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(14) At equilibrium at 25 °C an initially 0.1 M solution of $HFeNp(dmpe)_2$ in C_6D_6 is about 90% $DFeC_6D_5(dmpe)_2$ and only 10% $HFeNp(dmpe)_2$.[11]
(15) The mass spectrum of $HFeCH_2CN(dmpe)_2$ shows a weak parent ion peak (*m/e* 397) and a stronger M − 1 [*m/e* 396.099 08 vs. 396.0991 calcd for $FeCH_2CN(dmpe)_2^+$]. The base peak is *m/e* 356 [$Fe(dmpe)_2^+$].
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Iron Porphyrin Phenoxides: Models for Some Hemoglobin Mutants

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Abstract: Variously substituted phenoxides (L) react with $[Fe(PPIXDBE)]_2O$ (PPIXDBE is the protoporphyrin IX di-*tert*-butyl ester dianion) to produce five-coordinate high-spin complexes Fe(PPIXDBE)L which display spectroscopic properties similar to those of the Met form of the α mutant chain of HbM Boston. The addition of pyridine or 1-methylimidazole (L′) to Fe(PPIXDBE)L at 77 K produced low-spin six-coordinate complexes Fe(PPIXDBE)LL′ which were studied spectroscopically. With the strongly basic 2,6-dimethoxyphenoxide (L), the above reaction was studied at 298 K, where for L′ = 1-methylimidazole the binding constant was approximately 100 M^{-1} in CH_2Cl_2. The Fe(PPIXDBE)LL′ complexes were made in an attempt to mimic the Fe(III) in the α chain of Met HbM Iwate; however, the latter is high spin. With excess *p*-nitrophenoxide in CH_2Cl_2, Fe(PPIXDBE)(OC_6H_4-4-NO_2) forms Fe(PPIXDBE)(OC_6H_4-4-$NO_2)_2^-$, which exhibits a high-spin EPR spectrum at 77 K. Addition of phenoxides or fluoride to iron(II) protoporphyrin ester systems produces species such as Fe(PPIXDBE)X_2^{2-} (X = OR or F), similar to those found previously with methoxide and hydroxide ions. The addition of CO to a bisphenoxy species, in Me_2SO, results in a splitting of the Soret band at 438 nm into two bands at 434 and 413 nm, which are attributed respectively to a carbonyl (phenoxide) species and a carbonyl species which contains no phenoxide. The visible spectral data support the view expressed by others that upon reduction of HbM Iwate at pH 6.5 by $Na_2S_2O_4$ the iron–tyrosine bond is broken.

Introduction

One class of hemoglobin mutants HbM have their iron atoms, in either the α or β chain, permanently oxidized in vivo to Fe(III), and have the proximal or distal histidines in these chains replaced by tyrosines which are bound to the Fe(III) [e.g., HbM Boston ($\alpha_2^{\text{distal His-58}\rightarrow\text{Tyr}}\beta_2$),[3] HbM Iwate ($\alpha_2^{\text{proximal His-87}\rightarrow\text{Tyr}}\beta_2$),[4] HbM Hyde Park ($\alpha_2\beta_2^{\text{proximal His-92}\rightarrow\text{Tyr}}$),[4] and HbM Saskatoon ($\alpha_2\beta_2^{\text{distal His-63}\rightarrow\text{Tyr}}$)].[5] In HbM Milwaukee ($\alpha_2\beta_2^{\text{Val-67}\rightarrow\text{Glu}}$)[6] valine-67 is replaced by glutamic acid, a position four residues or one helical turn removed from the distal histidine, the glutamyl residue binding

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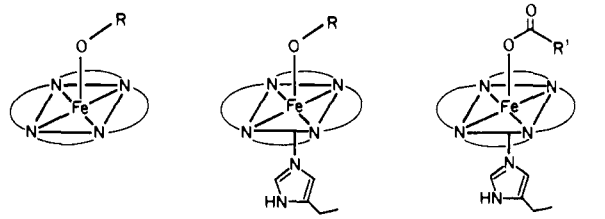

Figure 1. Ligands and geometries about the Fe(III) atoms of the mutant chains of some HbM. R–O represents the tyrosyl residue, R'COO the glutamyl residue, and N–N–N–N is protoporphyrin IX.

to the Fe(III) in the β chain. The α chain mutants have abnormally low oxygen affinity (resulting in cyanosis), almost no Bohr effect, and virtually no heme-heme interaction,[7-10] while the β chain mutants have normal oxygen affinity, a normal Bohr effect, but almost no heme-heme interaction.[9-12] Furthermore, the redox potentials of the mutant chains are said to be low,[3] hemoglobin reductase and ascorbic acid not reducing the Fe(III). Low-resolution X-ray crystal structure data indicate the ligands and geometries of the iron atoms[3,4,6] to be those shown in Figure 1.

Perutz et al.[3] consider that the out of plane displacement (0.24 Å) for the iron atom in HbM Boston is probably underestimated, and may be closer to the 0.455 Å found in methoxy iron(III) mesoporphyrin IX dimethyl ester.[13] The Fe(III) atom of HbM Milwaukee[6] also appears displaced from the porphyrin plane.

In this work we have attempted to model and study the chemistry of the compounds shown in Figure 1, and to make comparisons with the naturally occurring systems, using protoporphyrin IX diesters as the parent porphyrin.

Experimental Section

2,6-Dimethoxyphenol, *p*-methoxyphenol, 3,4-dimethylphenol, *p*-nitrophenol, and hemin chloride were obtained from Eastman Kodak, while 3,5-dimethoxyphenol and *p*-cresol were from Aldrich. 1-Methylimidazole (1-MeIm) (Sigma) and dicyclohexano-18-crown-6 (Aldrich) were distilled (the latter at 1.5 mm) before use. Toluene and dichloromethane were refluxed over and distilled from CaH_2 and P_2O_5, respectively. All other chemicals were reagent grade. Melting points are uncorrected.

Preparation of Sodium Salts of Phenoxides (4-Nitro, 4-Methyl, 4-Methoxy, 3,4-Dimethyl, 2,6-Dimethoxy, 3,5-Dimethoxy). The phenoxides were prepared by similar methods, of which the following is typical. To 2×10^{-2} mol of the appropriate phenol was added 2×10^{-2} mol of NaOH dissolved in 30 mL of EtOH. The solution was warmed for about 2 min and filtered, and the filtrate was evaporated to near dryness on a rotary evaporator. Dry Et_2O (50 mL) was added to precipitate the colorless or light tan product, which was washed with dry Et_2O and vacuum dried (yields 50–60%).

Iron(III) Protoporphyrin IX Di-*tert*-butyl Ester Complexes. PPIXDBE and PPIXDME are the abbreviations for the di-*tert*-butyl and dimethyl ester dianions, respectively.

A. [Fe(PPIXDBE)]$_2$O. H_2PPIXDBE[14] (2.02 g, 3×10^{-3} mol) was added to 200 mL of dry dimethylformamide to which 1.9 g (15×10^{-3} mol) of $FeCl_2$ was added. The solution was refluxed for 40 min, and then on cooling was added to 200 mL of a saturated solution of NaCl. The brownish precipitate was dried under vacuum and extracted with CH_2Cl_2 (100 mL) and the extract was filtered. The solution contained the brownish-red Fe(PPIXDBE)Cl. To this was added 40 mL of 1 M NaOH and the mixture was placed in a separatory funnel which was gently swirled for about 1.5 h. The now dark green CH_2Cl_2 layer was removed, washed with water (3×50 mL), and evaporated to dryness on a rotary evaporator. The residue was dissolved in 40 mL of CH_2Cl_2 and passed down an Al_2O_3 column. The greenish band was collected and the CH_2Cl_2 removed on a rotary evaporator until a few milliliters remained. Petroleum ether was added and the solution placed in a refrigerator overnight. Black crystals of the product were filtered off. A second crop can be obtained from the mother liquor, total yield 1.4 g. Anal. Calcd for $C_{84}H_{96}N_8Fe_2O_9$: C, 68.6; H, 6.6; N, 7.6. Found: C, 68.4; H, 6.7; N, 7.9.

B. Fe(PPIXDBE)(OC$_6$H$_4$-4-NO$_2$), Fe(PPIXDBE)(OC$_6$H$_3$-3,4-Me$_2$), Fe(PPIXDBE)(OC$_6$H$_3$-2,6(OMe)$_2$), and Fe(PPIXDBE)(OC$_6$H$_3$-3,5(OMe)$_2$). These were prepared by a modified literature procedure.[15] A typical example is given. To 0.196 g (0.13×10^{-3} mol) of [Fe(PPIXDBE)]$_2$O dissolved in 15 mL of dry toluene was added 2.2×10^{-3} mol of solid 3,4-dimethylphenol, and the mixture was warmed to 70 °C (in an oil bath) for 25 min. Molecular sieves were added to remove water. The brownish solution was filtered and most of the toluene removed on a rotary evaporator; about 40 mL of petroleum ether was added and the mixture placed in a refrigerator overnight. Purplish crystals (150 mg, 66% yield) of the product were collected and washed with petroleum ether. Anal. Calcd for $C_{50}H_{57}N_4O_5Fe$: C, 70.7; H, 6.75; N, 6.6. Found: C, 71.0; H, 6.65; N, 6.8. Mp 183 °C.

For the other complexes, 1.3×10^{-3} mol of the appropriate phenol was used, and after reaction the toluene volume was reduced to about 5 mL, to which 10 drops of petroleum ether (bp 120 °C) was added. After the solution was left in a refrigerator overnight, purplish-red products were collected, washed with diethyl ether, and vacuum dried (yields 50–60%).

Fe(PPIXDBE)(OC$_6$H$_4$-4-NO$_2$). Anal. Calcd for $C_{48}H_{52}N_5O_7Fe$: C, 66.5; H, 6.0; N, 8.1. Found: C, 66.0; H, 5.9; N, 8.3. Mp 171–172 °C.

Fe(PPIXDBE)(OC$_6$H$_3$-2,6(OMe)$_2$). Anal. Calcd for $C_{50}H_{57}N_4O_7Fe$: C, 68.2; H, 6.5; N, 6.4. Found: C, 68.4; H, 6.7; N, 6.6. Mp 200 °C.

Fe(PPIXDBE)(OC$_6$H$_3$-3,5(OMe)$_2$). Anal. Calcd for $C_{50}H_{57}N_4O_7Fe$: C, 68.2; H, 6.5; N, 6.4. Found: C, 67.3; H, 6.3; N, 6.4. Mp 138 °C.

C. Fe(PPIXDBE)(O$_2$CCH$_3$). [Fe(PPIXDBE)]$_2$O (0.17 g) was dissolved in a solution containing 20 mL of glacial acetic acid and 5 mL of acetic anhydride, and this was heated at 45 °C for 3 h. The solution was taken to dryness under vacuum. The dark solid was washed with 25 mL of petroleum ether (bp 120 °C) containing 0.5 mL of glacial acetic acid, then with petroleum ether, and finally vacuum dried (yield 160 mg, mp 196–198 °C). Anal. Calcd for $C_{44}H_{51}N_4O_6Fe$: C, 67.1; H, 6.5; N, 7.1. Found: C, 65.7; H, 6.5; N, 7.2.

Physical Measurements. Owing to the sensitivity of the majority of complexes to water and/or dioxygen, all manipulations and measurements were performed under pure dinitrogen. Electronic spectra of solutions were recorded on a Cary Model 14 or 17 spectrophotometer. Spectra of complexes at 77 K in toluene/methylcyclohexane glasses were obtained using a quartz Dewar having a flat quartz cell of ca. 1-mm path length. EPR measurements were made at 77 K with a Varian E-3 X-band spectrometer calibrated with DPPH and an NMR probe. A typical EPR sample consisted of 5–10 mg of complex, 0.4 mL of solvent, and ~30 equiv of base (1 drop from a Pasteur pipet). Magnetic susceptibilities were determined by the Faraday method. Cyclic voltammetry and polarography at a platinum sphere (0.15 cm^2) electrode were performed (at 25 ± 0.2 °C in solution deoxygenated by bubbling nitrogen or argon) using a PAR-173 potentiostat and PAR-176 *i/E* converter. Voltammetric data were recorded on a storage oscilloscope or X-Y recorder. The three-electrode cell included as the reference an Ag(s)|AgClO$_4$ (0.01 M), TEAP (0.1 M), CH_3CN electrode, whose potential is 0.30 V positive of the SCE in acetonitrile at 25 °C, and all potentials are thus referred to the SCE.

C, H, and N analyses were performed at the University of British Columbia by Mr. P. Borda. Acetonitrile for electrochemistry was distilled from P_2O_5 under N_2.

A ligand binding constant (K)[16] at 25 °C was obtained using an evacuated quartz cell of path length 1 cm. Ten milliliters of an approximately 10^{-4} M solution of Fe(PPIXDBE)(OC$_6$H$_3$-2,6(OMe)$_2$) in CH_2Cl_2 were titrated with 5-μL aliquots of 1-methylimidazole until a limiting spectrum was attained. Measurements were made at 609 nm, where the absorption decreases upon addition of amine; isosbestic points occur at 548 and 568 nm (see Figure 5).

Log $[(A_0 - A)/(A - A_\infty)]$ was plotted against log [L] (A_0 = initial absorbance of Fe(PPIXDBE)(OC$_6$H$_3$-2,6(OMe)$_2$), A = absorbance after addition of 1-methylimidazole, A_∞ = absorbance of final limiting spectrum, and [L] = concentration of ligand) to give a line of slope ~1.2, and a value for the binding constant of 100 ± 30 M^{-1} was obtained. The data are attributed to a 1:1 binding of the amine to give a six-coordinate (1-methylimidazole)(phenoxide) species with $K \sim 100$ M^{-1} (see eq 2).

Table I. Electronic Spectra,[a] EPR Data, and Magnetic Moments for High-Spin Fe^{III}PPIXDBE Complexes

species	λ_{max}, nm [ϵ, mM^{-1} cm^{-1}]				g^b	magnetic moment, μ_B
	α	β		γ (Soret)		
Fe(PPIXDBE)(OC_6H_4-4-NO_2)	617 [7.7]	528 [12]	500 [12.7]	404 [96.2]	5.17, 1.97[c]	5.91
Fe(PPIXDBE)(OC_6H_3-3,4-Me_2)	600 [11.7]	520 sh [11.3]	480 [14.0]	403 [100]	5.95, 5.07, 1.97[d] (5.8, 5.17, 2.0)[e]	5.91
Fe(PPIXDBE)(OC_6H_3-2,6-$(OMe)_2$)	609 [9.0]	520 sh [12.2]	496 [13.1]	402 [100]	7.9, 5.12, 2.06[d]	5.95
Fe(PPIXDBE)(OC_6H_3-3,5-$(OMe)_2$)	604 [11.5]	520 sh [11.2]	488 [13.8]	404 [104]	7.2, 5.17, 2.0[d]	5.87
Fe(PPIXDBE)(OAc)[f]	632 [5.58]	540 [9.7]	507 [9.98]	406 [104]	7.83, 5.28, 2.0[d]	
Fe(PPIXDBE)(OC_6H_4-4-$NO_2)_2^-$[g]	595 sh [7.0]	570 [7.7]			5.7, 4.3, 2.0	
$[Fe(PPIXDBE)]_2O$[h]	600 [12.6] (730 [0.3], 705 [0.4])	573 [14.6]		392 [138], 355 [94]		

[a] Toluene solution, unless otherwise stated. [b] At 77 K. [c] Toluene glass. [d] Toluene/dichloromethane (5:1 v/v) glass. [e] In 2-MeTHF. [f] In toluene/acetic acid (5:1 v/v). [g] By adding excess $NEt_4^+OC_6H_4$-4-NO_2^- to dichloromethane solution. Nitrophenoxide obscures Soret band. [h] Antiferromagnetic.

Results and Discussion

Synthesis of Iron(III) Complexes, Fe(PPIXDBE)(OR). Models for HbM Boston. Numerous five-coordinate iron(III) porphyrins Fe(Porp)L with L = halide, RS^-, RO^-, and RCO_2^- have been prepared previously.[15,17-19] The complexes of interest in this study were the phenoxides and the carboxylates, as these systems relate most closely to HbM. The method used for their preparation was similar to that of Tang et al.,[15] who first prepared Fe(PPIXDME)(OC_6H_4-4-NO_2). A series of complexes of the type Fe(PPIXDBE)(OR), where OR = 4-nitro-, 3,4-dimethyl-, 2,6-dimethoxy-, and 3,5-dimethoxyphenoxide, was synthesized by the addition of the appropriate phenol to $[Fe(PPIXDBE)]_2O$ in toluene at 70 °C (eq 1). PPIXDBE was chosen as the PPIX derivative to enhance the solubility of the complexes and to inhibit attack by excess phenoxide on the ester.

$$[Fe(PPIXDBE)]_2O + 2ROH \rightarrow 2Fe(PPIXDBE)(OR) + H_2O \quad (1)$$

Since the reverse of reaction 1 readily occurs, especially with complexes containing the more basic phenolates, the products are best kept in a vacuum desiccator. The acetate complex Fe(PPIXDBE)(O_2CCH_3) was also prepared by heating $[Fe(PPIXDBE)]_2O$ in glacial acetic acid/acetic anhydride.

High-Spin Derivatives of Fe(PPIXDBE)$^+$. The magnetic moments for the phenoxide complexes at room temperature show them to be high spin (Table I). Furthermore, these five-coordinate complexes almost certainly have a near square-pyramidal geometry, similar to that shown by Fe(PPIXDME)(SC_6H_4-NO_2)[15] and Fe(MPIXDME)(OCH_3)[13] (MPIXDME = mesoporphyrin IX dimethyl ester). In both these cases the Fe^{III} is about 0.45 Å from the porphyrin plane. HbM Boston has a similar geometry[3] for the Fe(III) in the mutant chain, where the phenolic group of tyrosine is bound to the iron (Figure 1a).

The electronic spectra for the 1:1 phenoxides show the α band at 600–617 nm, while the corresponding band for the acetate occurs at 632 nm (Table I). HbM Boston[3] and HbM Saskatoon[9] also display the α band around 600 nm. Methemoglobin at pH 7 has the α band at 631 nm.[6,10] Perutz et al.[6] have shown that HbM Milwaukee ($\alpha_2\beta_2^{Val\text{-}67\rightarrow Glu}$) with the α chain in the ferrous state also shows an α band at 630 nm. Hence, while serving to differentiate those mutants that have tyrosinate bound to Fe(III) from those that have glutamate, this band is not useful for distinguishing the glutamate mutants from methemoglobin A. The acetate complex thus models HbM Milwaukee with respect to the electronic spectrum, although structurally it is square pyramidal while the latter is distorted octahedral.

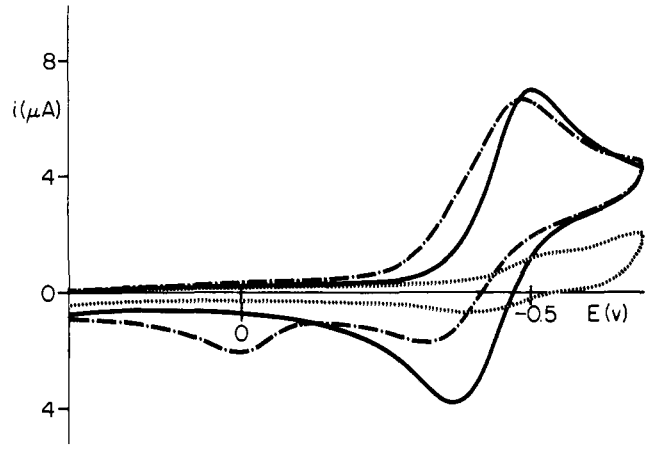

Figure 2. Cyclic voltammograms of 0.43 mM Fe(PPIXDBE)(OC_6H_4-4-NO_2) in CH_3CN/NEt_4ClO_4 (0.1 M) at 25 °C, without (· · ·) and with added $NEt_4^+OC_6H_4$-4-NO_2^-, 0.43 mM (——), 8.2 mM (· · ·).

The EPR spectra, measured in glasses at 77 K, are all of the high-spin type[20-23] (Table I). The Fe(PPIXDBE)(OC_6H_4-4-NO_2) complex shows axial symmetry with $g_\perp = 6.2$ and $g_\parallel = 2$, while the other phenoxides and acetate have rhombic symmetry signaled by a splitting of the $g = 6$ resonance. Such splitting is not observed in methemoglobin A[3,21] but is typical of HbM Boston,[3] although the splitting is smaller for the protein system.

Cyclic voltammetry (Figure 2) of 4.3×10^{-4} M Fe(PPIXDBE)(OC_6H_4-4-NO_2) in acetonitrile (0.1 M NEt_4ClO_4) revealed the $Fe^{III} \rightarrow Fe^{II}$ reduction process with $\frac{1}{2}(E_{p,c} + E_{p,a}) = -0.43$ V vs. the SCE. The non-Nernstian wave showed the presence of two reoxidizable species, suggesting that complex formation may not have been complete in both oxidation states. Indeed, when successive aliquots of NEt_4^+ OC_6H_4-4-NO_2^- in acetonitrile were added to the solution, the secondary oxidation peak at 0.0 V disappeared, and the ratio $i_{p,a}/i_{p,c}$ increased toward unity within the main wave. However, further addition of phenoxide caused the latter's currents to steadily diminish as well, as the wave was shifted to more negative potential via phenoxide complexation at iron. The optical spectrum of the solution containing 20-fold excess of phenoxide is essentially identical with the one shown in Figure 3, which was obtained by addition of solid $NEt_4^+OC_6H_4NO_2^-$ to Fe(PPIXDBE)(OC_6H_4-4-NO_2) in dichloromethane and is

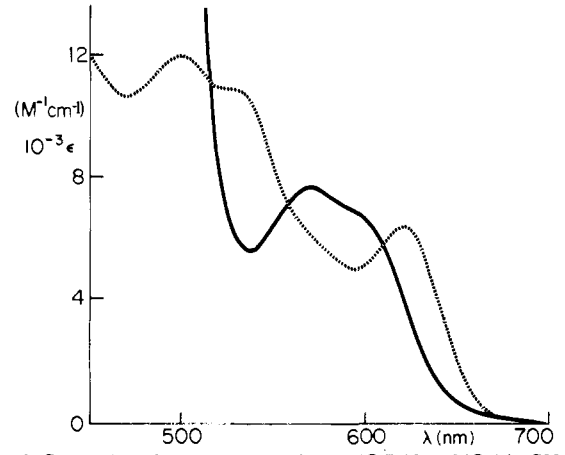

Figure 3. Conversion of 55 μM Fe(PPIXDBE)(OC_6H_4-4-NO_2) in CH_2Cl_2 (broken line) to Fe(PPIXDBE)(OC_6H_4-4-NO_2)$_2^-$ (solid line) by addition of excess $NEt_4^+OC_6H_4$-4-NO_2^-

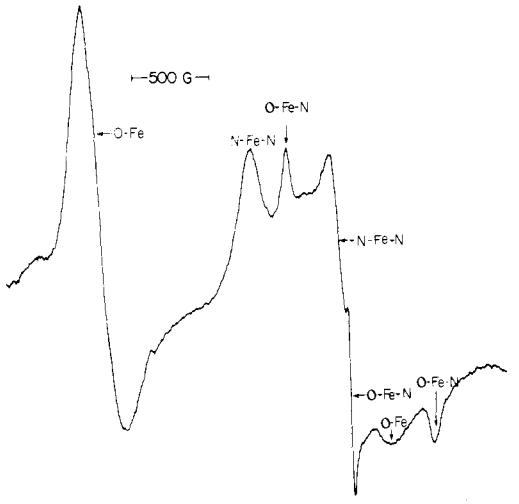

Figure 4. EPR spectrum at 77 K of reaction of Fe^{III}(PPIXDBE)(OC_6H_4-4-NO_2), (O-Fe), with 1-methylimidazole in toluene glass. N-Fe-N represents Fe^{III}(PPIXDBE)(1-methylimidazole)$_2^+$ and O-Fe-N represents (OC_6H_4-4-NO_2)Fe^{III}(PPIXDBE)(1-methylimidazole).

attributed to the bisphenoxide complex. The spectra of the monophenoxide complex in toluene or dichloromethane appear to be identical (450–700 nm) with that obtained by Tang et al.[15] for solution in 2-MeTHF (2-methyltetrahydrofuran). The bis complex, Fe(PPIXDBE)(OC_6H_4-4-NO_2)$_2^-$, yields an EPR spectrum (note g = 5.7, Table I) characteristic of a high-spin ($S > 1/2$) ferric porphyrin (dichloromethane, 77 K), which is known also for the case of the Fe(TPP)$(OH_2)_2^{2+}$ ion.[24] The optical spectrum of Fe(PPIXDBE)(OC_6H_4-4-NO_2) in acetonitrile (600 (shoulder), 568 nm (ε 7400)) was also very similar to that of the six-coordinate adducts, rather than to the characteristic spectra obtained for the five-coordinate species in nondonor solvents, clearly indicating ligation of the iron by CH_3CN.

EPR and Electronic Spectra of Low-Spin Fe(III) Complexes. Attempts at Models for HbM Iwate and Milwaukee. Compounds of the type Fe(PPIXDBE)(OR) discussed above allow for a study of the base addition reaction (eq 2, where L′ = 1-MeIm and pyridine (py)) and an attempt to model HbM Iwate (Figure 1b) and HbM Milwaukee (Figure 1c).

$$\text{Fe(PPIXDBE)(OR)} + \text{L}' \overset{K}{\rightleftarrows} \text{Fe(PPIXDBE)(OR)L}' \qquad (2)$$

The EPR was studied at 77 K after mixing the five-coordinate complex and amine at room temperature. For the *p*-nitrophenoxide with 1-MeIm, three distinct species were observed (Figure 4) and these are considered to be the starting complex (O-Fe), Fe(PPIXDBE)(1-MeIm)$_2^+$ or (N-Fe-N), and Fe(PPIXDBE)(phenoxide)(1-MeIm) or (O-Fe-N). The latter two showed spectra typical of rhombic low-spin hemes, with g values at or near 2.6, 2.2, and 1.9.[21,22] Some typical values are given in Table II. With the acetate/1-MeIm system only Fe(PPIXDBE)(1-MeIm)$_2^+$ was detected, while with the methyl- and methoxy-substituted phenoxide complexes only one product, low-spin (O-Fe-N), could be detected under the reaction conditions.

There were in most cases no visible spectral changes on mixing the Fe(PPIXDBE)(OR) complexes with 30 equiv of 1-MeIm or pyridine at room temperature, indicating that the forward reaction in eq 2 is exothermic. The spectrum of Fe(PPIXDBE)(OC_6H_3-3,4-Me_2)(1-MeIm) was recorded at 77 K, however, and showed bands at 570 and 540 nm of nearly equal intensity, similar to that of low-spin (2-MeTHF)Fe(PPIXDME)(SC_6H_4-4-NO_2) at 77 K[15] and ($C_6H_5CH_2S$)-Fe(PPIXDME)(Im) at 77 K.[25] When the phenoxide system was warmed to 24 °C, the spectrum recorded was essentially that of high-spin Fe(PPIXDBE)(OC_6H_3-3,4-Me_2) (Table I), the high-spin/low-spin conversion being reversible. Only with

Table II. EPR Spectra of Low-Spin Iron(III) Porphyrin Complexes, Fe(PPIXDBE)LL′

reactant Fe(PPIXDBE)L	added base (L′)	product g values[a]		
Fe(PPIXDBE)(OC_6H_4-4-NO_2)	1-MeIm[b]	2.61	2.24	1.86
	py[c]			
Fe(PPIXDBE)(OC_6H_3-3,4-Me_2)	1-MeIm	2.57	2.19	1.85
	py[d]	2.61	2.19	1.84
Fe(PPIXDBE)(OC_6H_3-2,6(OMe)$_2$)	1-MeIm	2.56	2.21	1.85
	py	2.63	2.21	1.83
Fe(PPIXDBE)(OC_6H_3-3,5(OMe)$_2$)	1-MeIm	2.55	2.20	1.86
	py[d]	2.61	2.19	1.84
Fe(PPIXDBE)(OAc)	1-MeIm[e]	2.90	2.30	1.57

[a] Measured at 77 K in toluene/dichloromethane glass (5:1 v/v); Fe:L′ ~ 1:30. [b] Starting material and [Fe(PPIXDBE)(1-MeIm)$_2$]$^+$ also observed. [c] Only the EPR spectrum of the starting material was observed. [d] EPR spectrum of starting material also observed. [e] EPR spectrum observed of [FePPIXDBE(1-MeIm)$_2$]$^+$ only.

the strongly basic phenoxides (e.g. 2,6-dimethoxy- and 3,5-dimethoxy-) was reaction 2 observed at room temperature, the spectra of the six-coordinate species (Table III, Figure 5) being similar to the one noted above at 77 K. The binding constant (K) at 24 °C for the reaction of Fe(PPIXDBE)(OC_6H_3-2,6(OMe)$_2$) with 1-MeIm in CH_2Cl_2 (see Experimental Section) was about 100 M^{-1}, which is similar to that obtained by Walker et al.[26] for the reaction of Fe(TPP)Cl with 1-MeIm (TPP is *meso*-tetraphenylporphyrin). Excess 1-MeIm with the dimethoxyphenoxide complex produced Fe(PPIXDBE)(1-MeIm)$_2^+$, identified by its spectrum (bands at 558 (shoulder) and 530 nm), which is the same as that of the PPIXDME analogue.[15]

We may thus compare the spectroscopic properties of high-spin model systems (O-Fe) and (O-Fe-O), and of low-spin (O-Fe-N), to the high-spin[27,28] ferric mutant α chains of HbM Iwate, for which Peisach and Gersonde obtained λ_{max} 595 nm for $\alpha_2^{MFe^{III}}\beta_2^{FeCO}$ at pH 6.5 in the presence of phytate.[28] The spectroscopic noncorrespondence of the mutated center to any of the three models, in combination with (1) the conclusion that a tyrosyl-iron linkage occurs and (2) Greer's inference[4] that a tyrosinate-iron-histidine (O-Fe-N) moiety pertains, can be rationalized if there is a stereochemical con-

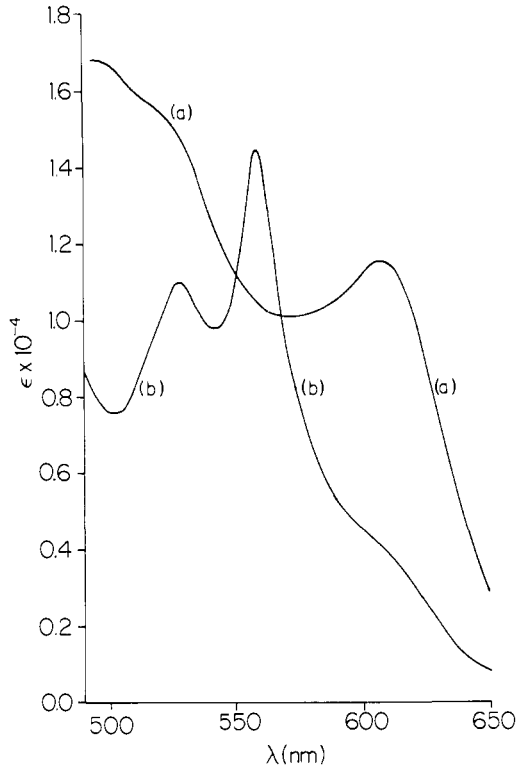

Figure 5. Electronic spectra of (a) $Fe^{III}(PPIXDBE)(OC_6H_3\text{-}2,6(OMe)_2]$ in CH_2Cl_2 and (b) $(OC_6H_3\text{-}2,6(OMe)_2)Fe^{III}(PPIXDBE)$ (1-methylimidazole) in CH_2Cl_2 at 24 °C.

Table III. Electronic Spectra of Low-Spin Iron(III) Porphyrin Complexes

compd	λ_{max}, nm [ϵ, mM^{-1} cm^{-1}]		
	α	β	γ
(1-MeIm)Fe(PPIXDBE)(OC_6H_3-3,4-Me_2)[a]	570	540	[b]
(1-MeIm)Fe(PPIXDBE)(OC_6H_3-2,6$(OMe)_2$)	558 [14]	528 [11]	411
(py)Fe(PPIXDBE)(OC_6H_3-2,6$(OMe)_2$)[c]	555[d]	524	407
(1-MeIm)Fe(PPIXDBE)(OC_6H_3-3,5$(OMe)_2$)[c]	557[d]	530	411

[a] Measured in toluene/methylcyclohexane glass at 77 K. [b] Not measured. [c] Measured at 296 K. [d] Ratio of α/β intensity is ≈0.9:1.

straint on the heme by the protein's tertiary structure, so that the iron(III) lies out of the porphyrin plane toward the tyrosinate. In HbM Milwaukee,[6] with a glutamate-Fe-proximal histidine (O-Fe-N) chromophore in the β chain, the Fe(III) can be either high spin or low spin, depending on whether the Fe(II) in the α chain is in the deoxy or oxy state, and again the structural constraint may be on the high-spin state. Unfortunately we could not prepare a model (acetate-Fe-imidazole) species for this system.

Interaction of Fe^{II}(PPIXDME) with Sodium Phenoxides. On the basis of Hill coefficient data for binding of ethyl isocyanide to mutant and normal hemoglobins, it has been suggested[27,29] that upon reduction of the iron(III) in the α hemes of HbM Boston, the tyrosine ligand probably becomes noncoordinated and is possibly replaced by the proximal histidine; this would then involve movement of the iron through the porphyrin plane. Reduction of HbM Iwate possibly breaks both the tyrosine and distal histidine bonds, since there is no cooperativity for the isocyanide affinity.[27,29] This point will be addressed again later. Dithionite reduction of [Fe(PPIXDBE)]$_2$O or Fe(PPIXDME)Cl in aqueous acetone, followed by removal of the solvents under vacuum, and then addition of the appropriate excess of sodium phenoxide dissolved in dicyclohexano-18-crown-6, gave solutions with visible spectra exemplified by Figure 6 (see Table IV). Similar spectra were obtained in Me_2SO (no crown ether required). Such spectra compare well with those[29,30] of $Fe(PPIX)(OH)_2^{2-}$ and a species which is probably $Fe(PPIXDME)(OCH_3)_2^{2-}$, and are attributed to high-spin diphenoxy species $Fe(PPIXDME)(OR)_2^{2-}$. There is a single Soret band at about 435 nm for the phenoxy species, and at 420 nm for a similarly formed fluoro species (Table IV). The μ-oxo iron(III) complexes are readily distinguished by their Soret band at about 390 nm.[17] Keilin[30] demonstrated formation of dihydroxy hematoheme species by pH titration ($\beta_2^{II} \sim 3.6$ M^{-2}), and

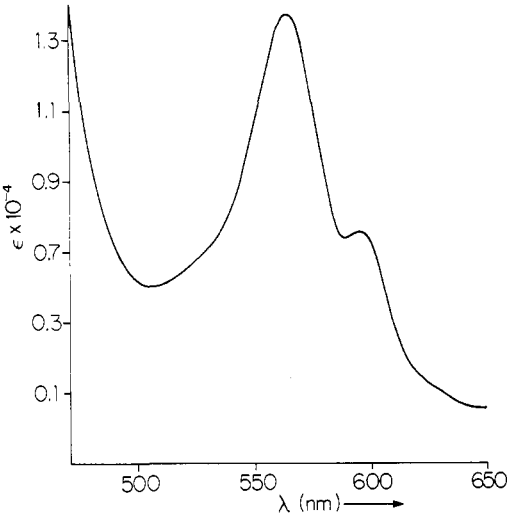

Figure 6. Electronic spectrum of $Fe^{II}(PPIXDME)(OC_6H_3\text{-}3,4\text{-}Me_2)_2^{2-}$ in toluene at 24 °C.

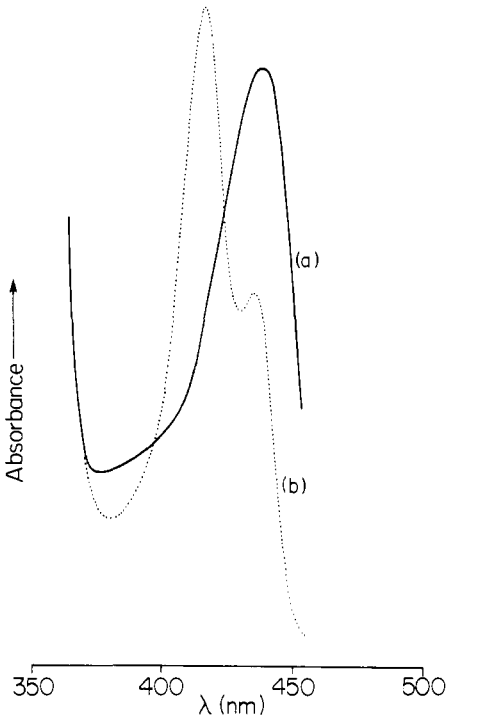

Figure 7. (a) The soret band for $Fe^{II}(PPIXDME)$ in the presence of excess $NaOC_6H_4\text{-}4\text{-}Me$ in dimethyl sulfoxide at 24 °C to give $Fe(PPIXDME)(OC_6H_4\text{-}4\text{-}Me)_2^{2-}$. (b) The addition of CO to (a) to give two new Soret bands.

Table IV. Electronic Spectra of Iron(II) Porphyrin Complexes

reaction[a]	solvent	products	λ_{max} nm [ϵ mM^{-1} cm^{-1}] α	β	γ
Fe(PPIXDBE)[b] + $NaOC_6H_3$-3,4-Me_2[c]	toluene	Fe(PPIXDBE)(OC_6H_3-3,4-Me_2)$_2^{2-}$	595 [7.6]	565 [13.7]	435
Fe(PPIXDBE) + $NaOC_6H_3$-3,4-Me_2[c] + CO	toluene	(CO)Fe(PPIXDBE)[d]	564[e]	535	414
Fe(PPIXDME)[f]	Me_2SO	Fe(PPIXDME) (solvated)	557 [21.3]	525 [16.9]	424
Fe(PPIXDME) + $NaOC_6H_3$-3,4-Me_2	Me_2SO	Fe(PPIXDME)(OC_6H_3-3,4-Me_2)$_2^{2-}$	593 [8.5]	562 [13.5]	436
Fe(PPIXDME) + $NaOC_6H_3$-3,4-Me_2 + CO	Me_2SO	(CO)Fe(PPIXDME)[d,g,h]	565[i]	535[i]	413
		(CO)Fe(PPIXDME)(OC_6H_3-3,4-Me_2)[d]	565	535	434
Fe(PPIXDME) + $NaOC_6H_3$-2,6$(OMe)_2$	Me_2SO	Fe(PPIXDME) (solvated)[h]	557	525	424
		Fe(PPIXDME)(OC_6H_3-2,6$(OMe)_2$)$_2^{2-}$	595	557	*j*
Fe(PPIXDME) + $NaOC_6H_4$-4-Me	Me_2SO	Fe(PPIXDME)(OC_6H_4-4-Me)$_2^{2-}$	593[k]	560	438
Fe(PPIXDME) + $NaOC_6H_4$-4-Me + CO	Me_2SO	(CO)Fe(PPIXDME)[d,g,h]	560[l]	535[l]	413
		(CO)Fe(PPIXDME)(OC_6H_4-4-Me)[d]	560[l]	535[l]	434
Fe(PPIXDME) + $NaOC_6H_4$-4-OMe	Me_2SO	Fe(PPIXDME)(OC_6H_4-4-OMe)$_2^{2-}$	593	560	438
Fe(PPIXDME) + $NaOC_6H_4$-4-OMe + CO	Me_2SO	(CO)Fe(PPIXDME)[d,g,h]	565[l]	535[l]	413
		(CO)Fe(PPIXDME)(OC_6H_4-4-OMe)[d]	565[l]	535[l]	433
Fe(PPIXDME) + KF[m]	CH_3OH	Fe(PPIXDME)F_2^{2-}	585	552	420

[a] Typically involved addition of 50–70 mg of the sodium phenoxide to 5–10 mL of a solution ~20 μM in Fe^{II}(PPIXDME) or Fe^{II}(PPIXDBE). [b] By reduction of [Fe(PPIXDBE)]$_2$O in acetone with aqueous $Na_2S_2O_4$ under N_2. After removal of solvents under vacuum, the residue was extracted into toluene and the resulting spectrum of the "bare heme" has bands at 569, 535, and 425 nm (α:β intensity ratio is 0.9:1). [c] Dicyclohexano-18-crown-6 was added to dissolve the salt. [d] Probably low spin. [e] 50% more intense than β band. [f] Generated by reducing an acetone solution of Fe(PPIXDME)Cl with aqueous $Na_2S_2O_4$ under N_2, removing solvents under vacuum, and extracting residue with Me_2SO. [g] Presumably solvated by Me_2SO. [h] Major product. [i] α, β bands of approximately equal intensity. [j] Minor product: band obscured. [k] 50% more intense than β band. [l] Broad bands. [m] 1 g of KF and 150 mg of dicyclohexano-18-crown-6 in 10 mL of CH_3OH containing 0.2 mg of reduced Fe(PPIXDME)Cl.

showed the dihydroxy uroheme species to be high spin in solution (μ = 5.67 μ_B). High-spin five-coordinate iron(II) porphyrin complexes containing 2-MeIm or mercaptide as axial ligand, as well as deoxymyoglobin itself, usually have a single broad band in the 520–570-nm region.[31-33]

We have not detected five-coordinate Fe(PPIXDME)(OR)$^-$ species. Addition of methoxide, phenoxides, hydroxide, or fluoride to a methanol solution of Fe(PPIXDME) all gave similar spectra, attributable to six-coordinate weak ligand field systems (cf. Table IV). These same ligands will certainly bind more strongly to iron(III) centers, and there is likely to be little or no interaction of tyrosinate with a fully reduced mutant chain in HbM, in which the low reduction potentials illustrate the preferred binding of the O donor to iron(III).

Iron(II)/Phenoxide/CO Systems. When CO was bubbled through a toluene solution of Fe(PPIXDBE)(OC_6H_3-3,4-Me_2)$_2^{2-}$, the spectrum changed to that characteristic[28] of Fe(PPIXDBE)CO (Table IV). In Me_2SO, however, the initial 438-nm Soret band of Fe(PPIXDME)(OC_6H_4-4-Me)$_2^{2-}$ was replaced by two bands at 413 and 434 nm (Figure 7); the former is considered due to Fe(PPIXDME)(CO), while the latter is attributed to (CO)Fe(PPIXDME)(OC_6H_4-4-Me). Addition of further phenoxide shifts the equilibrium in favor of the 434-nm band by increasing the concentration of the carbonylphenoxy species; purging with argon regenerates the bisphenoxy complex. The data for various phenoxides are summarized in Table IV, and are similar to those for the unsubstituted phenoxide studied by Peisach and Gersonde.[28]

Peisach and Gersonde[28] reported the spectrum of the reduced abnormal α chain of HbM Iwate at pH 6.5, in the presence of CO, to have a Soret band at 420 nm; this is similar to that found for the carbonmonoxy normal β chain (418 nm) and for carbonmonoxyhemoglobin A (419 nm) where the proximal histidine is coordinated, and hence it was suggested[28] that tyrosine is not bound in the carbonylated reduced chain but that distal histidine is the likely trans axial ligand. (The phenoxy-heme-CO species have a Soret band in the 433–436-nm region.) The effective local concentration of phenoxide would be higher at higher pH (the pK_a for the tyrosine OH is ~10), and studies of the effects of pH and protein conformation on the spectra of HbM Iwate are in progress.

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Activation Volume and Transition State Character in Several Proton and Hydride Transfer Reactions

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Abstract: Volumes of activation have been measured for the dedeuteration of chloroform, fluoroform, acetophenone, phenylacetonitrile, methyl phenylacetate, Me_2SO, indene, phenylacetylene, diphenylmethane, and 2-nitropropane. None of them has ΔV^* as low as -10 mL/mol, and it may be inferred that their transition states are product-like. In two examples of hydride transfer, reduction of acetophenone by borohydride and the Cannizzaro reaction of benzaldehyde, ΔV^* is substantially negative, which implies a central transition state. These results support a theoretical prediction by Swain.

Introduction

In many important organic reactions the rate is limited by a step in which a proton is removed from one of the reactants. There has been much interest in the character of the transition states for these reactions, and the subject has been extensively reviewed.[1,2] In a smaller but still very important group of reactions the rate is limited by transfer of hydrogen in the form of hydride ion. In this field too there is extensive literature on the question whether the transition state comes early or late.[3]

Experimental evidence for the location of the transition state on the reaction coordinate for proton transfers comes mainly from primary kinetic isotope effects,[4] solvent isotope effects,[5] Brønsted parameters,[6-8] stereochemistry,[9] and activation entropy.[6] For hydride transfers the evidence is based on the primary kinetic hydrogen isotope effect,[10] Hammett relationships,[11,12] activation entropy,[13] and numerous studies of steric effects which are described in a recent review.[14] Despite the great quantity and variety of evidence there is much difference of opinion about the nature of the transition state for both kinds of reaction. All of the authors cited above have been candid about the weak points of the various mechanistic tests. It is well known, for example, that moderate or small kinetic isotope effects are equally consistent with early and late transition states. The Brønsted parameters, on the other hand, are not two valued, but they can be very difficult to measure. The evaluation of β requires a measurement of catalysis by general bases which is often overshadowed by lyate catalysis, and the evaluation of α requires pK values for weak acids of very different strength in the same solvent. For deprotonation of nitroalkanes Bordwell[15] has reported α values substantially greater than one, and under the customary interpretation such values convey no meaning in terms of transition state structure. The Hammett ρ scale is also monotonic, but there is no firm basis for anticipating what value ρ should have for a highly product-like transition state. The observed values are usually considerably less than the theoretical maximum.

When the evidence is confusing or inconclusive it is probably better to have more kinds of evidence than a greater amount of the same kind. One method for investigating reaction mechanisms which has not yet been applied in any systematic way to the present problem is the measurement of the effect of pressure on reaction rate and the evaluation of the volume of activation. The utility of the method derives from the possibility of comparing the molar volumes of transition states with those of stable molecules. This gives information about changes in bonding and electrical polarization. More than 1000 measurements of activation volume have been reported to date, and the empirical basis for interpreting them is fairly secure.[16,17] The complete formation of a new bond causes a volume decrease which is usually in the range of 10–15 mL/mol, and the activation volume for a simple bond-forming step is usually a substantial fraction of the overall decrease. The case of bond breakage is reciprocal, and expansions on the order of 10–15 mL/mol are the rule. The formation of a pair of ions produces a contraction of about 20 mL/mol in water and 40 mL/mol in organic solvents. This phenomenon, which is called electrostriction, is considerably reduced by delocalization of charge as in carboxylate ions. For reactions of certain types the interpretation of activation volumes is complicated and uncertain. If there are strong contrary effects as in bond breakage accompanied by ionization it is difficult to estimate the amount of structural reorganization in the transition state unless the volume change is extremely negative. As it happens, most such reactions do have large negative activation volumes. There is also a problem if the rate-limiting step is preceded by an equilibrium. In such cases it is necessary to evaluate the volume change of the reversible reaction. A smaller uncertainty arises when an electronic charge in a reactant becomes more or less localized in the transition state and thus affects the volume of electrostricted solvent. This effect can often be anticipated and quantitatively estimated.

The isotopic exchange reaction of a neutral weak carbon acid catalyzed by an oxy anion is well suited to a study of activation volumes because ionic charge is conserved, and the first step is rate limiting. It is still necessary, however, to cope with changes in localization of the charge. The reduction of a ketone by sodium borohydride is also suitable. A recently proposed

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