Table VII. Resolved Rate Constants for the Reaction of $Ni^{II}(H_{-2}L)^-$ with Cyanide Ion

const	L = G_3^- [a]	L = Aib_3^-
K_1, M^{-1}	$>10^5$	$>10^4$
k_{1d}, s^{-1}		2.0×10^{-4}
k_2, $M^{-1}\ s^{-1}$	5.9	
k_2k_3/k_{-2}, $M^{-2}\ s^{-1}$	1.3×10^3	1.3×10^1
K_2, M^{-1}	17	not obsd [b]

[a] References 39 and 40. [b] This is not observed even though $[CN^-]$ is a factor of 1.5–36 higher than in the G_3 study.

strength occurs, which results in the shift of the d–d band to higher energy.

The crystal field stabilization energy (CFSE) for the square-planar d^8 nickel(II) complex should be greater than that for the d^9 copper(II) complex. This is reflected in shorter bonds for the nickel complex. Recent crystal structures[17] show that the average equatorial bond lengths are 1.83 Å for $Ni^{II}(H_{-2}Aib_3)^-$ compared to 1.92 Å for $Cu^{II}(H_{-2}Aib_3)^-$. The stability constant for the copper(II) complex is larger because electron-pairing energy is required to form the low-spin square-planar nickel(II) complex. The d^8 nickel(II) system shows a greater increase in stability constant for the Aib_3, relative to the G_3 complex, as compared to d^9 copper(II) system. This can be attributed to the larger gain in CFSE for the nickel(II) system as the donor strength of the ligand increases.

The nickel(III,II) and copper(III,II) reduction potentials have been measured for the complexes of Aib_3[6,16] and G_3.[33,34] The ratios of the stabilities of the complexes of divalent nickel and copper with Aib_3 and G_3 are now known. This information can be used to calculate the ratios (R, eq 20–22) of the stabilities of the

$$M^{II} + G_3^- \underset{-2H^+}{\overset{\beta_{1-21}}{\rightleftharpoons}} M^{II}(H_{-2}G_3)^- \overset{-e^-}{\underset{E^0}{\rightleftharpoons}} M^{III}(H_{-2}G_3) \quad (20)$$

$$M^{II} + Aib_3^- \underset{-2H^+}{\overset{\beta_{1-21}}{\rightleftharpoons}} M^{II}(H_{-2}Aib_3)^- \overset{-e^-}{\underset{E^0}{\rightleftharpoons}} M^{III}(H_{-2}Aib_3) \quad (21)$$

$$R = \frac{[M(H_{-2}Aib_3)][G_3^-]}{[M(H_{-2}G_3)][Aib_3^-]} \quad (22)$$

trivalent metal complexes of these two ligands. The R values follow the sequence Cu^{II} ($10^{1.8}$) < Ni^{II} ($10^{3.2}$) ≤ Ni^{III} ($10^{3.7}$) << Cu^{III} ($10^{6.2}$). In other words, $Ni^{III}(H_{-2}Aib_3)$ is over 5000 times more stable than $Ni^{III}(H_{-2}G_3)$, and $Cu^{III}(H_{-2}Aib_3)$ is almost 1.6 million times more stable than $Cu^{III}(H_{-2}G_3)$. Thus, the inductive effect of the six methyl groups on the copper(III) complex is enormous. The enhanced stability is consistent with previous observations of the increased thermal stability of the nickel(III) and copper(III) complexes of Aib_3, relative to that of the corresponding complexes of G_3,[6] and the large CFSE, which is expected for a d^8 trivalent metal ion with a square-planar geometry.[32]

The reaction of $Ni^{II}(H_{-2}Aib_3)^-$ with cyanide ion is also markedly slower than the corresponding reaction of $Ni^{II}(H_{-2}G_3)^-$. The values of the resolved rate constants for the reactions of both of these complexes with cyanide ion are given in Table VII. These complexes both rapidly form stable 1:1, cyanide ion mixed-ligand intermediates. The complexes react by a similar mechanism, with a rate step that depends on $[CN^-]^2$, which carries the major portion of the reaction. The third-order rate constant for this process, k_2k_3/k_{-2}, as defined in eq 19, is ~100 times smaller for the Aib_3 complex than the corresponding value for $Ni^{II}(H_{-2}G_3)^-$. In the case of the G_3 complex, there is kinetic evidence for the formation of significant concentrations of a second intermediate, $Ni^{II}(H_{-2}G_3)(CN^-)_2^{3-}$. In the present study, there is no evidence for the formation of such a species, despite the use of cyanide concentrations up to 36 times that used in the G_3 study. The failure to observe a second intermediate is presumably due to the more rigid steric restraints. The methyl groups on the Aib_3 peptide backbone limit the flexibility of the ligand, to the extent that the formation of the second mixed-ligand species is unfavorable.

The 1:1 mixed-ligand intermediate is probably an equatorial cyanide adduct, where cyanide replaces the carboxylate oxygen in the coordination sphere of the nickel atom. The main evidence in favor of an equatorial adduct, as opposed to an axial adduct, is the magnitude of the association constant (K_1 as defined in eq 11). Studies on axial adducts of nickel(III)–peptide complexes[14] give association constants that are all smaller than that obtained for K_1 in this study. It is expected that the nickel(II) complexes would have smaller axial association constants than the nickel(III) complexes, due to the smaller charge of nickel(II) and its preference for square-planar geometries. The rate of the opening of the carboxylate chelate ring has been estimated as less than 50 s^{-1} for $Cu^{II}(H_{-2}Aib_3)^-$.[18] The rate of formation of the $Ni^{II}(H_{-2}Aib_3)(CN)^{2-}$ intermediate is significantly faster than this, which suggests that the observed process is the cyanide-assisted displacement of the carboxylate oxygen from the nickel.

In general, all of the observed reactions of $Ni^{II}(H_{-2}Aib_3)^-$ except electron transfer are significantly slower than the corresponding reactions of $Ni^{II}(H_{-2}G_3)^-$. The slowness of the reactions is a reflection of the enhanced stability of the Aib_3 complex, as compared to that of the G_3 complex. The electron-donating effect of the methyl groups on the Aib_3 backbone give rise to increased donor strength of the peptide nitrogens, which is effective in stabilizing the complexes. The stabilizing effect is even greater for the trivalent metal complexes than for the divalent metal complexes of copper and nickel.

Acknowledgment. This work was supported by Public Health Service Grant No. GM-12152 from the National Institute of Medical Sciences.

Registry No. CN^-, 57-12-5.

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Electron Self-Exchange in Bis(imidazole)iron Porphyrins

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Received November 26, 1984

Self-exchange rate constants have been measured for a series of bis(imidazole)iron porphyrins. The rate constants in CD_2Cl_2 at −21 °C for bis(1-methylimidazole)-ligated tetraphenylporphyrins are as follows ($M^{-1}\ s^{-1}$): tetraphenylporphyrin (TPP), 8.1×10^7; 3-MeTPP, 5.3×10^7; 4-MeTPP, 9.7×10^7; 4-OMeTPP, 6.8×10^7; 2,4,6-Me_3TPP, 1.6×10^8. Increasing the steric bulk either at the heme edge or on the axial imidazole has little effect on the rate constant for electron self-exchange. However, complexes with axial imidazoles bearing an N–H substituent have self-exchange rate constants that are a factor of 2–3 smaller than those with *N*-alkyl substituents. The rate constants measured in this study are only slightly larger than those observed for short cytochromes. ^1H NMR spectral assignments of the $Fe^{II}TPP(RIm)_2$ complexes are reported.

Heme proteins are ubiquitous electron-transfer agents in biological processes. The role of the protein in controlling the rate or specificity of electron transfer is currently the subject of intense study.[1-9] A variety of factors have been postulated to be im-

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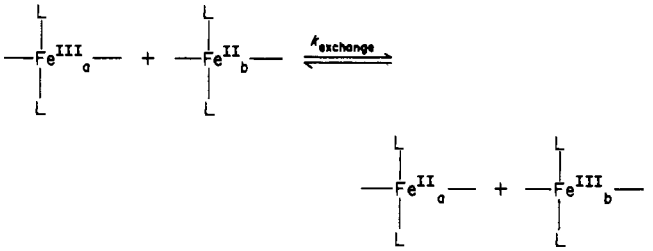
portant, including the arrangement of charged amino acids on the surface of the protein,[7,9,10] the extent of exposure of the heme,[11,12] the presence of aromatic acids close to the heme,[13] ionization of one of the heme propionates,[14,15] hydrogen bonding of the N_1H of the axial histidine,[16,17] and the overall dipole moment of the protein.[18] Accurate assessment of the role of the protein demands a knowledge of the factors that control electron transfer in the hemes themselves.[19,20]

This paper reports rate constants for electron self-exchange in a series of bis(imidazole)iron porphyrins:



The goals of this work were twofold: to determine how changes in the heme and imidazole affect electron exchange in the models and to elucidate the role of the protein by comparing electron self-exchange rate constants in model hemes with those in heme proteins.

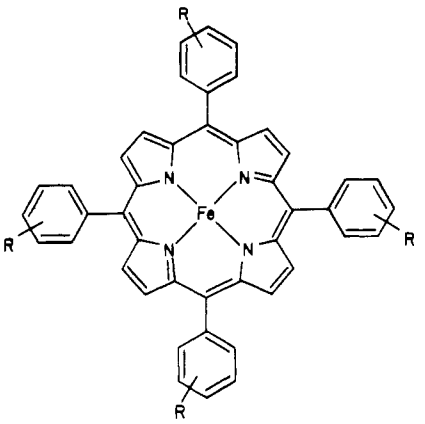
The self-exchange rate constants were measured in NMR line-broadening experiments. There are two advantages to this technique. First, because the reaction is electron self-exchange, the rate constant does not depend on the redox potential of the complex. It is thus not necessary to know either the redox potential of the complex or the rate constants for self-exchange in any partner complexes, as it would be if cross-exchange reactions and Marcus theory were used to calculate the rate constants.[20] Second, the measurements are easily made in nonaqueous solutions, which means that studies can be performed on hemes without charged substituents.

The Fe(II) and Fe(III) species are in fast exchange. Therefore, the observed chemical shift of a given resonance is the weighted average of the chemical shifts of the nonexchanging species. The line width of the resonance is the weighted average of the line widths of the nonexchanging species plus an additional term that reflects the chemical exchange:[21,22]

$$W_{DP} = f_D W_D + f_P W_P + f_D f_P 4\pi(\delta\nu)^2/kc \qquad (1)$$

where W_{DP}, W_P, and W_D are the peak widths at half-height for the exchanging peak and nonexchanging paramagnetic and diamagnetic peaks, f_P and f_D are the fractions of paramagnetic and diamagnetic species, $\delta\nu$ is the difference in chemical shift between the two nonexchanging peaks, k is the rate constant for self-exchange, and c is the total concentration of iron porphyrin.

This study has utilized tetraphenylporphyrin derivatives. These have simple spectra, are easily synthesized with various groups at the heme periphery, and have less tendency to aggregate than do the natural hemes.[23] The self-exchange rate constants for iron tetraphenylporphyrin derivatives are similar to those of the natural hemes, protoheme and deuteroheme.[19b] We have measured the rate constants as a function of changes in the substituent pattern on both the phenyl ring of the heme and the imidazole.



R = H
3-Me
4-Me
4-OMe
2,4,6-Me_3

R' =	R =
H	Me
H	n-Bu
Me	t-Bu
Me	H
t-Bu	H

Experimental Section

Porphyrins were either purchased from Midcentury Chemicals or synthesized by pyrrole–benzaldehyde condensation in a propionic acid reflux.[24] Tetramesitylporphyrin (TMP) was synthesized according to

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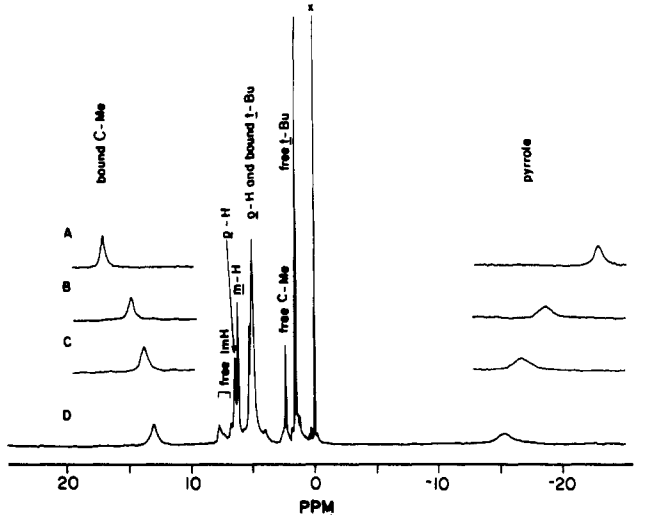

Figure 1. ^1H NMR spectra of FeTPP(1-*t*-Bu-5-MeIm)$_2$ in CD_2Cl_2 at −21 °C: (A) Fe(III); (B) 14% reduced; (C) 20% reduced; (D) 25% reduced.

the procedure of Groves and Nemo.[25] Iron was inserted with use of $FeCl_2$ in DMF.[26] Hemin purity was checked by TLC and by UV/vis and ^1H NMR spectroscopy. $Na_2S_2O_4$ (Baker), D_2O (Aldrich), and CD_2Cl_2 (MSD Isotopes) were used as received. Imidazole (Aldrich) was recrystallized three times from benzene. The purity of 1-MeIm (Aldrich), 5-MeIm (Aldrich), and 1-*n*-BuIm (Fluka) was checked by ^1H NMR. 1-*tert*-Butyl-5-methylimidazole was prepared from the aldimine of *tert*-butylamine and acetaldehyde ($CH_3CH{=}NC(CH_3)_3$)[27] and tosylmethyl isocyanide (Aldrich) by the procedure of van Leusen et al.:[28] bp 118–120 °C (20 mmHg) (lit.[28] mp 42–44 °C); ^1H NMR ($CDCl_3$) δ 1.6 (s, 9 H), 2.4 (s, 3 H), 6.7 (d, J = 1 Hz, 1 H), 7.5 (d, J = 1 Hz, 1 H). 5-*tert*-Butylimidazole was prepared from pinacolone and formamide according to the procedure of Jönsson:[29] bp 155–158 °C (9 mmHg) [lit.[29] bp 140–145 °C (7 mmHg)]; ^1H NMR (CD_3OD) δ 1.23 (s, 9 H), 6.67 (s, 1 H), 7.49 (s, 1 H).

^1H NMR Spectra. Proton NMR spectra were recorded on a JEOL FX-100 spectrometer operating at 99.54 MHz. The spectral width was 3–5 kHz. Typical spectra for the Fe(III) species, or exchanging mixtures, had 2–8K data points, an acquisition time of 0.4–1.3 s, and 100–500 scans. Line widths for the diamagnetic peaks were measured without any line broadening. Line widths for the paramagnetic peaks were measured with 5–10-Hz line broadening. The temperature of the probe (−21 °C) was measured with a methanol thermometer.[30]

Sample Preparation and Data Collection. The heme was dissolved in CD_2Cl_2 in a screw-top NMR tube (Wilmad). The tube was bubbled for 5–10 min with nitrogen or argon. Reduction was effected by adding aliquots of saturated aqueous (D_2O) $Na_2S_2O_4$.[31] Usually 10 μL of a solution reduced approximately 5% of the heme, which was 3–12 mM in 500 μL of CD_2Cl_2. Typical runs had six to eight spectra; at least four were recorded in each case. Every kinetic run except that of the mesityl porphyrin was duplicated. The rate constants measured were independent of the concentration of the heme or the number of equivalents of the imidazole (6–20 equiv).

CD_2Cl_2 was chosen as a solvent for these studies because it was necessary to cool the heme solutions to the point where the imidazole did not exchange (see Results). It was chosen in preference to $CDCl_3$ because the latter can react with Fe(II) hemes.[31b] CD_2Cl_2 had the additional advantage that much of the work on Fe(III) equilibrium and rate constants for binding of imidazoles has been done in $CHCl_3$ (see below) and the values should be similar in the two solvents.

Table I. Electron Self-Exchange Rate Constants for Bis(imidazole)iron Tetraphenylporphyrin Complexes (CD_2Cl_2, −21 °C)

porphyrin	ligands	rate const, 10^7 M^{-1} s^{-1}
TPP	1-MeIm	8.1 ± 0.7
3-MeTPP		5.3 ± 0.6
4-MeTPP		9.7 ± 0.8
4-OMeTPP		6.8 ± 0.6
2,4,6-Me_3TPP		16 ± 5
TPP	1-*n*-BuIm	7.0 ± 0.9
	1-*t*-Bu-5-MeIm	6.8 ± 0.4
3-MeTPP	5-MeIm	2.3 ± 0.3
	5-*t*-BuIm	1.6 ± 0.2

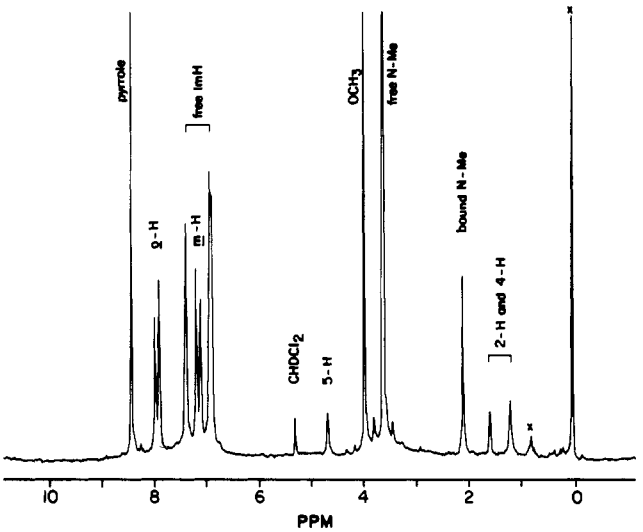

Figure 2. ^1H NMR spectrum of Fe^{II}(4-OMeTPP)(1-MeIm)$_2$ in CD_2Cl_2 at −21 °C.

Results

Electron-Transfer Rates. Partial reduction of a solution of Fe^{III}TPP(RIm)$_2$Cl in CD_2Cl_2 with aqueous $Na_2S_2O_4$ led to a mixture of the Fe(III) and Fe(II) species, which showed a single set of resonances, as described above. An example is given in Figure 1, where the shifting and broadening of both the pyrrole and bound 5-Me peaks can be seen easily. The self-exchange rate constants measured from the line broadening in these experiments are given in Table I. The reported values were measured on the pyrrole resonance, but in some instances these were checked by measuring the line broadening of the methyl resonances in the bound imidazoles. The rate constants did not depend on which signal was used for the calculation.

Assignment of Spectra. Most ^1H NMR studies of iron porphyrins have examined Fe(III) paramagnetic complexes. For this work, however, it was necessary to assign the resonances of the diamagnetic Fe(II) species as well (Table II). To make the $Fe^{II}L_2$ species, it was generally necessary to add a solution saturated in both $Na_2S_2O_4$ and the imidazole to the Fe(III) hemin. Figure 2 shows the ^1H NMR spectrum of the Fe^{II}(4-OMeTPP)(1-MeIm)$_2$ complex. The *N*-alkylimidazole Fe(III) complexes were fully reduced in 10–15 min. The N–H imidazole complexes, however, reduced more slowly, and it was necessary to let the tube sit for 1–2 h to achieve complete reduction.

Table III gives the imidazole resonances for various diamagnetic metalloporphyrins bearing either one or two imidazoles as axial ligands. Three points are noteworthy. First, the chemical shifts of the bound imidazole resonances depend on both the metal atom and the macrocycle, although the total span for a given resonance is generally <1 ppm. Second, there is essentially no dependence of chemical shift on temperature. Goff has investigated the Co(III) porphyrins and found less than a 0.03 ppm difference in

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Table II. Chemical Shifts of Heme Resonances in Diamagnetic Tetraphenylporphyrin Complexes

heme	ligand[a]	β-H	o-H	m-H	p-H
$Fe^{II}TPP$	1-MeIm	8.33	8.02[b]	7.64[b]	7.64[b]
Fe^{II}(3-MeTPP)	1-MeIm	8.41	7.83	7.53 (H) 2.53 (CH_3)	7.46
Fe^{II}(4-MeTPP)	1-MeIm	8.41	7.92 (d, J = 7.6 Hz)	7.49[c]	2.61 (CH_3)
Fe^{II}(4-OMeTPP)	1-MeIm	8.44	7.95 (d, J = 8 Hz)	7.17 (d, J = 8 Hz)	4.01 (CH_3)
Fe^{II}(2,4,6-Me_3TPP)	1-MeIm	8.08	1.84 (CH_3)	7.17	2.51 (CH_3)
$Fe^{II}TPP$	1-*n*-BuIm	8.40	8.01	7.64	7.64
$Fe^{II}TPP$	1-*t*-Bu-5-MeIm	8.37	8.00[b]	7.67[b]	7.67[b]
Fe^{II}(3-MeTPP)	5-MeIm	8.32	7.77	7.58 (H) 2.51 (CH_3)	7.51
Fe^{II}(3-MeTPP)	Im	8.38	7.79	7.52	7.46
Fe^{II}(3-MeTPP)	5-*t*-BuIm	8.36	7.80	7.45	7.45
Ni^{II}(3-MeTPP)[d]		8.55	7.65	7.39 (H) 2.56 (CH_3)	7.39
Zn^{II}(3-MeTPP)[d]		8.75	7.86	7.39 (H) 2.59 (CH_3)	7.39
In^{III}(4-*i*-PrTPP)Cl[e]		9.13	8.18[b]	7.63[b]	
TiO(4-*i*-PrTPP)[e]		9.23	8.26[b]	7.68[b]	
$Co^{III}TPP(Cl)$[f]	1-MeIm	9.09	8.03	7.78	7.78
free base, 3-MeTPP[d]		8.66	7.87	7.42 (H) 2.56 (CH_3)	7.42

[a] Two equivalent axial ligands. [b] Center of multiplet. [c] Presumably a doublet with upfield half under 2-H of free 1-MeIm. [d] Reference 32. [e] Reference 33. [f] Reference 34.

Table III. Chemical Shifts (δ) of Imidazoles Bound to Diamagnetic Metalloporphyrins

	imidazole position				line width, Hz				
complex	1	2	4	5	1	2	4	5	conditions
$Fe^{II}TPP(1\text{-MeIm})_2$	2.13 (CH_3)	1.65	1.29	4.72	3.2	3.6	3.6	4.0	CD_2Cl_2
$Fe^{II}TPP(1\text{-}n\text{-BuIm})_2$	2.38 (α-CH_2)	1.66	1.36	4.73	J = 5.5 Hz, t			4.0	CD_2Cl_2
$Fe^{II}TPP(1\text{-}t\text{-Bu-5-MeIm})_2$	0.39 (*t*-Bu)	*a*	1.15	0.91 (CH_3)			3.2	2.8	CD_2Cl_2
Fe^{II}(3-MeTPP)$(5\text{-MeIm})_2$		1.57	1.02	0.70[b] (CH_3)		6.0	8.4	13.2	CD_2Cl_2
Fe^{II}(3-MeTPP)$(Im)_2$		1.82	1.28	4.81[b]		5.2	4.0	10.3	CD_2Cl_2, −62 °C
Fe^{II}(3-MeTPP)$(1\text{-MeIm})_2$	2.14 (CH_3)	1.65	1.27	4.73	4.7	4.1	4.1	5.3	CD_2Cl_2
Fe^{II}(3-MeTPP)$(5\text{-}t\text{-BuIm})_2$		1.74	0.99	0.15 (CH_3)		2.9	*c*	4.4	CD_2Cl_2
Fe^{II}(4-MeTPP)$(1\text{-MeIm})_2$	2.12 (CH_3)	1.63	1.26	4.70	2.9	4.1	4.1	4.1	CD_2Cl_2
Fe^{II}(4-OMeTPP)$(1\text{-MeIm})_2$	2.13 (CH_3)	1.62	1.24	4.71	2.5	4.4	5.9	4.4	CD_2Cl_2
Fe^{II}(2,4,6-Me_3TPP)$(1\text{-MeIm})_2$	2.05 (CH_3)	*d*	1.55	4.64	2.6		4.1	4.1	CD_2Cl_2
Fe^{II}PPDME(CO)(1-MeIm)[e]	1.99 (CH_3)	1.05	0.36	4.86					Me_2SO
Ru^{II}MPDME(CO)(Im)[f]		0.67	0.25	3.91					$CDCl_3$, 4 °C
$Co^{III}TPP(1\text{-MeIm})_2Cl$[g,h]	2.13 (CH_3)	0.65	0.14	8.40					$CDCl_3$, −22 °C

[a] Only one of 2-H and 4-H seen; presumably 2-H is under free *t*-Bu peak at 1.58. [b] Center of multiplet. [c] Shoulder of free *t*-Bu signal. [d] Only one of 2-H and 4-H seen; presumably 2-H is under CH_3 at 1.84. [e] Reference 35. [f] Reference 36. [g] Reference 34. [h] Chemical shifts at 26 °C differ by no more than ±0.03.

chemical shift between −22 and +26 °C.[34] We have observed less than 0.06 ppm difference for the shifts of Fe^{II}(3-MeTPP)$(Im)_2$ between −62 and −21 °C. The peaks are slightly broader for the bound Im resonances of this complex at the higher temperature (e.g. 5.2–7.3 Hz for the resonance at 1.82 ppm and 4.0–6.5 Hz for the resonance at 1.28 ppm). This continued narrowing of the lines below −20 °C was not observed for complexes with substituted imidazoles.

Finally, the complexes Fe^{II}(3-CH_3TPP)$(5\text{-MeIm})_2$ and Fe^{II}(3-CH_3TPP)$(Im)_2$ show multiple resonances for 5-CH_3 and the 5-H, respectively (Figure 3). In the former case the bound 5-Me group had three major peaks (relative height 1.2:1.5:1) with a separation of ~5.3 Hz ($\Delta\nu_{1/2}$(total) = 13.2 Hz). In the latter case the 5-H proton was a broad multiplet with at least five peaks ($\Delta\nu_{1/2}$ = 10.3 Hz). This phenomenon of multiple resonances was seen only with 3-MeTPP Fe(II) complexes of imidazoles bearing N–H groups. Thus, in Fe^{II}(TPP)$(Im)_2$ the bound 5-H proton was a narrow singlet, indicating that the 3-Me substituent plays a role in the multiple resonances. On the other hand, the complex of Fe^{II}(3-CH_3TPP) with two 1-MeIm ligands showed a singlet for the bound Me ($\Delta\nu_{1/2}$ = 4.0 Hz) and narrower peaks for the 2-H and 4-H resonances [4.1 and 4.1 vs. 6.0 and 8.4 Hz for Fe^{II}(3-MeTPP)$(5\text{-MeIm})_2$]. This indicates that the N–H substituent also plays a role in the observation of multiple resonances.

We ascribe these multiple resonances to complexes arising from restricted rotation of the phenyl groups of the heme.[37] This has been studied in a variety of systems.[32,33] For metalloporphyrins with one ortho or one meta substituent on each ring (all rings the same) there are four atropisomers. In the case of ortho substituents, the isomers often show different NMR resonances. Fewer studies have been made on the meta-substituted metalloporphyrins; whether or not splitting is observed depends on the system.[32,38] The fact that splitting is more commonly observed in the ortho-substituted porphyrins probably reflects larger differences in chemical shifts of the isomers due to the proximity of the substituents. It may be a function of slower rates of rotation as well. However, even when the phenyl group itself is symmetrical with respect to rotation (unsubstituted or parasubstituted), restricted rotation has been observed to give splitting of the phenyl

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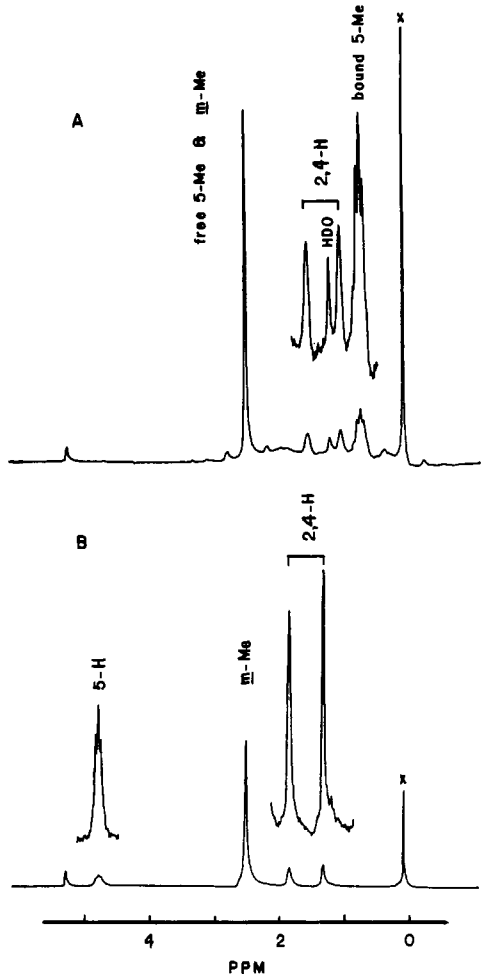

Figure 3. ^1H NMR spectra of (A) Fe^{II}(3-MeTPP)(5-MeIm)$_2$ in $CDCl_2$ at −21 °C and (B) Fe^{II}(3-MeTPP)(Im)$_2$ in CD_2Cl_2 at −21 °C.

resonances for metalloporphyrins with two different axial ligands. ΔG^* for phenyl rotation in *p*-alkyl-substituted Ru(CO)(L), InCl, and TiO porphyrins is 15–18 kcal mol^{-1}.[33]

The difference between the N–H and *N*-alkyl imidazoles in these Fe(II) hemes may be due to hydrogen bonding of the N–H group either to another imidazole or to water.[39] This would serve to make the ligand effectively bulkier and thus more likely to interact with the meta substituent. Although the equilibrium constant for this hydrogen bonding is probably low (vide infra), the large excess of water and imidazole necessary to make the $Fe^{II}(Im)_2$ species would drive the equilibrium toward the hydrogen-bonded form.

Imidazole Exchange. Equilibrium constants for binding of imidazoles to $Fe^{III}TPP^+$ derivatives are high enough (binding of two Im (β_2) $\geq 10^4$ M^{-2} at room temperature[40-45]) that ≥6 equiv of base gives a low-spin, hexacoordinate $Fe^{III}TPP(L)_2^+X^-$ species. However, to calculate rates of electron exchange, it was important to ensure that none of the line broadening was due to ligand exchange. Imidazoles exchange on the NMR time scale at ambient temperatures, but for most of the complexes cooling solutions to <0 °C slows the rate enough that no line broadening due to ligand exchange is seen. The preexchange lifetimes for the 1-MeIm and 5-MeIm complexes of $Fe^{III}TPP(Cl)$ in $CDCl_3$ at −21 °C are 9 and 70 s, respectively.[46]

The Fe(II) system is similar in many respects. At room temperature the off rate of imidazole from hexacoordinate bis(imidazole) hemes is ~1500 s^{-1} in toluene or benzene.[47,48] For $Fe^{II}TPP$ and 1-MeIm, imidazole exchange was fast on the NMR time scale at room temperture but slowed as the temperature was lowered. Separate signals were seen below 0 °C, and no further line narrowing of the bound signal was seen below ~−15 °C. Similar results were found for the other imidazoles substituted at either nitrogen or carbon. The parent imidazole itself was an exception; the line widths at −62 °C were somewhat narrower than those at −22 °C. In addition, complexes of imidazole itself with $Fe^{III}TPP^+$ derivatives tended to have more than one pyrrole signal, and no electron-exchange studies were done on this system.

Discussion

Table I shows that the self-exchange rate constants do not vary in a regular fashion with steric hindrance on the heme periphery. The total span of rate constants was less than a factor of 2. The observation that changes in steric hindrance at the heme periphery have almost no effect on the rate of electron transfer has also been found in $Fe^{III/II}TPP(CN)_2^{-/2-}$ derivatives.[19b]

The situation is similar for substitution at the N_1 position on the imidazole ring. Thus, the complexes with 1-MeIm, 1-*n*-BuIm, and 1-*t*-Bu-5-MeIm ligands all transfer electrons at approximately the same rate, showing that there is no steric effect on the rate constant for electron transfer in these systems.

The complexes with 5-MeIm and 5-*t*-BuIm, however, have slower self-exchange rate constants. In addition, the N–H complexes also reduced noticeably more slowly in the presence of aqueous dithionite than did the *N*-alkyl complexes. The observation that the rate constant for electron exchange is somewhat smaller in complexes involving substituted imidazoles indicates that the N–H bond may play a role in controlling electron transfer. We ascribe this difference to stronger hydrogen bonding of the N–H in the Fe(III) as compared with that in the Fe(II) species. This implies a necessity to rearrange the hydrogen-bound species as the oxidation state of the metal changes. This is an increase in the outer-sphere reorganization energy,[49,50] which leads to a decrease in rate.

The difference between imidazoles bearing an N–H and those bearing an *N*-alkyl group is seen in the ligation of Fe(III) porphyrins by imidazoles.[40-45] For most imidazoles, the equilibrium constant for binding the second imidazole, K_2, is larger than that for binding the first, K_1, and only the overall reaction, with $\beta_2 = K_1K_2$, is seen.[40,41] The ratio β_2(Im)/β_2(1-MeIm) decreases from 1000 to 5 as the solvent is changed from $CHCl_3$ to Me_2SO.[40,41,45] The effect is presumably due to the hydrogen bonding of the counterion to the N–H of the coordinated imidazole in the less polar $CHCl_3$, resulting in charge delocalization and increased stability of the N–H imidazole complex as compared with that of the N–R complex. Aromatic solvents are anomalous in that the equilibrium for imidazole itself involves not two, but four imidazoles. The structure of the complex has been postulated to consist of two bound imidazoles, each hydrogen bonded to another imidazole.[40,51]

The binding constants of ferrous porphyrins are not as sensitive to the presence of an N–H in the imidazole as the ferric porphyrins

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Table IV. Electron Self-Exchange Rate Constants of Cytochromes

cytochrome	class[a]	no. of amino acids	charge on Fe(III) protein[b]	methionine orientation[c]	pD	buffer	temp, °C	k_{NMR},[d] M^{-1} s^{-1}	ref
Paracoccus denitrificans c-550	L	134	−6	*R*	7.5	10 mM phosphate + 100 mM NaCl	25	1.6×10^4	70
Rhodospirillum rubrum c_2	L	112	1	*R*	6.9		30	slow[e]	71
Saccharomyces cerevisiae c I	M	108	10	*R*	7.0	0.1 M phosphate	30	10^4	72, 73
Crithidia oncopelti c-557		112	4	*R*	7.3		26	slow[e]	74
Candida krusei c	M	109	4	*R*	7	0.1–1 M salt	40	10^2–10^3	73, 75
horse heart *c*	M	104	7.5	*R*	6.6	0.1 M phosphate + 0.1 M KCl	~25	5×10^4	76a
					7	<0.05 M HEPES–0.4 M salt	30	3×10^2–1×10^4	76b
Euglena gracilis c-552	S*	87	−8	*R*	7.0	50 mM phosphate	29	5×10^6	77
Chlorobium thiosulfatophilum c-555		86	7		7.0		13	slow[e,f]	78
Desulfovibrio desulfuricans c-553		86	−2	*S*	7.7		8	slow[e,f]	79
Alcaligenes faecalis c-554		~86			8	10 mM phosphate + 100 mM NaCl	40	$\sim 3 \times 10^8$	80
Rhodopseudomonas gelatinosa c-551 I	S	85	5	*S*	7.4		8	10^5	81
Desulfovibrio vulgaris c-553		82	2	*S*	6.2		12	slow[e,f]	79
Pseudomonas aeruginosa c-551	S	82	−2	*S*	7.0	50 mM phosphate	42	1.2×10^7	82
Pseudomonas mendocina c-551	S	82	−1	*S*	6.2	CD_3OD/D_2O	−6	10^6–10^7	71
Pseudomonas stutzeri c-551	S	82	−2	*S*	6.8		30	4×10^6	71
cyt *c* undecapeptide		11			10.1	0.5 M cyanide	57	1.3×10^7	83
cyt *c* octapeptide		8				>2 equiv of pyridine		$>10^5$	84

[a] Reference 88. [b] Calculated from sequence except for *R. rubrum*, *C. krusei*, and horse.[85] [c] Fe(II) proteins, see ref 79. [d] From NMR saturation transfer, T_1, and line shape measurements. Self-exchange rate constants k_{app} calculated from the rates of cross reactions with organometallic reagents are as follows: *C. krusei c*, 2.2×10^3–2.2×10^5 M^{-1} s^{-1};[85] horse heart *c*, $6.2 - 2.4 \times 10^5$ M^{-1} s^{-1};[85,86] *E. gracilis c*-552, 2.1×10^3–1.3×10^4 M^{-1} s^{-1};[85] *Ps. aeruginosa c*-551, 2.0–1.4×10^7 M^{-1} s^{-1}.[85,86] [e] Both the Fe(II) and Fe(III) oxidation states can be seen in a mixture of the two. [f] Subambient temperature was presumably used to slow electron exchange and thereby prevent excessive line broadening. The value of k_{NMR} is probably $>10^5$ M^{-1} s^{-1}.

are. For example, β_2 is 7×10^8 M^{-2} for imidazole (benzene)[52] and 8.4×10^9 M^{-2} for 1-MeIm (toluene).[48] For $Fe^{III}TPP(Cl)$ the β_2 values are 4.6×10^4 M^{-2} (imidazole in benzene, $\beta_4^{1/2}$) and 5.8 M^{-2} (1-MeIm in toluene).[40a] Thus, equilibrium studies in models indicate that hydrogen bonding to N–H imidazoles bound to Fe(III) porphyrins is greater than that to imidazoles bound to Fe(II) porphyrins.

It must be remembered that the rate constants reported in this work are those for electron self-exchange and thus that any changes in rate constant with changes in the structure of the complex are not due to differences in the redox potential of the complexes. In any cross-exchange reactions, such as those of importance in biological electron transfer, the rate constant for electron transfer will be affected by changes in redox potential of the heme. Hydrogen bonding of the axial imidazole is expected to alter the redox potential of a heme. This has been shown recently in model systems by Sweigart and co-workers.[17] Hydrogen bonding of $Fe^{III}TPP(Im)_2^+$ with imidazole in solution increased the redox potential by 60 mV; that with phenanthroline increased it by 100 mV.

Hydrogen Bonding and Deprotonation of the Axial Histidine in Heme Proteins. One of the many factors proposed to control heme protein reactivity is hydrogen bonding of the axial imidazole N–H.[16,17] Many heme protein X-ray structures indicate that the proximal imidazole N–H is hydrogen bonded to a residue in the amino acid chain. In addition, studies on a number of heme proteins have shown that complete deprotonation of the imidazole to give the imidazolate form is possible under physiological conditions.

In models and in hemoglobin and myoglobin the pK_a of the axial imidazole N–H proton is 9–10.5, more acidic than imidazole itself by 4–5 pK_a units.[53–56] Exogenous imidazole bound to leghemoglobin has an even lower pK_a, ~7.[56,57] In addition, the proton lost in cytochrome *c'* at pH 7–9 is thought to come from the axial imidazole.[58,59]

The rate of exchange of the proximal N–H proton with solvent has been studied in NMR experiments.[57–64] The N–H exchange rates in heme proteins vary greatly, from too slow to measure ($k < h^{-1}$) to too fast to measure ($k > 10^4$ s^{-1}). La Mar and co-workers have shown that the exchange is base catalyzed and therefore involves deprotonation and not the following sequence: cleavage of the N–Fe bond, protonation of N_3, deprotonation of N_1, reprotonation of N_1, deprotonation of N_3, and reformation of the N–Fe bond.[61–63] Thus, both the equilibrium and kinetic measurements indicate that the axial imidazole can be found as the imidazolate in biological systems. The role of the hydrogen-bonded imidazole or imidazolate species in electron transfer in heme proteins is not yet clear. It is possible that a decrease in hydrogen bonding of an Fe(III) heme or increase in hydrogen bonding of an Fe(II) heme would promote electron transfer between the two species.

Electron Self-Exchange in Heme Proteins. It is generally thought that electron transfer occurs through the exposed heme edge. Many studies of cytochrome *c* have shown that modification of lysines near the heme edge produces a larger change in the

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electron-transfer rate constant than modification of lysines at a distance from the exposed heme edge.[7,10] Cross-linking[65] and derivatization[66] experiments have provided further support for this hypothesis. Computer graphics simulations of complex formation between cytochromes *c* and b_5,[67] cytochrome *c* and cytochrome *c* peroxidase,[13] and cytochrome b_5 and methemoglobin[68] have produced good fits between the charged surfaces when the proteins are oriented heme edge to heme edge with the hemes parallel.

If heme electron transfer does occur through the heme edge, then in the simplest picture the rate constant for electron transfer in the proteins will be substantially smaller (a factor of 10^{-3}–10^{-4}) than that in the model hemes, because most of the heme is inaccessible in the protein.[11,12] Protein self-exchange rate constants from the literature are given in Table IV. They were obtained in two ways. The first involved direct measurement by NMR. The second involved calculation of the self-exchange rate constants from measurements of the rates of cross reaction between the heme protein and organometallic or inorganic reagents.[85] These latter rate constants span a wide range, thought to reflect different mechanisms and distances for electron transfer from different reagents.[87] Comparing self-exchange rate constants measured by NMR for the hemes in Table I with those of the proteins measured by NMR in Table IV shows that the model compounds have rate constants only approximately 10-fold larger than those of the small (80 to 90 amino acids) cytochromes.

Table IV also shows that the self-exchange rate constants measured to date for proteins by NMR range from $\sim 10^2$ to $\sim 10^8$ M^{-1} s^{-1}. Dickerson has divided the *c*-type cytochromes into four classes based on the following sequences: long, medium, short, and short*.[88] The rates of electron self-exchange generally correlate with these classes; fast rates are found in the S and S* classes and slow rates in the M and L classes.

Assessing the basis of this correlation is difficult, because, of the cytochromes listed in Table IV, X-ray structures have been reported only for *Paracoccus denitrificans c*-550,[89] *Rhodospirillum rubrum* c_2,[90] horse heart cytochrome *c*,[91] *Chlorobium thiosulfatophilum c*-555,[92] and *Pseudomonas aeruginosa c*-551.[93] It appears that heme exposure is not the major determining factor. For example, for horse heart cytochrome *c*, ϕ, the fraction of the surface area of the protein that is heme, is 0.6%[12] and the exchange rate constant is 10^3–5×10^4 M^{-1} s^{-1}. For *P. aeruginosa c*-551, ϕ is 3%[94] and the rate constant is 1.2×10^7 M^{-1} s^{-1}. Thus, these two proteins differ by a factor of 5 in heme exposure but $\sim 10^4$ in the rate constant for electron transfer. Heme exposure calculations have utilized the crystal structure coordinates.[12] In solution there will be more motion of the amino acid backbone, but experiments indicate that the heme is still almost completely buried.[95,96]

If the steric (heme exposure) effect is indeed 10^3–10^4 in favor of the models, then one or more factors serve to either decrease the rate of electron transfer in models or increase the rate in the small proteins, because the observed difference between the two is only approximately a factor of 10. Electron self-exchange measurements on the models and proteins thus raise two questions: why do the models transfer electrons only approximately 10 times more rapidly than the small cytochromes, and why do the cytochromes show such a wide range of electron self-exchange rate constants? Controlling factors for electron transfer include inner-sphere reorganization, outer-sphere reorganization, electrostatic interactions, and, for the proteins, heme exposure, complex formation, and sequence-specific effects.

The difference in the inner-sphere reorganization energies[49,50] between proteins and models is very small, because both bond lengths and force constants are very similar in the two.[97,98] Outer-sphere reorganization energy[49,50] in the models is significant, a contribution of 2–3 kcal to $\Delta G^{\ddagger}$.[19] In the Marcus theory formalism outer-sphere reorganization energy is proportional to $1/D_{op} - 1/D_s$, where D_{op} is the optical dielectric constant (refractive index squared, ~ 1.8) and D_s is the static dielectric constant. The internal dielectric constant of the protein (ϵ usually taken as 2–4) is similar to that of methylene chloride ($\epsilon = 9$). Thus, the outer-sphere reorganization energy of the protein should be approximately half that found in methylene chloride if the protein is taken as a dielectric continuum. However, changes in the geometry of specific amino acids may contribute to the outer-sphere reorganization energy. Churg et al. have recently pointed out that one of the catalytic advantages of a heme protein compared to a free heme in water may be a low reorganization energy in the protein.[99]

Electrostatic interactions are not important in the bis(imidazole) model hemes because one of the reactants, the Fe(II) species, is uncharged. For the proteins, however, electrostatic interactions may be very important. Cytochrome *c* may orient itself in the electric field of its redox partner as the two approach.[18] This orientation presumably would produce a favorable geometry for electron transfer. For self-exchange, where the heme proteins are not natural partners, orientation could just as well produce geometries unfavorable for electron transfer. Electrostatic interactions may also govern the rate constant for electron self-exchange in cytochromes by influencing the distance between the two hemes. The X-ray structures of tuna cytochrome *c*[100] and *P. aeruginosa* cytochrome *c*-551[93] show that there are more positively charged lysines closer to the exposed heme edge in the former protein. This

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may cause the two heme edges to be further away in cytochrome *c* than in *c*-551, even when the orientation is correct for electron transfer. The greater the distance between the two hemes, the slower the rate of electron transfer, consistent with the larger self-exchange rate constant in *c*-551. A similar effect has been invoked to explain the observation that cytochrome b_5 reconstituted with protohemin dimethyl ester has a large self-exchange rate constant than the native protein (7.6×10^2 and 11 M^{-1} s^{-1}, respectively).[101] The esterified hemin has neutral, rather than negatively charged, propionate side chains.

The discussion above has assumed that electron transfer takes place only through the exposed heme edge. However, electron transfer has been shown to occur through the protein in some instances. For example, electron transfer between cytochrome *c* peroxidase and cytochrome *c* occurs with a second-order rate constant of $\sim 10^8$ M^{-1} s^{-1}. A computer-graphics-generated model for the electron-transfer complex shows that the two hemes are parallel, with an edge separation of ~ 17 Å. This is the closest possible approach due to the location of the peroxidase heme in the interior of the protein. Thus, in this case an electron is transferred rapidly over a long distance.[13]

Electron transfer through the protein has also been demonstrated in a number of studies on derivatized heme proteins. Gray[102] and Isied[4,103] and their co-workers have investigated ruthenium derivatives of cytochrome *c* and shown that intramolecular electron transfer takes place over ~ 12 Å (k = 30–50 s^{-1}, $\Delta G^\circ \simeq -4.5$ kcal mol^{-1}). Hoffman and co-workers have replaced one of the hemes in hemoglobin with a zinc porphyrin and shown that electron transfer occurs between the Zn and Fe centers (photoexcitation of ZnP, $k \approx 60$ s^{-1}, $\Delta E' = 0.6$ V, heme edge-to-edge distance ~ 20 Å).[104] Simolo et al. have shown intramolecular electron transfer within the $\alpha_2^{Zn}\beta_2^{Fe^{III}CN}{}_{Hb}$/ferricytochrome b_5 complex ($k \approx 8 \times 10^3$ s^{-1}, heme edge-to-edge distance ~ 7 Å) and Zn(cytochrome *c*)/cytochrome b_5 complex ($k \approx 4 \times 10^5$ s^{-1}, heme edge-to-edge distance ~ 8 Å).[105] Direct comparison with the self-exchange studies is difficult, because $\Delta G^\circ \neq 0$ in these systems. However, the observation that electron transfer can occur through the protein may in part explain the observation that electron exchange in model hemes is only approximately 10-fold faster than that in the small cytochromes.

Conclusions

Bis(imidazole)iron prophyrins have self-exchange rate constants that do not depend on variations in steric bulk on either the heme periphery or axial imidazole. The rate constants are smaller for complexes with imidazoles bearing an N–H, rather than an *N*-alkyl, substituent. Hydrogen bonding or complete deprotonation of the axial imidazole nitrogen atom may play a role in controlling electron transfer in heme proteins. The rate constants for model hemes (10^7–10^8 M^{-1} s^{-1}) are only approximately a factor of 10 larger than those found in the small cytochromes. This observation, together with data on the proteins themselves, argues that heme exposure is not the major determinant in controlling the rate constant for electron self-exchange in cytochromes.

Acknowledgment. We thank the National Institutes of Health (Grants AM 30479 and BRSG S07 RR07054) for support of this work.

Registry No. Fe(TPP)(1-MeIm)$_2$, 54032-54-1; Fe(3-MeTPP)(1-MeIm)$_2$, 74964-83-3; Fe(4-MeTPP)(1-MeIm)$_2$, 85538-93-8; Fe(4-OMeTPP)(1-MeIm)$_2$, 85529-45-9; Fe(2,4,6-Me$_3$TPP)(1-MeIm)$_2$, 93110-26-0; Fe(TPP)(1-*n*-BuIm)$_2$, 71768-83-7; Fe(TPP)(1-*t*-Bu-5-MeIm)$_2$, 96666-14-7; Fe(3-MeTPP)(5-MeIm)$_2$, 96666-15-8; Fe(3-MeTPP)(5-*t*-BuIm)$_2$, 96688-71-0; Fe(3-MeTPP)(Im)$_2$, 96666-16-9.

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Characterization of (Tetrabenzoporphinato)iron

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Received November 1, 1984

(Tetrabenzoporphinato)iron chloride (ClFeTBP) was characterized by cyclic voltammetry, by EPR, optical absorption, and magnetic circular dichroism (MCD) spectroscopy, and by conductance measurements. ClFeTBP is a pentacoordinated iron(III) high-spin-state complex. However, the extent of dissociation of the Fe–Cl bond is larger than that in (*meso*-tetraphenylporphinato)iron(III) chloride (ClFe^{III}TPP). As with other iron(III) porphyrins, electrochemical or chemical reduction of ClFe^{III}TBP seems to produce stepwise iron(II) and iron(I) complexes and chemical oxidation gives the Fe^{III}TBP π cation radical. Unlike most porphyrins, optically pure and stable mono- and bis(imidazole) complexes are obtained; they are both iron(III) low-spin-state complexes. EPR data suggest ClFeTBP in pyridine exists as a mixture of iron(III) high- and intermediate-spin complexes. The results are examined in comparison with those for iron porphyrins and hemes previously studied and are of interest since tetrabenzoporphyrins are structurally intermediary between general porphyrins and phthalocyanines.

Introduction

Iron porphyrins (FePor's) and phthalocyanines (FePc's) have been the subjects of extensive studies. However, there is little information[1-3] on FeTBP, a structural intermediate of FePor's and FePc's, because of the difficulty in its synthesis.[4] Even the oxidation and spin states of the central iron have not been clarified. Since FePor's generally favor the iron(III) state and FePc's the iron(II) state under air,[5] such studies of FeTBP are of fundamental importance. Other thermodynamic and spectroscopic properties have also been expected to be between those of normal FePor's and FePc's.[6] As will be described below, Fe^{III}TBP exhibited, in many respects, properties intermediate between those of normal

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