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Heteronuclear Imidazolate-Bridged Complexes of Iron(III) Porphyrins and Copper(II). Toward Modeling of Cytochrome *c* Oxidase

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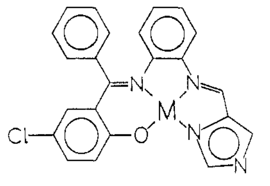
There is considerable interest in the identity of the bridging group that antiferromagnetically couples high-spin heme iron ($S = {}^5/_2$) and copper(II) ($S = {}^1/_2$) ions in the enzyme cytochrome *c* oxidase to give a $S = 2$ system. Imidazolate[1] and thiolate[2] have been suggested as possibilities, and several model compounds containing these bridges have been prepared and characterized.[3-10] Thermodynamics of the bond formation between iron porphyrins and imidazoles, as well as other nitrogenous donors, have been studied in solution.[11-13] This paper reports the formation of an imidazolate-bridged Fe(III)-Cu(II) complex in solution and the thermodynamics of the bond formation between an iron(III) porphyrin and a copper imidazolate species. Formation of the aforementioned complex was accomplished by reaction of an iron(III) porphyrin with a neutral metal chelate containing an imidazolate ring, MIm (I). Axial ligation of the iron porphyrin by MIm yields imidazolate-bridged M(II)-Fe(III) centers.



I

M = Cu or Ni

The reaction of Fe(TPP)Cl (TPP is the dianion of tetraphenylporphyrin) with an axial base, B, in a noncoordinating solvent can be represented by the following stepwise equilibria to give the $[\mathrm{Fe(TPP)B_2}]^+\mathrm{Cl}^-$ ion pair:

$$\mathrm{Fe(TPP)Cl} + \mathrm{B} \overset{K_1}{\rightleftharpoons} \mathrm{Fe(TPP)BCl} \tag{1}$$

$$\mathrm{Fe(TPP)BCl} + \mathrm{B} \overset{K_2}{\rightleftharpoons} [\mathrm{Fe(TPP)B_2}^+]\mathrm{Cl}^- \tag{2}$$

With most bases only the overall formation constant, β_2, is observed since, in general, $K_2 \gg K_1$.[11]

$$\mathrm{Fe(TPP)Cl} + 2\mathrm{B} \overset{\beta_2}{\rightleftharpoons} [\mathrm{Fe(TPP)B_2}]^+\mathrm{Cl}^- \tag{3}$$

The overall reaction in DMSO, in which the chloride ion is initially dissociated, is represented as

$$\mathrm{Fe(TPP)(DMSO)_2}^+ + 2\mathrm{B} \overset{\beta_2}{\rightleftharpoons} \mathrm{Fe(TPP)B_2}^+ + 2\mathrm{DMSO} \tag{4}$$

Spectroscopic studies are reported here for the Fe(porphyrin)Cl + MIm system in DMSO. Formation of the bis adduct in this case yields the trinuclear system MIm-Fe$^{\mathrm{III}}$-ImM, in which imidazolate links the Fe(III) atom to both M(II) atoms. The present study investigates the thermodynamics of the reaction of CuIm and Fe(TPP)Cl in DMSO to give a product that contains the Cu$^{\mathrm{II}}$-Im-Fe$^{\mathrm{III}}$ fragment of the imidazolate-bridged model of cytochrome *c* oxidase. The electronic dependence of the reaction of Fe(*p*-XTPP)Cl (*p*-XTPP is the dianion of the tetrakis(*p*-X-substituted)tetraphenylporphyrin) with 1-methylimidazole (1-MeIm) in DMSO was also investigated and found to obey the Hammett equation (5) with a positive ρ value. $\beta_{2\mathrm{X}}$ and $\beta_{2\mathrm{H}}$ are

$$\log \beta_{2\mathrm{X}} = \log \beta_{2\mathrm{H}} + 4\sigma\rho \tag{5}$$

the formation constants of the adducts of Fe(*p*-XTPP)Cl and Fe(TPP)Cl with 1-MeIm, respectively, σ is the Hammett constant[14] for the *p*-X substituent, and ρ is the sensitivity of the reaction to electronic effects.

Experimental Section

Reagents. 1-Methylimidazole (1-MeIm) was obtained from Aldrich, vacuum distilled, and stored over molecular sieves prior to use. Fe(TPP)Cl and Fe(OEP)Cl (OEP is the dianion of octaethylporphyrin) were obtained from Aldrich and stored in the dark at −5 °C until needed. Spectrophotometric grade dimethyl sulfoxide (DMSO, Aldrich) and dimethylformamide (Baker) were used as received.

M[CBP-PHEN-4-CHO-Im]. The metal imidazolate complexes, MIm, were prepared by the literature procedure[15] and recrystallized from ethanol before use.

$\mathbf{H_2}$(*p*-XTPP). The para-substituted tetraphenylporphyrins (X = $\mathrm{CH_3O}$, $\mathrm{CH_3}$, F, and Cl) were prepared by the method of Adler[16] by the reaction of the appropriately substituted benzaldehyde (Aldrich) with pyrolle in propanoic acid.

Fe(*p*-XTPP)Cl. Iron was inserted into the porphyrin by the method of Adler,[17] as described previously.[18] The complexes were chromatographed before use in subdued light on dry alumina with chloroform as eluent. The chloroform solutions were treated with HCl gas and evaporated to dryness. The complexes were then stored in the dark at −5 °C until needed.

Spectra. Visible spectra were obtained on a Perkin Elmer Lamda 3B spectrophotometer equipped with a Model 3600 data station and a thermostated cell compartment attached to a circulating constant-temperature bath or on a Perkin Elmer Model 576 spectrophotometer with a temperature controller. Absorbance data were obtained as follows. A fixed aliquot of Fe(*p*-XTPP)Cl or Fe(OEP)Cl and variable amounts of axial base were mixed and diluted to 2.5 mL giving a total metalloporphyrin concentration of $\sim 4.0 \times 10^{-5}$ M. The absorbance at 529 nm (Fe(TPP)Cl) or 552 nm (Fe(OEP)Cl) was monitored as a function of base concentration.

Calculations. Equilibrium constants were determined from the x intercepts of plots of $\ln [(A - A_0)/(A_c - A)]$ vs $\ln [\mathrm{B}]_{\mathrm{eq}}$ where A_0 is the absorbance of the porphyrin, A_c is the absorbance of the adduct, A is the absorbance of an intermediate solution, and $[\mathrm{B}]_{\mathrm{eq}}$ is the equilibrium concentration of base. Enthalpies and entropies of reaction were determined from a least-squares fit of $\ln \beta_2$ vs $1/T$ over the temperature range 20.0-35.0 °C.

Results and Discussion

The reaction of Fe(TPP)Cl with nitrogenous donors is easily followed by monitoring its visible spectrum in the 750-450-nm region. In noncoordinating solvents, such as chloroform, di-

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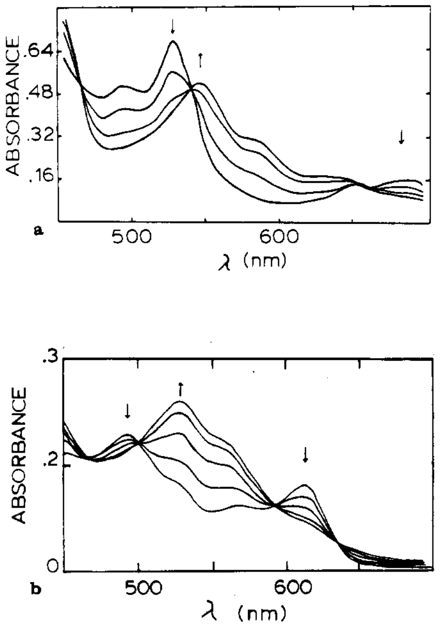

Figure 1. Spectral changes upon addition of CuIm to Fe(por)Cl in DMSO: (a) [Fe(TPP)Cl] = 4.9 × 10^{-5} M and [CuIm] = 0, 1.6 × 10^{-4}, 2.3 × 10^{-4}, and 3.1 × 10^{-4}M, with isobestic points at 659, 541, and 465 nm; (b) [Fe(OEP)Cl] = 4.1 × 10^{-5}M and [CuIm] = 0, 6.6 × 10^{-5}, 1.3 × 10^{-4}, 2.0 × 10^{-4}, and 2.6 × 10^{-4}M with isobestic points at 635, 592, 501, and 460–473 (poorly intersecting) nm.

chloromethane, and ethyl acetate, the low-spin bis adduct produced with 1-MeIm has a spectral maximum at ~548 nm, which is red shifted from 510 nm.[19] Evidence for axial ligation of Fe(TPP)Cl by CuIm was not observed in chloroform or toluene probably due to the relatively small values of β_2 in these solvents and to solubility limitations of CuIm. The spectrum of Fe(TPP)Cl in DMSO differs from that observed in noncoordinating solvents, but the spectral changes (Figure 1a) that occur on addition of CuIm or 1-MeIm to Fe(TPP)Cl are nearly identical with one another and ultimately resemble the spectrum of the bis adducts observed in noncoordinating solvents. Likewise, the spectral changes (Figure 1b) that occur on addition of CuIm to Fe(OEP)Cl in DMSO also resemble those obtained with 1-MeIm as axial base. Thus, the product of the reaction of Fe(por)Cl with CuIm (eq 4) is formulated as the low-spin bis adduct, [CuIm–Fe(por)–ImCu]$^+$, by analogy to the nearly identical spectral changes observed in the reaction of 1-MeIm with Fe(por)Cl and as demonstrated by the spectrophotometric titrations described below. The isolation and characterization of these products in the solid state have not been achieved to date due to the low volatility of DMSO and the high solubility of these products in this solvent and the absence of product formation in solvents of greater volatility such as toluene or chloroform.

Spectrophotometric titrations of Fe(TPP)Cl and Fe(OEP)Cl with 1-MeIm or CuIm were conducted at 529 and 552 nm, respectively. Plots of ln $[(A - A_0)/(A_c - A)]$ vs ln $[\mathrm{B}]_{\mathrm{eq}}$ gave slopes of 2.0, indicating bis ligation. Equilibrium constants were obtained from the x intercepts. Enthalpies and entropies for the reaction of Fe(TPP)Cl with 1-MeIm and with CuIm were determined from van't Hoff plots as shown in Figure 2. These data and those of related reactions are given in Table I.

The enthalpy of the axial ligation of Fe(TPP)Cl is nearly the same with both 1-MeIm and CuIm. Consequently, the large differences in the formation constants for the bis adducts of Fe(TPP)Cl at 25 °C with 1-MeIm and CuIm are attributed to entropy effects. The same observation was made in comparison of the thermodynamic terms for the reaction of 1-MeIm and CuIm with Zn(TPP) in toluene.[20] A possible explanation for the disparity in the entropy terms is that the CuIm complex may be partially dimerized in solution. The overall structure of CuIm[21] is similar to those of related tetradentate Schiff base complexes[15] but differs in that it is weakly dimerized through its phenolic oxygen atom. A similar attraction, although weaker, may exist in solution. This effect is probably also responsible for the differences in the formation constants at 25 °C of the bis adducts of Fe(OEP)Cl with 1-MeIm and CuIm.

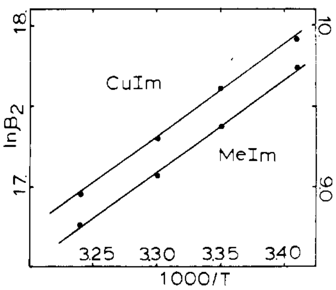

Figure 2. Plot of ln β_2 vs $1/T$ for reaction of 1-MeIm and CuIm with Fe(TPP)Cl in DMSO. Values are given as ln β_2(1-MeIm), ln β_2(CuIm), $1/T$: 9.74, 17.93, 0.003 41; 9.38, 17.63, 0.003 35; 9.07, 17.31, 0.003 30; 8.77, 16.97, 0.003 24.

Table I. Selected Thermodynamic Data for Reactions of Iron(III) Porphyrins

$$\mathrm{Fe(por)Cl} + 2\mathrm{B} \overset{\beta_2}{\rightleftharpoons} \mathrm{Fe(por)B_2{}^+Cl^-}$$

porphyrin	B	β_2[a]	ΔH, kJ/mol	ΔS, eu	solvent	ref
TPP	1-MeIm	1.4 × 10^3	−109	−37	$CDCl_3$	12
	1-MeIm	1.28 × 10^4	−42.7	−15.4	DMSO[b]	13
	1-MeIm	1.20 × 10^4	−47.8	−19.6	DMSO	this work
	CuIm	4.40 × 10^7	−47.5	−3.0	DMSO	this work
OEP	1-MeIm	6.75 × 10^3			$CHCl_3$	11
	1-MeIm	2.49 × 10^3			DMSO	this work
	CuIm	8.81 × 10^7			DMSO	this work

[a] 298 K. [b] Ionic strength adjusted to 0.04 M.

The enthalpies for the reaction of Fe(TPP)Cl and 1-MeIm in noncoordinating[12] ($CDCl_3$) and coordinating (DMSO) solvents are significantly different. Although the enthalpy change is more favorable in chloroform than in DMSO, it is offset by an extremely unfavorable entropy term, resulting in larger formation constants in DMSO. The more favorable entropy values obtained in DMSO may arise from the release of two solvent molecules from the solvated iron(III) porphyrin in DMSO (eq 4) compared to a single chloride ion from the undissociated Fe(TPP)Cl in noncoordinating solvents (eq 3).[13]

The spectral changes that are observed on addition of NiIm to Fe(TPP)Cl or Fe(OEP)Cl in DMSO differ from those observed on addition of CuIm or 1-MeIm. Addition of NiIm to Fe(TPP)Cl initially results in an increase in the intensity of the 529-nm band, followed by a decrease in absorbance at this wavelength as the base concentration is increased, eventually producing the spectrum of the bis adduct. The same type of behavior is observed in the Fe(OEP)Cl–NiIm system. Similar results were obtained for the reaction of Fe(TPP)Br or Fe(TPP)I with 1-MeIm in $CHCl_3$ by Walker et al.[11] and are interpreted as indicating stepwise formation of the bis adduct (eq 6 and 7). This is in contrast to the reactions

$$\mathrm{NiIm} + \mathrm{Fe(por)(DMSO)_2{}^+} \overset{K_1}{\rightleftharpoons} \mathrm{Fe(por)(NiIm)(DMSO)^+} \tag{6}$$

$$\mathrm{NiIm} + \mathrm{Fe(por)(NiIm)(DMSO)^+} \overset{K_2}{\rightleftharpoons} \mathrm{Fe(por)(NiIm)_2{}^+} \tag{7}$$

of Fe(TPP)Cl and Fe(OEP)Cl with both 1-MeIm and CuIm, which show uniform changes throughout the concentration range employed due to apparent overall addition of two ligands. Direct

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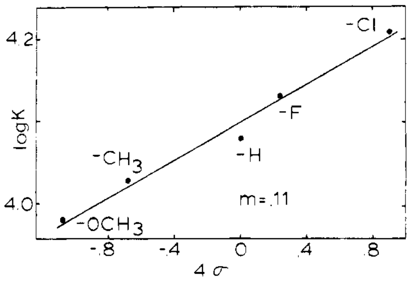

Figure 3. Hammett plot for the reaction of Fe(*p*-XTPP)Cl and 1-MeIm in DMSO at 25 °C. X (4σ, log β_2): Cl (0.908, 4.21), F (0.248, 4.15), H (0.0, 4.08), CH_3 (−0.68, 4.05), OCH_3 (−1.072, 3.98).

comparison of the reactivities of CuIm and NiIm as was done in their reactions with Zn(TPP)[20] is not possible in this case because of the obvious mechanistic differences. The spectral parameters in the NiIm case are such that determination of K_1 and K_2 and subsequent calculation of β_2 cannot be accomplished by methods employed for related systems[22] that undergo stepwise formation. It appears that these mechanistic differences between the reaction of 1-MeIm or CuIm with Fe(por)Cl (eq 4) and NiIm with Fe(por)Cl (eq 6 and 7) are solvent dependent. In DMF, no significant differences in spectral changes and isosbestic behavior were observed for the reaction of Fe(TPP)Cl ($\sim 10^{-5}$M) with 1-MeIm, CuIm, and NiIm.

The electronic effect of varying substituents on the porphyrin periphery on the axial ligation of Fe(*p*-XTPP)Cl by 1-MeIm in DMSO was examined as part of this study to determine conditions for optimum formation of the bis-ligated products in this solvent and for comparison with literature values obtained in noncoordinating solvents. The electronic dependence with CuIm is expected to be similar since this dependence is largely a function of the metalloporphyrin, not the axial base, and a recent Hammett study of its reaction with Zn(*p*-XTPP)[23] shows that CuIm behaves similarly to other nitrogenous donors. The Hammett plot for the reaction of Fe(*p*-XTPP)Cl with 1-MeIm in DMSO, shown in Figure 3, is linear with $\rho = +0.11$. This value is opposite in sign to values obtained in $CHCl_3$ by spectrophotometric means[11] (−0.39) and in $CDCl_3$ by NMR[12] (−0.41). There are two effects operative in determining the value of ρ in the axial ligation reactions of Fe(*p*-XTPP)Cl: (1) the Lewis acidity of Fe(*p*-XTPP)Cl, which predicts an increase in β_2 with increasing electron-withdrawing ability of the substituents to give a positive dependence, and (2) the charge stabilization of an ion-pair product, which produces a negative dependence. From the data presented here, it is concluded that the former effect is more important in DMSO with charge stabilization being accomplished through solvation. These results are consistent with reports which state that the chloride ion is dissociated in DMSO.[24]

The purpose of the present work has been to synthesize a complex for the imidazolate-bridged model of cytochrome *c* oxidase. The reaction between Fe(por)Cl and CuIm in DMSO succeeds in producing imidazolate-bridged Fe(III) and Cu(II) centers although it fails in producing an acceptable model of the enzyme in that the resultant complex is trinuclear and the iron atom is low spin. The reaction of CuIm with sterically hindered Fe(III) porphyrins is currently being investigated as a method of synthesizing the mono CuIm adduct. The magnetic properties of these adducts, in which the iron atom is expected to be high spin, will be of considerable interest in an investigation of the ability of the imidazolate bridge to foster antiferromagnetic coupling between the iron and copper centers as postulated for cytochome *c* oxidase.

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Registry No. CuIm, 103690-64-8; Fe(TPP)Cl, 16456-81-8; Fe(OEP)Cl, 28755-93-3; Fe(*p*-XTPP)Cl (X = CH_3O), 36995-20-7; Fe(*p*-XTPP)Cl (X = CH_3), 19496-18-5; Fe(*p*-XTPP)Cl (X = F), 60250-86-4; Fe(*p*-XTPP)Cl (X = Cl), 36965-70-5; 1-MeIm, 616-47-7; cytochrome *c* oxidase, 9001-16-5.

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