence of a proton source. The hexacoordinate low-spin species are, however, oxidized by an outer sphere mechanism that is acid dependent. NMR and visible spectra of the low-spin adducts and the influence of axial ligands upon the rates of oxidation are consistent with a rate-limiting step that entails dissociation of a protonated 1:1 iron porphyrin–quinone π complex. The redox reaction of the low-spin complexes is reversible, and reduction by hydroquinone is also an outer sphere process. The pattern of the influence of axial ligand upon the rate of oxidation and reduction of the low-spin complexes by quinone and hydroquinone is the same. The rate of both processes increases with expected metal–porphyrin d–π interaction in the various complexes. The results indicate two mechanisms for the oxidation of iron(II) porphyrins that are dependent upon axial ligation and spin state. High-spin (presumably) pentacoordinate hemes are oxidized by an axial inner sphere process, the low-spin hexacoordinate species by a peripheral π transfer. The reduction of these latter occurs by a similar mechanism. The reactivity of the iron(II) and iron(III) proteins myoglobin and cytochrome *c* to these reagents matches the reactivity of the corresponding iron porphyrins and accords well with theory. Hydroquinone is a specific probe for the peripheral π-transfer capacity of hemoproteins in solution.

Quinones and hydroquinones may play an important role in the electron-transport sequence[1] manifest in mitochondria. Moreover, quinones are one of the bond types noted to oxidize high-spin iron(II) porphyrins in homogeneous solution.[2] As a follow-up to these initial findings, we report here our studies of the oxidation and reduction of iron porphyrins by quinones and hydroquinones. The work is a part of our effort to develop the organic redox chemistry of these iron units such that mechanistically defined reagents may be used as substrates to plumb the influence of a protein upon the reactivity of its heme in a variety of hemoproteins. Our general approach has been summarized, and a brief account of a portion of the quinone work has been presented.[3]

Results

Stoichiometry. (a) High-Spin Iron Porphyrins. Solutions of deuteroheme in 1:1 v/v *N*-methylpyrrolidone–acetic acid[7,19] were employed as primary scanning reagent. The visible spectrum of the iron(II) complex (λ_{max} 540 nm) is typical of a high-spin heme.[4] A wide array of quinones were examined for oxidative reactivity. All quinones oxidized the heme rapidly at room temperature in this solvent, benzene (containing 1–2% acetic acid), or methanol. The stoichiometry (eq 1) was established for benzoquinone in methanol by direct spectral observation of the hydroquinone at 246–250 nm and by flame ionization gas chromatography. The reaction is quantitative. Generally, it was not possible to gas chromatograph the air-sensitive hydroquinone products, and visible spectral verification was also beclouded by the spectra of the iron(II) and iron(III) porphyrin. Consequently, many of the hydroquinone products could be demonstrated only by direct thin-layer chromatography of the reaction mixture under nitrogen (cf. the Experimental Section). In all cases, the conversion of iron(II) to iron(III) porphyrin was quantitative and, except for the porphyrins, no organics except the quinone and hydroquinone were visible in the product mixture. Table I lists the quinones examined. In no case, including ubiquinone or vitamin K_1, did the reverse reaction occur.





L = MeOH, Cl^-, OAc^-

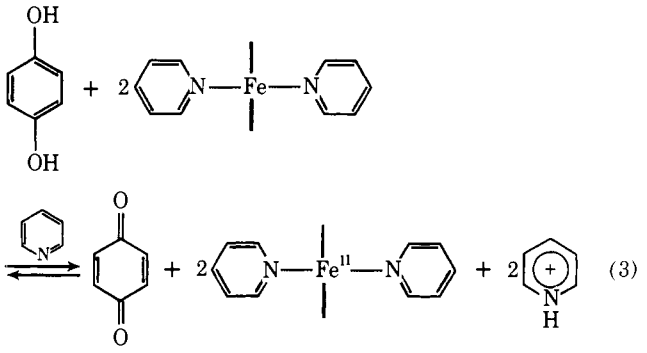
We conclude *high-spin iron(III) porphyrins* **are not** *reduced by* hydroquinones at room temperature, and this accords with estimates of the overall thermodynamics (see below). The quinone–hydroquinone pair was used to further plumb the influence of porphyrin, axial ligand, and solvent on these reactions.

(b) Low-Spin Iron Porphyrins. In contrast to the behavior exhibited by the high-spin iron complexes, their low-spin counterparts in pyridine are cleanly reduced to the iron(II)

Table I. Hydroquinones from the Oxidation of Iron(II) Deuteroporphyrin IX by Quinones in 1:1 v/v in *N*-Methylpyrrolidone-Acetic Acid at 25 °C

Quinone	Hydroquinone characterization		
1,4-Benzoquinone	TLC[a]	VPC[b]	mmp[c]
Tetramethyl-1,4-benzoquinone	TLC	VPC	
Tetrachloro-1,4-benzoquinone	TLC		
2,3-Dicyano-5,6-dichloro-1,4-benzoquinone	TLC		
1,4-Naphthoquinone	TLC	VPC	
1,2-Naphthoquinone	TLC	VPC	
Tetrahydroxy-1,4-benzoquinone	TLC		
Phenanthroquinone	TLC		
Bianthrone	TLC		
Hypericin	TLC		
Ubiquinone-30	TLC		
Vitamin K	TLC		

[a] Thin-layer chromatography under N_2. [b] Gas chromatography. [c] Mixture melting point.



(3)

adducts by hydroquinone at room temperature (eq 3). Thus, a large excess of hydroquinone (~0.01 M) with iron(III) octaethylporphyrin (10^{-4} M) in pyridine under argon yields the iron(II) dipyridyl product spectrum quantitatively in ~1 min. At lower concentrations of hydroquinone, the reaction, while longer, appears essentially complete (>99.5%). However, under these conditions a careful examination of the α,β bands of the iron(II) complex indicates they are not quite fully developed. Thus, addition of 0.5 μL of hydrazine to a 3-mL reaction causes a very slight increase in intensity of these bands. More significantly, analyses of the reaction at higher concentration (0.03 M porphyrin, 0.16 M hydroquinone) by both visible and NMR spectroscopy in pyridine-d_5 show that while the visible spectrum would indicate the reaction is nearly complete, there is *no* NMR signal for the porphyrin. The equilibrium mixture of iron(II) and iron(III) porphyrin generated by hydroquinone[5a] reduction undergoes an electron exchange reaction that broadens the signals of the diamagnetic iron(II) adduct.[5b] In agreement with the final sharpening upon hydrazine addition observed in the visible spectrum, addition of this reductant to an NMR tube causes the immediate development of a sharp NMR spectrum of the iron(II) bispyridyl adduct. Moreover, when this reaction is conducted with chloroiron(III) octaethylporphyrin-*meso*-d_4 in pyridine-d_5, brought to near completion with hydroquinone and then treated with hydrazine, the resulting NMR shows *no* absorption due to the meso H proton. Thus, a hydrogen atom *is not* transferred to the meso position upon reduction by hydroquinone (or hydrazine). It should be emphasized here that hydrazine is functioning solely as a reductant and not as an axial ligand. The final visible spectrum accords exactly with the bispyridyl adduct spectrum obtained by dissolving pure bispiperidyl iron(II) octaethylporphyrin in pyridine or by reducing the iron(III) complex with a trace of aqueous dithionite

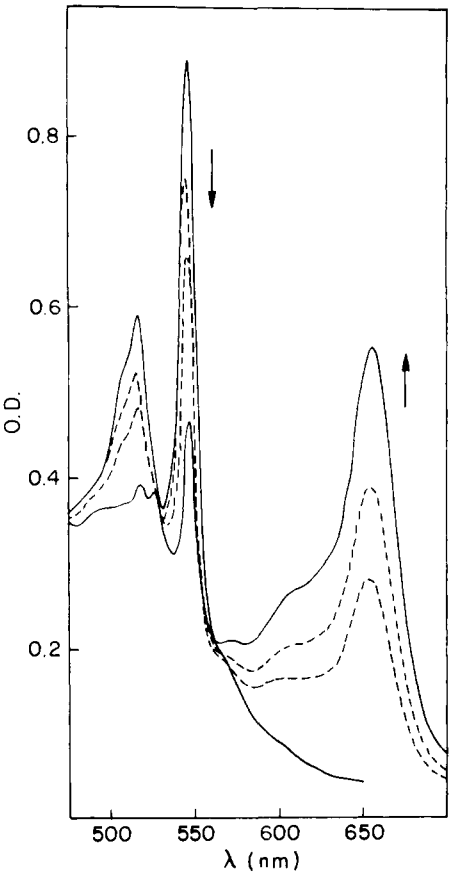


Figure 1. Reduction of bis(β-imidazolylamide) of mesohemin IX in pyridine by hydroquinone in the presence of oxygen.

in situ or a large excess of hydroquinone. The nonconjugated amine ligated iron(II) complexes all show a different intensity pattern in the α,β band region. The relevant spectra are presented elsewhere.[6] Many artifacts can occur in these systems, and it should be noted that tight anaerobic conditions are essential. Thus, reduction with hydroquinone in the presence of oxygen results in the development of a green substance with λ_{max} at 654 nm. The sequence $Fe^{III} \rightarrow Fe^{II} \rightarrow \lambda$ 654 is illustrated in Figure 1 for the diimidazole porphyrin. It is a typical response with all low-spin iron porphyrins, and it occurs with any of a series of reductants in the presence of oxygen.

In apparent contradiction to these findings, the pure iron(II) porphyrin in neat pyridine is *inert* to benzoquinone. The visible spectrum of the iron(II) adduct in pyridine remains unaltered for days upon addition of benzoquinone. However, oxidation proceeds immediately upon addition of even very low concentrations of a proton source (pyridinium chloride). Thus, the reverse reaction (eq 3) will readily occur, but it is acid dependent (cf. Thermodynamics, below).

Kinetics

High-Spin Iron. Reduction by hydroquinone does not occur with these pentacoordinate Fe^{III} species. The rate of oxidation of the high-spin iron(II) porphyrins by any of the quinones employed in this work in *N*-methylpyrrolidone-acetic acid, benzene (1% acetic acid), or methanol is very rapid and beyond the limits of conventional spectroscopic analysis. A lower limit estimate of the rate of oxidation by benzoquinone is given in Table II.

Low-Spin Iron. (a) Reduction by Hydroquinone. Integrated rate plots for these reactions were difficult to interpret. A typical example is given in Figure 2. While the pseudo-order plots of the data do more closely resemble a process that is first

Table II. Rate Constants for the Oxidation and Reduction of Iron Porphyrins by Quinones and Hydroquinones at 25 °C

Iron porphyrin	Solvent	Axial ligands	Oxidn. of Fe^{II} porphyrin by quinone, k_{ox}, L^2 mol^{-2} s^{-1} [a]	$\sim k_{ox}^{rel}$	Redn. of Fe^{III} porphyrin by hydroquinone, k_{red}, L mol^{-1} s^{-1} [a]	$\sim k_{red}^{rel}$
Deuteroporphyrin IX	NMP[e]-HOAc (1:1, v/v)		$\lesssim 10^8$	>800-1000[g]	0	0
Octaethylporphyrin	*tert*-Butylamine	*tert*-Butylamine	Fast[f]	?	80	60
Octaethylporphyrin	Pyridine	Pyridine	1.3×10^5	1	1.3	1
Octaethylporphyrin	4-*tert*-Butylpyridine	4-*tert*-Butylpyridine	3.5×10^4	0.3		
Meso IX diimidazole[b]	Pyridine	Imidazole	1.6×10^7	100	63	46
Meso IX monoimidazole[c]	Pyridine	Imidazole, pyridine	1.4×10^7	100	59	46
Meso IX dimethyl ester[d]	Pyridine	Pyridine	3.0×10^5	2.4	1.2	1
Octaethylporphyrin + CO (satd)	Pyridine	Pyridine, CO	0	0		

[a] Reproducibility ±10%. [b] Bis(β-imidazolylethylamide) of mesoporphyrin IX. [c] β-Imidazolylethylamide of mesoporphyrin IX. [d] Mesoporphyrin IX dimethyl ester. [e] *N*-Methylpyrrolidone-acetic acid in methanol or benzene (1% HOAc). [f] The amine adds to the quinone. [g] The very fast rate also obtained in methanol or benzene (1% HOAc).

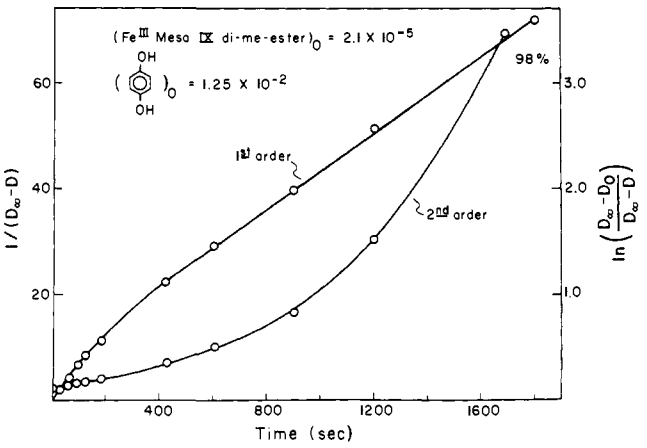


Figure 2. The reduction of mesohemin IX dimethyl ester in pyridine by hydroquinone.

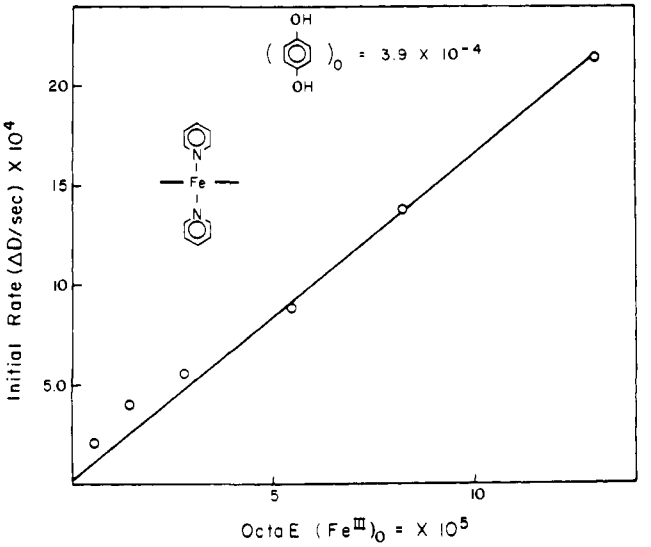


Figure 3. Initial rate of reduction of iron(III) octaethylporphyrin by hydroquinone as a function of iron(III) OEP. The initial rate is plotted as change in optical density ($D_0 - D$) at 547 nm.

order in iron, clearly they are curved. We attribute this general phenomena to the fact that the D_∞ reading can, depending upon conditions, be reduced by the occurrence of the reverse reaction and or the destruction of the α band by a combination of oxygen (leak) and hydroquinone to produce the green substance noted above. Consequently, an initial slopes treatment of the kinetic data was employed throughout.

All porphyrins showed a linear dependence on the concentration of iron(III) complex and hydroquinone over the entire concentration range (usually ~0.5–20 × 10^{-5} M in Fe^{III} and ~0.5–25 × 10^{-3} M in hydroquinone). Illustrative plots for the reduction of iron(III) octaethylporphyrin in pyridine are presented in Figures 3 and 4. The rate law for the hydroquinone reduction is:

$$\text{rate} = -\frac{d(Fe^{III})}{dt} = k(Fe^{III}\ \text{Porp}\ L_2)(p\text{-}(HO)_2C_6H_4)$$

The lack of reactivity of the high-spin pentacoordinate iron(III) species noted above was more clearly manifest by an examination of the influence of benzene upon the rate of reduction in pyridine. Setting the rate in neat pyridine as 100% reactivity, Figure 5 shows the percent of the maximum rate as a function of the mole fraction of pyridine in benzene. The percent spectral change at 635 nm represents the difference in the spectrum of chloroiron(III) octaethylporphyrins in pyridine and in benzene. Clearly, the rate parallels the distribution of the chloride and dipyridyl adducts of iron(III) with solvent composition. The fractional rate is directly proportional to the concentration of the bispyridyl iron(III) complex. Rate constants for the reduction of a series of porphyrins by hydroquinone are presented in Table III. The influence of axial ligand upon the rate is:

$$\text{Fe}^{III}\left(\text{H}_2\text{N}-\text{C}(\text{CH}_3)_3\right)_2 > \text{Fe}^{III}\left(\text{imidazole}\right)_2$$

$$\sim \text{Fe}^{III}\left(\text{imidazole}, \text{pyridine}\right) > \text{Fe}^{III}\left(\text{pyridine}\right)_2$$

$$\gg \text{Fe}^{III}\text{Cl},\ \text{Fe}^{III}(\text{OAc})$$

(b) Oxidation by Quinone. In similar fashion, the rate law for oxidation of the corresponding iron(II) adducts was found to be:

$$\frac{-d(Fe^{II})}{dt} = k(Fe^{II}\ \text{Porp}\ L_2)((p\text{-}O_2C_6H_4)(H^+)$$

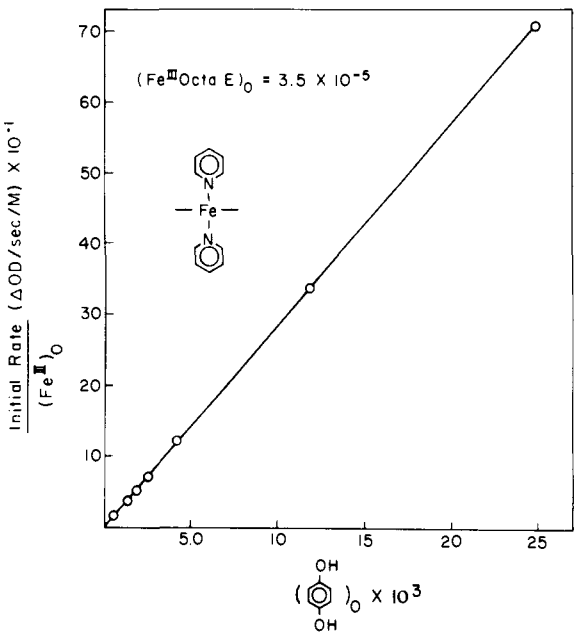


Figure 4. Initial rate of reduction of iron(III) octaethylporphyrin by hydroquinone as a function of hydroquinone. The initial rate plotted as the change in optical density ($D_0 - D$) at 547 nm with time. Initial rates are corrected for the slightly different $(Fe^{III}\ OEP)_0$.

Table III. Activation Parameters for the Reactions in Pyridine at 30 °C

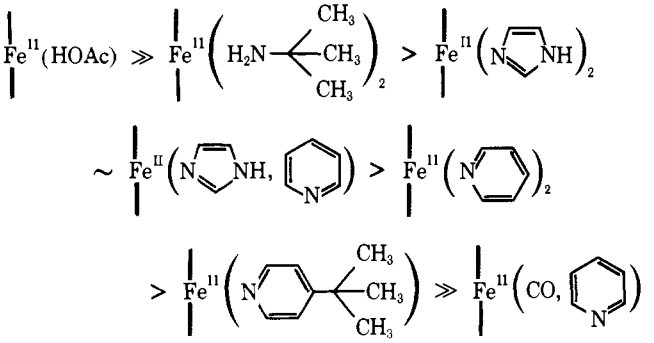
Reaction	$\Delta H^{\ddagger}$, kcal/mol	$\Delta F^{\ddagger}$	$\Delta S^{\ddagger}$, eu
Fe^{III} Octa E[a] + hydroquinone	9.7	18	−26
Fe^{III} Meso IX diimidazole + hydroquinone	8.8	16	−23
Fe^{II} Octa E + quinone	6.9	10	−11

[a] Octa E = octaethylporphyrin.

Typical kinetic data are presented for three different porphyrins in Figures 6-8 and the rate constants are summarized in Table II.

The addition of imidazole to solutions of iron(II) octaethylporphyrin in pyridine enhances the rate, and the faster process is proportional to the concentration of the diimidazole adduct (Figure 8). It will be noted that imidazole can also function as a weak proton source.

The reactivity of hemes toward oxidation by benzoquinone stands in the order:



This sequence parallels exactly that encountered for the oxidation of these same species by oxygen.[6] Moreover, for the hexacoordinate low-spin adducts of iron(III), the same pattern of reactivity obtained in the reduction by hydroquinone (see above).

Activation Parameters. The Arrhenius plot for the reduction of meso diamide by hydroquinone in pyridine is shown in Figure 9. Respective rate laws were valid at all temperatures. The activation parameters are summarized in Table III for the reactions in pyridine.

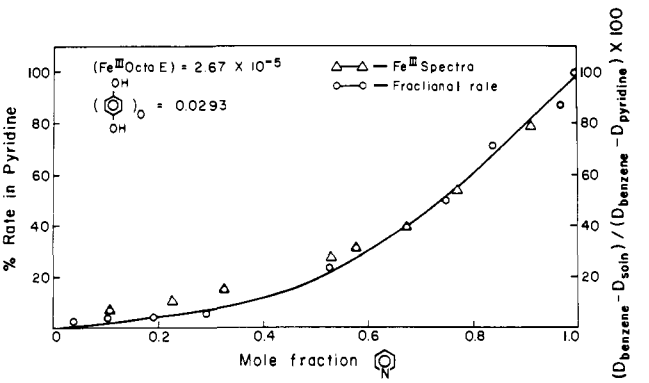


Figure 5. Fractional rate of reduction of chloroiron(III) octaethylporphyrin by hydroquinone as a function of pyridine-benzene solvent composition and state of ligation.

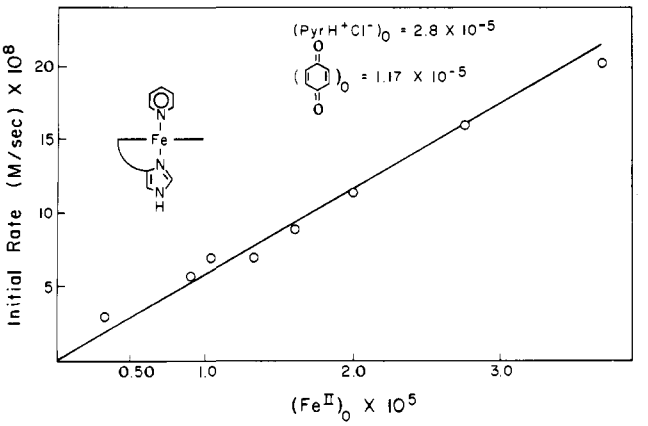


Figure 6. Rate of oxidation of iron(II) β-monoimidazolylethylamide of mesoporphyrin IX by benzoquinone in pyridine as a function of iron(II) porphyrin.

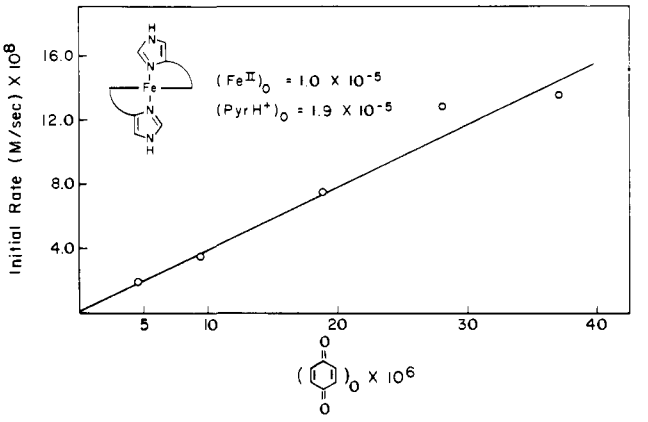


Figure 7. Rate of oxidation of iron(II) bis(β-imidazolylethylamide) of mesoporphyrin IX by benzoquinone in pyridine as a function of benzoquinone.

The Hemoproteins. The rate constants for oxidation and reduction of the corresponding iron(II) and iron(III) complexes of cytochrome *c* and myoglobin by quinone and hydroquinone are presented in Table IV. The rates of reduction of iron(III) cytochrome *c* by hydroquinone obey clean second-order kinetics over a wide range of initial concentrations (Figures 10 and 11) and show a considerable influence of ionic

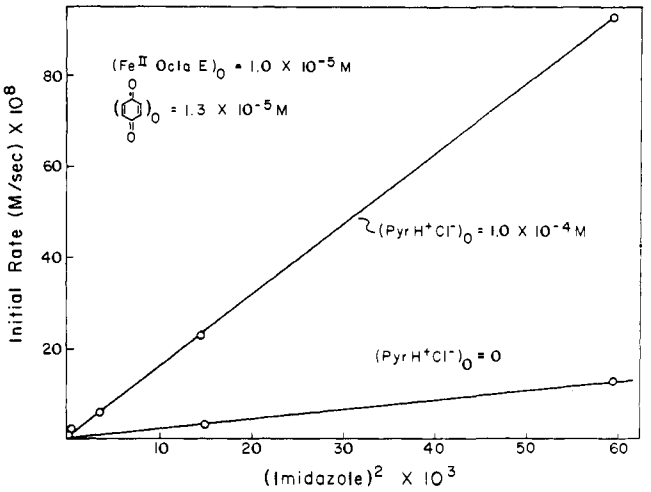


Figure 8. Rate of oxidation of iron(II) octaethylporphyrin by benzoquinone in pyridine as a function of added imidazole and pyridinium chloride.

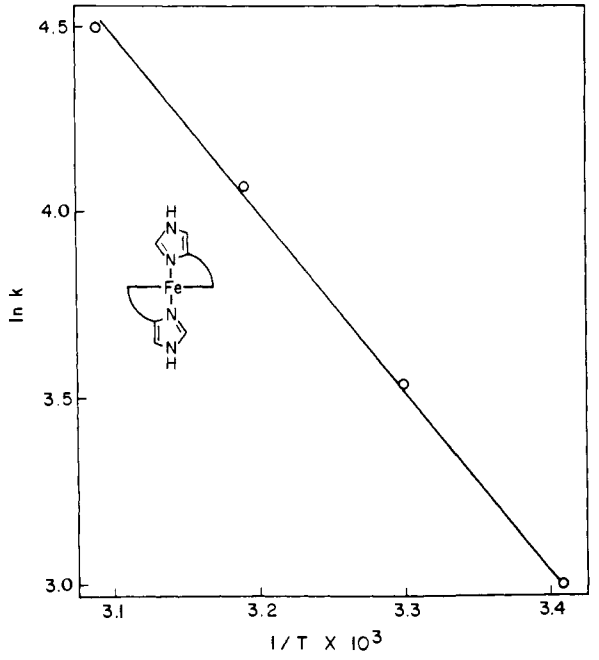


Figure 9. Arrhenius plot for the reduction of iron(III) bis(β-imidazolylethylamide) of mesoporphyrin IX by hydroquinone in pyridine.

strength (Figure 12). The rate constant (45 L mol^{-1} s^{-1}) is considerably smaller than that reported by Yamazaki and Ohnishi,[8] but these authors have based their calculations upon the semiquinone radical as the only reducing species. Our results show that hydroquinone (presumably the monoanion) itself is a reductant. Thus, for the above rate law to obtain, semiquinone concentration would have to be directly proportional to that of hydroquinone (rather than the expected half-order). It will be noted that myoglobin is inert to reduction by hydroquinone.

Thermodynamics. It is difficult to formally align the above results with the general thermochemistry of the overall redox processes or the driving force associated with discrete steps (e.g., eq 4, 5, or 8). While all of the reactions represent the interaction of a closely related iron complex with the same quinone-hydroquinone pair, the actual potentials for the half-cell reactions in the benzoquinone system[9-13] as well as those associated with the iron(II)-iron(III) porphyrin couple[14,15] can vary markedly with solvent composition and structure. Moreover, the quinone-hydroquinone couple may not be reversible in organic solvents although reversibility is

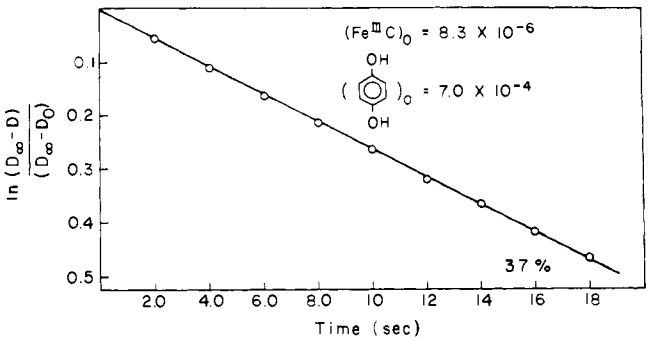


Figure 10. Rate of reduction of cytochrome *c* by hydroquinone; initial phase of the reaction in aqueous 0.01 M phosphate buffer (pH 7.4), 0.1 M KCl.

Table IV. Rate Constants for the Oxidation and Reduction of Myoglobin and Cytochrome *c*

Protein	$k_{ox}(p\text{-}O_2C_6H_4)$, L mol^{-1} s^{-1} [a]	$k_{red}(p\text{-}(HO)_2C_6H_4)$, L mol^{-1} s^{-1}	E^0,[b] V
Fe^{II} Mb	12 X 10^2		+0.23
Fe^{II} cyt *c*	20 X 10^2		+0.03
Fe^{III} Mb		0	−0.23
Fe^{III} cyt *c*		45	−0.03

[a] At pH 7.4, 0.01 M phosphate buffer, 0.1 M KCl. [b] Hemoprotein redox potentials have been measured at pH 7.0. The quinone-hydroquinone half-cell was calculated at this pH to be +0.28 V; cf. ref 9 and H. R. Mahler and E. H. Cordes, "Biological Chemistry", Harper and Row, New York, N.Y., 1966, p 568.

apparently restored (one 2-e$^-$ process) upon addition of a proton source.[12b,13,14] Given the assumptions necessary to make an extrapolation to our reaction systems, a rough thermodynamic ordering can be made for the oxidation of iron(II) porphyrins by benzoquinone. The driving force for these reactions stands in the order high-spin iron(II) porphyrin solvate ($E \sim 0.8$ V) > low-spin bisimidazole iron(II) porphyrin ($E \sim 0.4$ V) > low-spin bispyridyl iron(II) porphyrin ($E \sim 0.3$ V). The differences in the approximate relative emf's arise primarily from changes in the quinone potential in going from NMP-HOAc to pyridine containing pyridinium chloride.[12,16] The high-spin heme and the low-spin bisimidazole adduct have about the same potential.[17] The kinetic reactivity sequence for the oxidation of hemes by benzoquinone qualitatively follows the approximate driving force of these reactions; however, the corresponding reactivity for the reverse reaction does not.[18] While all low-spin iron(III) porphyrins are more rapidly reduced than the high-spin complexes by hydroquinone, the differences in reactivity among the various low-spin species do not follow the thermodynamics. On the other hand, the relative rates for the homogeneous reduction by hydroquinone do follow rather closely the relative rates observed[14] for the heterogeneous electrochemical reduction of these species. These latter do parallel the basicity of the axial ligand and we presume the extent of metal-porphyrin d-π interaction.

The potentials for the quinone and hemoprotein in aqueous solutions are known[9] and can be corrected for pH. The appropriate E^0 values for the overall reactions are given in Table IV along with the rate observations. The reduction rates by hydroquinone follow the thermodynamic order. The corresponding oxidations by benzoquinone do not.

Discussion

A large difference in reactivity and reaction characteristics is apparent in the behavior of the pentacoordinate high-spin and the hexacoordinate low-spin iron porphyrin complexes toward oxidation or reduction by the quinone-hydroquinone

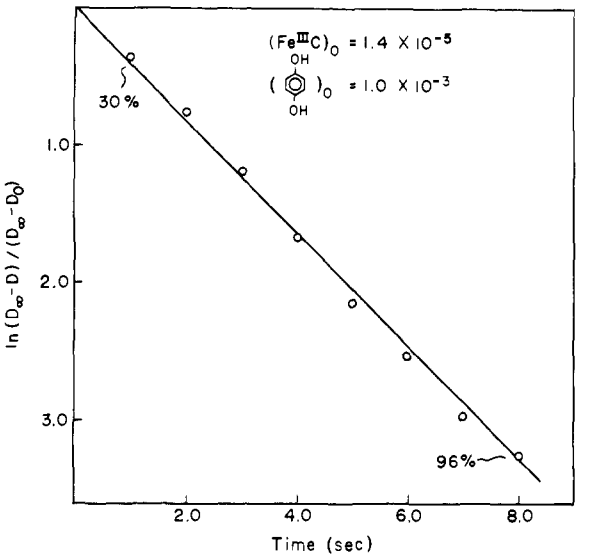


Figure 11. Rate of reduction of cytochrome *c* by hydroquinone; middle and end of reaction.

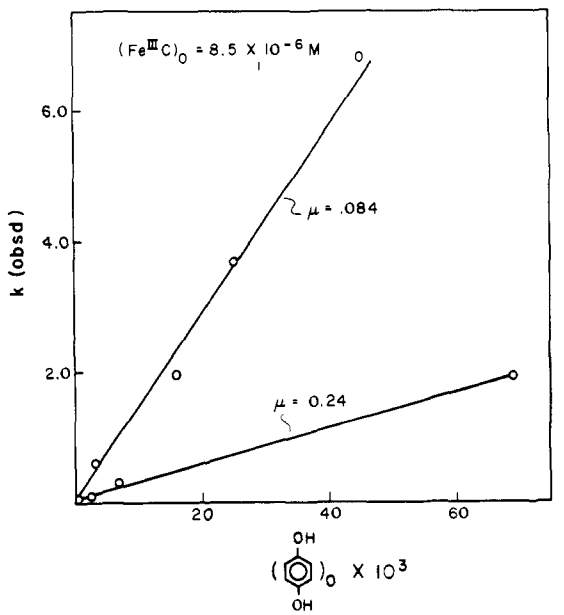
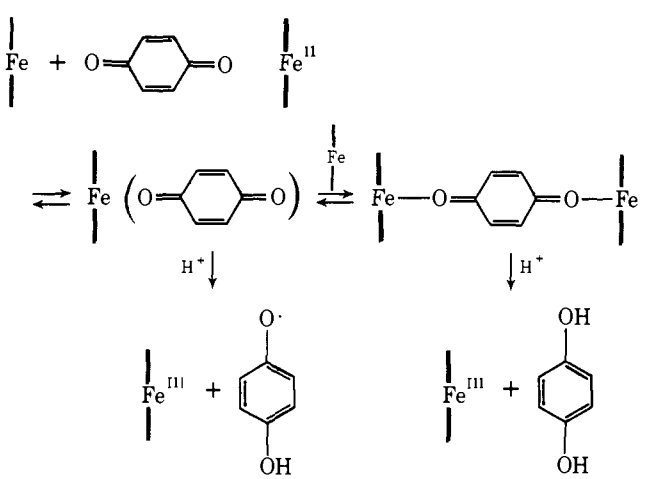


Figure 12. Rate of reduction of cytochrome *c* by hydroquinone in aqueous buffer as a function of hydroquinone and ionic strength.

pair. The oxidation of the high-spin hemes by benzoquinone (eq 1) is extremely rapid in any solvent, it is the most thermodynamically favored reaction, and it is irreversible. In contrast, the oxidation of the low-spin hemes by quinone (eq 2), while still exothermic, is much slower and it is reversible. Some indication of the marked inhibition of the oxidation by axial ligands can be obtained by comparing the rates of oxidation of the hemes by quinone in pyridine containing pyridinium chloride and benzene containing acetic acid (Table II). The axial pyridines diminish the rate 800- to 1600-fold at least.We take this large inhibition and the lack of reversibility of the reaction (eq 1) to indicate a different mechanistic path of oxidation for the high- and low-spin complexes.

The High-Spin Pathway. An Axial Inner Sphere Process. Most reasonably, the high-spin pathway entails a transition state in which the quinone has entered the inner coordination



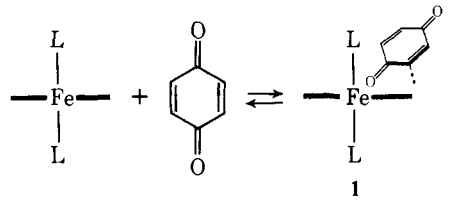
sphere of iron. The scheme above is similar to that found for the oxidation of hemes by nitro aromatics[19] and analogous to the mechanism advocated for the oxidation of high-spin iron(II) porphyrins by oxygen.[20] Electron transfer may well proceed from either a 1:1 or 2:1 iron porphyrin–quinone adduct.[21] The salient point is that the process is inhibited by good axial ligands. Thus, we characterize it as axial inner sphere. This characterization, however, is subject to an inherent ambiguity.[22]

The Low-Spin Pathway. An Outer Sphere Process. Both thermodynamic and kinetic observations support an outer sphere mechanism that is acid dependent for oxidation of the low-spin hemes by quinone. The arguments are the same as those presented for the oxidation of these species by oxygen[6] and they are only briefly outlined here.

(1) Neither the NMR nor visible spectrum of the low-spin iron(II) complexes is altered by the addition of pyridinium chloride or other amine salts. Thus, (i) the concentrations of any pentacoordinate species must be exceedingly low under reaction conditions; (ii) the porphyrin ring is not protonated prior to reaction.

(2) The visible spectrum of the iron(II) complexes of octaethylporphyrin or mesoporphyrin IX dimethyl ester in pyridine is not altered by benzoquinone. Thus, (iii) any interaction of the quinone with the porphyrin complex must be feeble and (iv) axial ligation of the quinone is not observed.

(3) The rate of oxidation by benzoquinone in pyridine is enhanced by the addition of imidazole and it is proportional to (imidazole^2). Moreover, the porphyrins with built on imidazoles react 100 times more rapidly than the dipyridyl species. The carbonyl adduct is inert. The reactivity sequence is opposite to what would be expected if dissociation of an axial ligand were essential for reaction.[23] Thus, (v) the axial ligands remain affixed during reaction; (vi) the process is outer sphere. We favor a peripheral transfer process for these reactions (eq 4 and 5). Electron transfer may ensue subsequent to or concomitant with protonation of the 1:1 π adduct **1**. An alternative scheme would entail protonation of the quinone prior to reaction 6. While not unreasonable, if there is any charge transfer at all from metal to quinone in the 1:1 adduct, this species should be the most basic. While we have formulated the 1:1 adduct (**1**) as a π complex with the periphery of the porphyrin we have no evidence for its existence at these concentrations. Moreover, our data do not eliminate a π transfer through the axial ligand. The only small decrease in rate encountered in *tert*-butylpyridine (although it is a more hindered ligand than pyridine) is not large enough to warrant conclusion. The influence of axial ligand upon the speed of these reactions, and in particular the inertness imparted by carbon monoxide coordination and the enhanced rate in *tert*-butylamine, fit very well with what has been predicted for reactivity in a peripheral redox process.[24] The bis(*tert*-butylamine) and carbonyl adducts of iron(II) porphyrins are classic examples of the influence of axial ligand upon metal-porphyrin d-π interaction.



(4)

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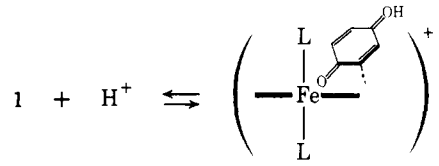

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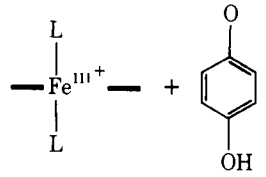
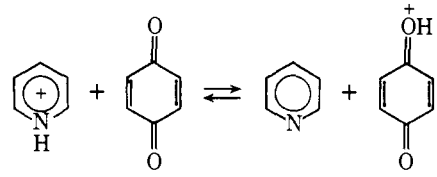


However, the inertness of the carbonyl compound can as well be ascribed to the thermodynamic instability of the corresponding iron(III) adduct. Hence, an axial through ligand process cannot be eliminated as a mechanism for oxidation with the evidence at hand.



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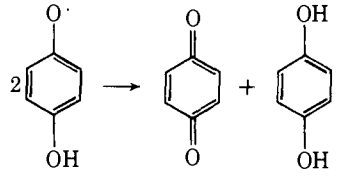
The Fate of the Semiquinone. We presume the semiquinone generated in these processes undergoes disproportionation (eq 7). Rates for this process have been ascertained[8] and would be

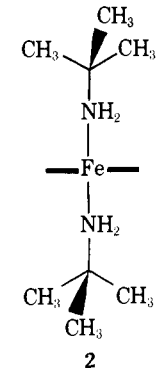


(7)

consistent with our findings. On the other hand, oxy radicals are very rapidly reduced by high-spin iron(II) porphyrins.[25] While we have no direct evidence indicating such a reaction with the hexacoordinate low-spin species, there is no reason to suppose a process analogous to eq 4 and 5 could not occur with the semiquinone. The only constraint our data would place on such a reaction is that it must be more rapid than the competing reaction with quinone, and this is reasonable.

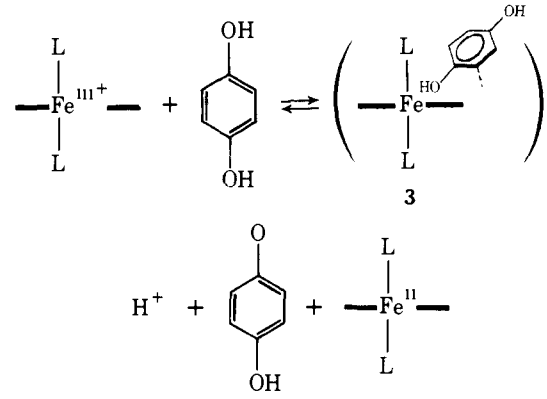
In contrast to the high-spin derivatives, the quinone oxidation of the low-spin species is reversible. We believe the reduction of the low-spin iron(III) adducts proceeds by a similar mechanism.

Thus, the same factors that enhance metal-porphyrin d-π conjugation should increase the rate of both oxidation and reduction by peripheral processes.[24] Clearly such is the case. Indeed, in this series we take the *tert*-butylamine adduct **2** to be an unambiguous example of a peripheral π transfer for the reduction. Thus, there are no vacant orbitals on the amino ligand to accept electrons from the metal or a reductant. Moreover, this amine is seriously hindered from access, yet the complex is most rapidly reduced by hydroquinone. In short, the heightened reactivity of this diamine adduct is in perfect keeping with theory, and an axial through ligand process is



2

impossible. For all porphyrins we prefer a peripheral path very similar to the oxidation (eq 8). Again, we envision the 1:1 adduct **3** as a π complex. While we have no direct spectral evidence for this intermediate, we note that at least some interaction of nitro aromatics with iron(II) porphyrins has been observed.[26] It is a weak interaction and no solid derivatives of iron(III) complexes could be isolated.[27] With hydroquinones some degree of hydrogen binding to the amine ligands may also stabilize the 1:1 adduct.



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(8)

We eliminate a "σ-meso addition mechanism" for these reductions[28] as an alternate peripheral route by virtue of the fact that no H for D exchange occurs at the meso position of iron(II) octaethylporphyrin-*meso*-d_4 upon reduction with hydroquinone. The process is a peripheral π transfer for *tert*-butylamine, but again we cannot eliminate a through-axial ligand route for electron transfer with the imidazole and pyridyl adducts.

Finally, we note that the small entropy loss associated with both the oxidation and reduction would be in keeping with our formulation of transition states for these processes, and may reflect a less ordered solvent about the free hexacoordinate ground-state complex as compared to that of a protonated 1:1 adduct **1** or **3**.

Hemoproteins. A preliminary account of our work with hemoproteins has appeared.[3] We wish to emphasize here that the redox reactivity toward quinone and hydroquinone (Table III) is in agreement with prediction[24] and the mechanistic pathways outlined above. Quinone can oxidize by either an axial inner sphere process or by a peripheral π transfer. The reactions of the high-spin iron(II) myoglobin and low-spin cytochrome *c* reflect these two processes and again underscore the modulating constraint of the protein conformation upon reactivity. The G conformation of myoglobin allows access to

G C

the metal but not without some hindrance to approach by the relatively large quinone, and the rate of the high-spin path is correspondingly attenuated. However, the availability of the porphyrin periphery in cytochrome *c* allowed by the C conformation enables the peripheral π-transfer process to take place unimpeded. Thus, in contrast to the iron porphyrins, the low-spin pathway (with cytochrome *c*) is faster than the (more thermodynamically favorable) high-spin route with myoglobin.

The rates of reduction for the two iron(III) proteins also correspond with the iron porphyrin observations. Iron(III) *c* reduces at about the same rate as the corresponding low-spin iron(III) porphyrins (cf. the bisimidazole adduct in Table II). Myoglobin is inert as it should be. That is, the iron(III) complex of myoglobin is no exception to the general observation that an inner sphere reduction by hydroquinone does not occur with high-spin iron(III) complexes. This same consideration renders an axial inner sphere mechanism impossible for the reduction of iron(III) *c*. A substitution into the inner coordination sphere of this protein should displace[29] the weaker sixth ligand and result in an iron(III) porphyrin with imidazole and hydroquinone (anion) as axial ligands. The identical inner coordination sphere must obtain in myoglobin and the heme is enshrouded in a similar environment. Clearly, reduction does not ensue from an iron(III) complex of this kind whether it is in or out of a protein. Taken together, the results with hydroquinone demonstrate that it is, in fact, an excellent probe for the peripheral π-transfer capacity of hemoproteins.

Experimental Section

Materials. All porphyrins and the preparation of the iron complexes have been previously described.[6,25,30,33] Quinones were carefully sublimed before use. Hydroquinone was recrystallized. Horse heart myoglobin and cytochrome *c* (Sigma type III) were handled as previously described.[31]

Detection of the Hydroquinone Products. Thin-layer chromatograms were conducted under nitrogen in a carefully evacuated and purged drybox that was suitably equipped. Spots of the reaction mixture, the quinone, and hydroquinone solutions were placed on the same silica gel G thin-layer plate. Chromatograms were eluted with 5:4:1 toluene–ethyl formate–formic acid and developed by spraying with potassium ferricyanide and ferric chloride.[32] The hydroquinone solutions were obtained by reducing the corresponding quinones in the drybox with sodium borohydride in 50% aqueous ethanol. The quinone and hydroquinone in any reaction mixture corresponded exactly in R_f to that of the authentic compounds. Gas chromatography of some of the hydroquinones and quinones was possible on a 1 ft × $\frac{1}{8}$ in. 20% S.E. Gum on fire brick.

Oxidation of Deuteroheme Dimethyl Ester by Benzoquinone. By the method previously described,[33] 90 mL of an 8.1×10^{-3} M heme solution in 1:1 *N*-methylpyrrolidone–acetic acid was reacted under argon with 10 mL of a 2.0×10^{-2} M solution of benzoquinone in the same solvent. The red (heme) to brown (hemin) color change occurred immediately upon addition of the quinone solution. After standing 1 h the reaction mixture was poured into 300 mL of ether. The ether solution was extracted with water. The aqueous solution was concentrated in a rotary evaporator until hydroquinone began to crystallize. The solution was cooled and filtered. The crude weight of hydroquinone was 15 mg (73%), mp 166 °C. The substance was recrystallized from ethanol–benzene, mp and mmp 170 °C.

Kinetics. Reactions with iron porphyrins were conducted in the manner previously described.[6] Usually 1–5 μL of stock quinone or hydroquinone was added via hypodermic syringe to a spectrophotometric cell that contained the requisite iron porphyrin solution. For benzoquinone oxidation, reaction could also be initiated by addition of pyridinium chloride. Oxidation or reduction was followed by monitoring the disappearance or appearance of the α band in the 550-nm region (cf. ref 6 and 30 for spectra). Note: *rubber septums cannot be employed* in the above transfers. Iron(III) porphyrins in pyridine are reduced by rubber stopples. Reactions with hemoproteins were handled in similar fashion except that an aqueous saline buffer (usually 0.1 M phosphate (pH 7.4)–0.1 M KCl) was employed as solvent. The reduction of iron(III) cytochrome *c* by hydroquinone was examined with a standard Durrum stopped flow unit.

Acknowledgment. This work was supported in part by a research grant (AM-17936) from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

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(17) Studies of the iron protoporphyrin system by cyclic voltammetry[14] would set the peak potentials of the iron(II)–iron(III) conversion in dimethylacetamide at about +0.2 V for the high-spin solvate as well as for the low-spin bis(4-aminopyridine) and bisimidazole adducts. The value for the dipyridyl adduct in this solvent (+0.09) and the $E_{1/2}$ in neat pyridine (+0.06) are lower.
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