Dynamics and Thermodynamics of Axial Ligation in Metalloporphyrins. 5. Affinity of Ferric Porphyrins for Nitrogenous Bases and the Stoichiometry and Spin States of the Product Complexes

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Abstract: Proton NMR has been employed to characterize the association of substituted imidazoles and pyridines with synthetic ferric porphyrin halides. For tetraarylporphyrins and unhindered bases the only observable reaction is described by: $PFeX + 2L \rightleftharpoons PFeL_2^+X^-$, with the equilibrium given by $\beta_2 = [PFeL_2^+X^-]/[PFeX][L]^2$. β_2 was found to increase with decreasing FeX bond strength and decreasing electron withdrawing properties of the porphyrin substituents. β_2 also decreased significantly upon introducing substituents at the N-position. The increased polarity of the N–H bond, which is thought to stabilize the coordination of imidazole, is confirmed by the observation of strong hydrogen bonding between coordinated and free base. Determination of solution susceptibilities and direct integration of the proton NMR spectra for complexes with sterically hindered bases (2-methyl- and 1,2-dimethylimidazole, 1-methylbenzimidazole) demonstrate that, contrary to previous ESR and Mössbauer data, primarily low-spin bis complexes are formed.

The characterization of a wide variety of hemoproteins[2-3] has revealed the confirmed or suspected existence of at least one histidyl imidazole–iron bond in the native proteins. Although the one iron–imidazole linkage appears ubiquitous, there is a great diversity in the ligands trans to the imidazole, ranging from none in deoxyhemoglobin[2] (Hb) and -myoglobin[2] (Mb), for example, to thioethers[3a] in cytochrome *c*, a second imidazole[3a] in cytochromes b_2, b_5, water[2] in met-Hb, -Mb, and the coordinated substrate[2] in ligated Hb, Mb, and peroxidases,[3b] etc. Thus the iron–imidazole bond is almost as characteristic of hemoproteins as the dominant heme moiety.

The special role of histidine in hemoproteins has therefore drawn considerable attention to the iron–imidazole bond and numerous studies have been directed towards elucidating the physical properties of model iron porphyrin complexes with imidazole[4-17] and the relationship between the degree of axial coordination, spin state, and stereochemistry about the iron. There is also renewed interest in the factors which influence the affinity of heme iron for axial ligands inasmuch as two allosteric effectors (Bohr proton and organic phosphates) operate in vivo to modulate the affinity of hemoglobin towards oxygen.[2] How these two effectors alter the metal acidity with respect to both the kinetic and thermodynamic properties of oxygen binding is incompletely understood, but both cis porphyrin perturbations[18] and trans imidazole perturbations[19] have been proposed. The understanding of these phenomena in hemoproteins requires that the thermodynamics of ligand coordination in model complexes be clarified. The simplest system which could serve as a model is the reaction of ferric porphyrin halides with imidazoles.

We had previously observed[12] by proton NMR that addition of imidazole to a solution of ferric porphyrin halides yielded spectra which were the superposition of the traces of the individual unligated and ligated species in solution. This slow interconversion between the various forms permitted a direct determination of the relative amounts of all species in solution and suggested a novel method for characterizing the thermodynamics of imidazole coordination.

Although a few studies of such reactions had appeared at the time this study was initiated,[4,5] there existed no systematic study of the role of porphyrin and imidazole substituents on the thermodynamics of imidazole ligation, and virtually no data on the dynamics of imidazole ligation. More recently several more reports of imidazole coordination have appeared[7-9,12,13] and a systematic study has just been completed.[20] However, all of these studies but one[12] utilize optical spectroscopy to monitor the amounts of all species in solutions.

We pursued our present NMR study of the coordination of imidazole to ferric porphyrin for a number of reasons. Although thermodynamic data have been obtained by optical spectroscopy,[7-9,13,21] the large extinction coefficients require very dilute (0.001 to 0.1 mM) porphyrin solutions and the stoichiometry of the reaction must be evaluated by indirect means. Furthermore, the optical spectrum for an adduct, particularly in the presence of other porphyrin species, cannot be unambiguously interpreted in terms of its electronic structure or spin state. In the NMR experiment, we have shown[12] that the peaks for individual species are clearly resolved such that apparent equilibrium constants are obtainable. It is therefore of interest to compare the thermodynamic data obtained by optical spectroscopy on very dilute solutions with our NMR data derived from relatively concentrated samples (5–20 mM) and to evaluate this new method for obtaining equilibrium constants of ferric porphyrins. In addition, under the favorable circumstance of slow imidazole exchange,[12,22] it is possible to establish directly the stoichiometry of the species from the relative areas of the peaks for the coordinated imidazole and porphyrin.

Lastly, and most importantly, in addition to providing thermodynamic data and the reaction stoichiometry, the NMR experiment provides data on the spin state of the complex in the same solution in which the stoichiometry is determined. In the past, elucidation of the spin state of ferric porphyrin complexes with sterically hindered imidazoles have had to rely on ESR measurements[14] to differentiate the characteristic spectra of high spin (HS) and low spin (LS) complexes. Since the resolution of low-spin ESR spectra requires low temperatures, the spin state was necessarily defined in the frozen glass.[14] As we will illustrate below, this has led not only to incorrect conclusions as to the solution structure of certain complexes, but has resulted in the generally accepted principle that certain substituted hindered imidazoles are incapable of forming bis complexes[10,11,14,16,24] and therefore stabilize a five-coordinate, high-spin geometry for steric reasons.

The reaction we will consider is that of a high-spin ferric porphyrin halide (PFeX) with a heterocyclic base (L). The processes can occur in two steps, of which the first is:

$$\mathrm{PFeX\ (HS) + L \overset{K_1}{\rightleftharpoons} PFeX(L)\ (HS?)} \quad (1)$$

with

$$K_1 = [\mathrm{PFeX(L)}]/[\mathrm{PFeX}][\mathrm{L}] \quad (2)$$

The second step is given by

$$\mathrm{PFeX(L)\ (HS?) + L \overset{K_2}{\rightleftharpoons} PFeL_2{}^+X^-\ (LS)} \quad (3)$$

with the product a tight ion pair,[7,8] and

$$K_2 = [\mathrm{PFeL_2{}^+X^-}]/[\mathrm{PFeX(L)}][\mathrm{L}]. \quad (4)$$

Since the change in spin state occurs upon addition of the second imidazole,[15] K_2 is generally much larger than K_1, so that in some favorable cases only the overall process need be considered,[20] i.e.,

$$\mathrm{PFeX + 2L \overset{\beta_2}{\rightleftharpoons} PFeL_2{}^+X^-} \quad (5)$$

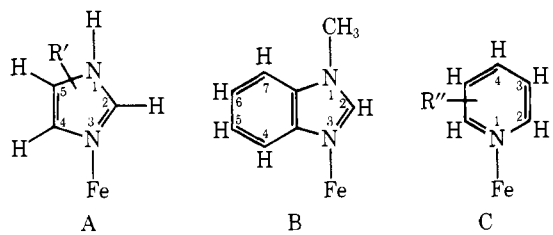
with $\beta_2 = K_1K_2$, or

$$\beta_2 = [\mathrm{PFeL_2{}^+X^-}]/[\mathrm{PFeX}][\mathrm{L}]^2 \quad (6)$$

Our preliminary NMR measurements showed[12] that, for sterically unhindered imidazoles, the condition $K_2 \gg K_1$ holds so that only PFeX and $PFeL_2{}^+X^-$ are detectable in solution, indicating that eq 6 is the only measurable equilibrium constant. Similarly, only the 2:1 complexes of imidazoles were detected[25] in a separate study of the coordinated ligand isotropic shifts for both synthetic and natural porphyrins. However, the question as to the spin state and stoichiometry of complexes with sterically hindered bases remains open.

Our intent in this study, therefore, is to establish the validity of NMR for obtaining equilibrium constants and to characterize the thermodynamics of coordination of unhindered heterocyclic bases to ferric porphyrins. In the case of sterically hindered bases[10,11] we will define both the coordination stoichiometry and spin state of the resulting solution complexes. In the companion study,[22] we will report on the dynamic properties of axial ligation of these heterocyclic bases.

We employ here only the high spin ferric porphyrin halide complexes of synthetic porphyrins, PFeX, since the analogous natural porphyrins aggregate appreciably at the concentrations required for the NMR work. The complexes of interest are the *meso*-tetraarylporphyrins[26] (R-TPPFeX, X = halide) and octaethylporphyrin[27] (OEPFeX); the heterocyclic bases are various substituted imidazoles (R′-Im, A), 1-methylbenzimidazole (1-CH_3Bzim, B), and substituted pyridines (R″-py, C). The numbering system[28] adopted for imidazoles is that for the coordinated ligand depicted in A.



A B C

Experimental Section

Substituted *meso*-$\alpha,\beta,\gamma,\delta$-tetraphenylporphyrins were prepared by the methods of Adler et al.[26] and purified as previously described. The ferric chloride complexes were also synthesized according to Adler.[26] Bromide or iodide complexes were obtained from (*p*-R)TPPFeCl or OEPFeCl by metathesis as described elsewhere.[29] The purity of these complexes was determined optically and by proton NMR. The complexes used were at all times greater than 99% pure. Octaethylporphyrin[27] was a gift from Professor H. H. Inhoffen.

The various axial bases, imidazole (Im), 2-methylimidazole (2-CH_3Im), 1-methylimidazole (1-CH_3Im), 5-methylimidazole, (5-CH_3Im), 1-methylbenzimidazole, (1-CH_3Bzim), and 1-benzylimidazole (1-BzIm), were purified by previously published methods.[25] 1-Methyl-5-chloroimidazole (1-CH_3-5-ClIm) was obtained as a gift from Professor F. A. Walker in a highly purified form as determined from the proton NMR spectrum.

4-Methylpyridine (4-CH_3py) and pyridine (py) were obtained as spectroquality solvents (MCB). They were distilled as clear liquids onto activated molecular sieves (Baker, 3A) and stored under nitrogen in sealed brown bottles. The purity of all the heterocyclic bases was determined to be greater than 99% from proton NMR spectra.

The bis-substituted base complexes ($PFe(L)_2{}^+X^-$) were prepared in chloroform-*d* solutions. These solutions were typically 0.02 M in ferric porphyrin complex and varied between 0.04 and 0.10 M in the concentration of the heterocyclic base, with the concentration accurately determined.

Peak assignments for the coordinated, substituted imidazole ligand protons have been published elsewhere.[25] The assignments of the protons of coordinated pyridine and 4-methylpyridine ligands have similarly been determined and will be described in a forthcoming publication (vide infra).

Proton NMR spectra were obtained using a JEOL-PS100 FT NMR spectrometer operating at 99.5 MHz. Between 200 and 2000 transients were accumulated using a 20-μs 90° pulse; points were collected over bandwidths of 4000 or 6250 Hz. All temperatures were calibrated to an accuracy of ±1 °C using a Leeds-Northrup potentiometer equipped with an iron–constantan thermocouple. During variable temperature runs the probe temperature was measured both before and after data accumulation. Solution susceptibilities were determined by the method of Evans[23] on a sample containing 0.020 M complex and an appropriate excess of base. The anulus contained PFeX, base, and 1% Me_4Si in $CDCl_3$, while the capillary contained the identical amounts of the free porphyrin (PH_2), base, and 5% Me_4Si to minimize differences in diamagnetic susceptibilities. The magnetic moment of the paramagnetic adduct was obtained from the Me_4Si peak separation at different temperatures in a standard manner.

Equilibrium constants were determined[30] by integration of a HS (typically *m*-H of R-TPP$FeL_2{}^+$) and a LS (typically methyl of X-CH_3Im), correcting for the fact that the HS peak reflects eight protons, while the LS peak contain only six. At least six determinations were made in each case. Thermodynamic parameters were obtained by fitting the data in a ln β_2 vs. T^{-1} plot to a straight line using a least-squares computer program.

Results

The solution complexes of the unhindered imidazoles with TPPFeCl have been previously shown[25] to be solely the 2:1 adducts, $TPPFeL_2{}^+Cl^-$, at ambient temperatures, as verified by integration of the assigned imidazole and porphyrin pyrrole-H peaks. Both solid state[15] and solution NMR[7] susceptibility measurements give $\mu \sim 1.8\ \mu_B$, which is consistent with the low-spin, $S = \frac{1}{2}$ state. Thus the stoichiometry of the product complexes involving Im, 1-CH_3Im, 5-CH_3Im, and 1-BzIm are established. The proton trace (not shown) for the 1-CH_3-5-ClIm complex exhibits a coordinated 1-CH_3 peak indicative of the 2:1 stoichiometry below 20 °C; fast ligand exchange[22] precludes an integration at higher temperatures. The stoichiometry and spin state of adducts with hindered imidazoles will be discussed later (vide infra).

The proton trace of the 1-CH_3Bzim complex is illustrated in Figure 1. Although this ligand can be classified as sterically hindered due to the 4-H on the six-membered ring, earlier studies[16] had suggested 2:1 coordination. In Figure 1, the assigned 1-CH_3 peak intensity relative to that of the upfield pyrrole-H clearly confirms the bis nature of the complex. Although ligand exchange is too fast to permit integration at higher temperature, the characteristic low-spin pyrrole-H peak is observed up to 25 °C, though with reduced intensity due to the shift of the equilibrium in eq 5 to the left.

Although the proton NMR spectra of the pyridine complexes of natural porphyrins have been reported and evidence

Table I. Effect of Axial Base on β_2 for $TPPFeL_2^+Cl^-$[a]

Axial base L	$[L]_o$[b]	pK_a	$[TPPFeCl]_o$[b]	T[c]	β_2[d]
1-CH_3Im	43.9	7.33[e]	20.9	25	$(1.38 \pm 0.20) \times 10^3$
	22.9	7.33[e]	9.80	25	$(1.40 \pm 0.22) \times 10^3$
	21.9	7.33[e]	7.85	25	$(1.37 \pm 0.28) \times 10^3$
5-CH_3Im	10.8	7.22[e]	11.1	25	$\gtrsim 10^6$
Im	10.2	6.65[e]	18.8	25	$\gtrsim 10^6$
1-BzIm	51.0		21.2	25	$(3.5 \pm 0.6) \times 10^2$
1-CH_3-5-ClIm	285.	4.75[e]	23.8	9	34 ± 7
1-CH_3Bzim	523.	5.6[e]	58.3	−45	25 ± 5
4-CH_3py	114.	6.10[e]	21.9	−16	35.2 ± 7.0
	57.0	6.10[e]	11.0	−16	36.9 ± 7.4
	114.	6.10[e]	21.9	−25	54 ± 10
Py	193.	5.17[e]	22.0	−25	9.3 ± 0.9

[a] In $CDCl_3$ solution. [b] Concentrations in millimolar (mM). [c] Temperature in °C. [d] β_2 as defined in eq 5, in units of M^{-2}. [e] Taken from ref 41 and 42.

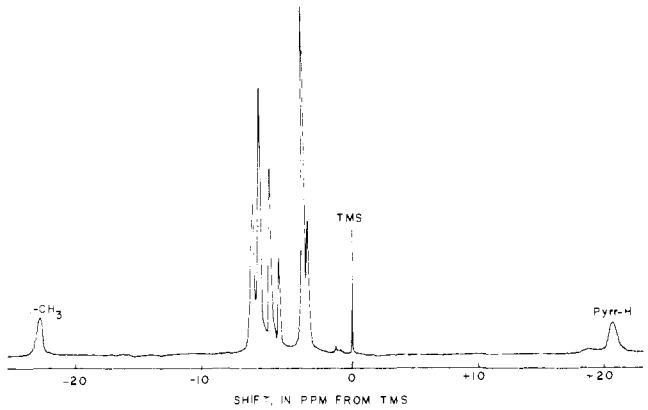


Figure 1. Proton NMR trace of $TPPFe(1\text{-}CH_3Bzim)_2^+Cl^-$ in $CDCl_3$ at −56 °C. The region 0 to −10 ppm contains both the free and some unassigned coordinated base peaks. $[TPPFeCl]_o = 0.020$ M, $[1\text{-}CH_3Bzim]_o = 0.10$ M.

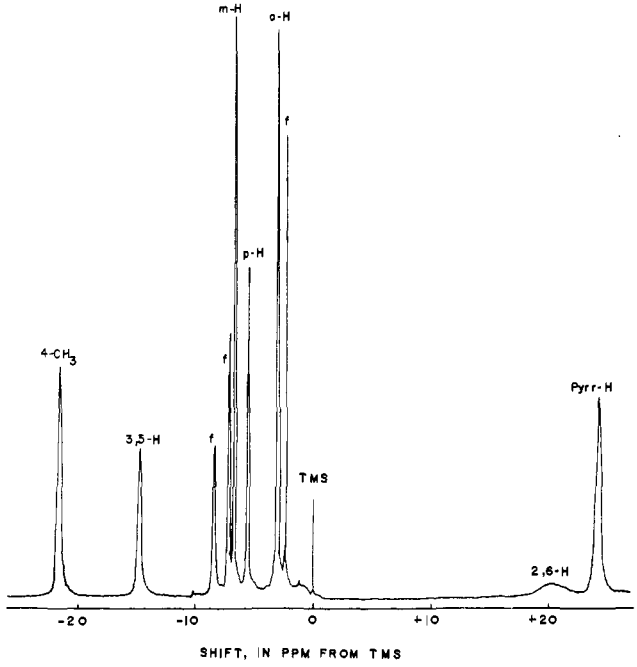


Figure 2. Proton NMR trace of $TPPFe(4\text{-}CH_3py)_2^+Cl^-$ in $CDCl_3$ at −61 °C; f indicates free 4-CH_3py peaks. $[TPPFeCl]_o = 0.02$ M, $[4\text{-}CH_3py]_o = 0.042$ M.

for the 2:1 stoichiometry presented,[31,32] these complexes of synthetic porphyrins have not been previously characterized. The proton trace of $TPPFe(4\text{-}CH_3py)_2^+Cl^-$ at −30 °C is shown in Figure 2. The 2:1 area ratio of pyrrole-H relative to the pyridine meta-H clearly establishes the bis stoichiometry.

In all cases involving unhindered imidazoles, the proton traces yielded exclusively peaks which arose from either the unligated high-spin reactant, PFeX, or the 2:1 adduct $PFeL_2^+X^-$, indicating that the reaction can be described by eq 5. The equilibrium constants, β_2, were computed from the relative areas of the HS meta-H doublet and the low-spin 1-CH_3, 5-CH_3, or 5-H imidazole peaks and 4-H or 4-CH_3 pyridine peaks, corrected for the different number of protons in each resonance. The effects of the axial base on β_2 are listed in Table I, while Table II gives β_2 as a function of porphyrin substituent and halide. Typical proton traces as a function of temperature are illustrated in Figure 3 for TPPFeCl and 1-CH_3Im. The log β_2 vs. T^{-1} plots for this system, as well as for TPPFeCl and 1-CH_3Bzim, are shown in Figure 4.

The effects of the excess ligand (5-CH_3Im and 1-CH_3Im) on the methyl shift at the coordinated ligands of $TPPFeL_2^+Cl^-$ in $CDCl_3$ are depicted in Figure 5.

The low-temperature proton trace of the complex with the sterically hindered base, 2-CH_3Im, is shown in Figure 6. At this temperature, the integration of the coordinated ligand 1-H peaks establishes the stoichiometry as $TPPFeL_2^+Cl^-$. The solution susceptibility[23] of this complex, as well as those of $TPPFeL_2^+Cl^-$ with L = 1,2-$(CH_3)_2$Im, Im, 1-CH_3Bzim and py, and TPPFeCl are listed in Table III.

Discussion

The confirmation that unhindered imidazoles yield only the bis, low-spin complexes in solution is not unexpected inasmuch as representative samples had been well characterized in the solid state by x-ray crystallography,[17] magnetic susceptibility,[15] Mössbauer,[5,16] and ESR[14] studies. Rapid imidazole exchange[22] in a number of cases precluded integration of the coordinated ligand peaks at ambient temperatures. However, the characteristic low-spin pyrrole-H peak follows the expected Curie law[33] from the low temperatures where the integration can be performed right up to ambient temperatures, confirming the integrity of the low-spin bis complexes for the imidazoles listed in Table I and the porphyrins listed in Table II.

It is somewhat surprising that the bis-pyridine adducts do not exhibit any spin equilibrium,[31,32] as evidenced by both the solution susceptibility data in Table III and the rigorous adherence to the Curie law of the pyrrole-H peak[34] up to 10 °C.

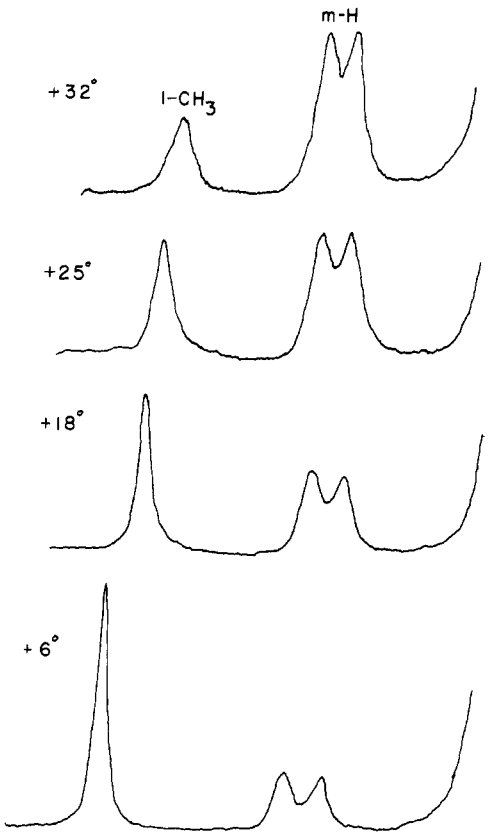


Figure 3. Part of the proton NMR trace where the LS 1-CH_3 peak and the HS meta-H peaks of TPPFe(1-CH_3Im)$_2^+Cl^-$ resonate; $[TPPFeCl]_o$ = 0.0209 M, $[1\text{-}CH_3Im]_o$ = 0.0439 M, in $CDCl_3$.

Table II. Effect of Porphyrin Substituents and Halide on β_2 for $PFeL_2^+X^-$ [a]

Complex	Ligand	T, °C	β_2
p-CH_3OTPPFeCl	1-CH_3-Im	25	$(4.0 \pm 0.6) \times 10^3$
p-CH_3TPPFeCl		25	$(3.0 \pm 0.4) \times 10^3$
TPPFeCl		25	$(1.4 \pm 0.7) \times 10^3$
p-ClTPPFeCl		25	$(0.65 \pm 0.10) \times 10^3$
p-CH_3TPPFeBr		25	$\gtrsim 10^6$
p-CH_3TPPFeI		25	$>10^6$
OEPFeCl		25	$(0.66 \pm 0.13) \times 10^3$
TPPFeCl	1-CH_3-Bzim	−45	25 ± 5
p-CH_3TPPFeBr	1-CH_3-Bzim	−45	$(2.4 \pm 0.5) \times 10^3$

[a] In $CDCl_3$ solution and $[PFeX]_o \sim 20$ mM in each case.

This behavior contrasts that of the presumed bis-pyridine complexes of natural porphyrins,[31,32] which exhibit a spin-equilibrium even at reduced temperatures. The detailed analysis of the bonding of pyridines will be treated elsewhere.[34]

The analysis of the proton spectra of solutions containing both PFeX and an unhindered base L revealed at most only two porphyrin species in all cases, the unreacted PFeX and the bis adduct $PFeL_2^+X^-$ (indirect kinetic evidence[22] for the existence of traces of a third species at high temperatures and for hindered bases is obtainable from analysis of line width and will be considered in the companion report). The existence of measurable quantities of only PFeX, $PFeL_2^+X^-$, and free L for the unhindered bases in the limit of slow-ligand exchange is supported by two lines of evidence; in no case was it possible to detect signals arising from any other species in solution, and the sum of the areas of the pyrrole-H peak in PFeX and $PFeL_2^+X^-$ remained constant as the amount of L is varied,

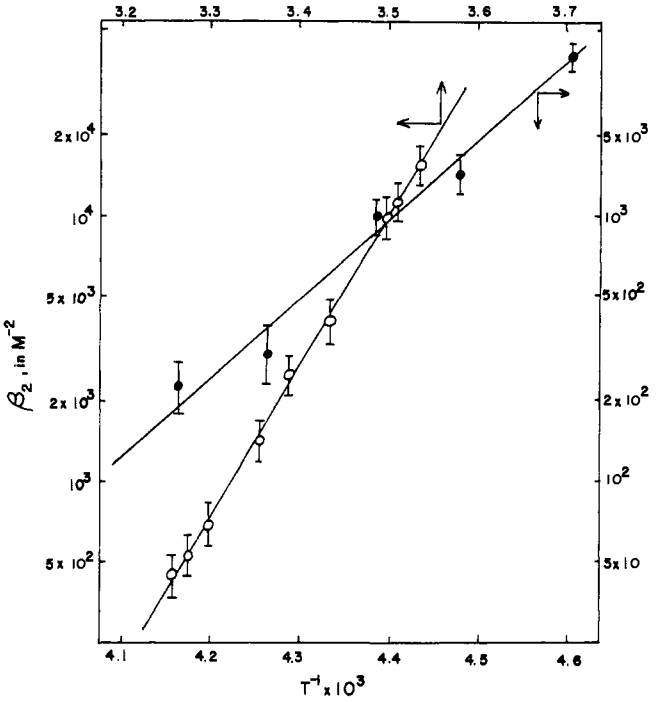


Figure 4. Semilog plot of β_2 vs. T^{-1} for TPPFeCl and 1-CH_3Im (○) and for TPPFeCl and 1-CH_3Bzim (●).

Table III. Solution Susceptibility Data

Complex	T[a]	μ_{eff}[b]	$[L]_o/[PFeX]_o$	2:1 integration[c]
TPPFeCl	25	5.93		
	−25	5.76		
TPPFe(Im)$_2^+$	25	1.76	10	
	−25	1.70	10	
	−58	1.71	10	
TPPFe(2-CH_3Im)$_2^+$	−25	1.72	10	Below −20 °C
	−58	1.75	3–10	
TPPFe(1,2-$(CH_3)_2$-Im)$_2^+$	−50	1.81	~25	Below −50 °C
TPPFe(1-CH_3-Bzim)$_2^+$	−25	1.80	10	Below −25 °C
TPPFe(py)$_2^+$	−30	1.77	10	Below −20 °C

[a] T in °C. [b] μ_{eff}, in μ_B. [c] Verification of the bis stoichiometry.

indicating that, within experimental error, all of the porphyrin exists in these two species.

The validity of the β_2 for R-TPPFeL$_2^+X^-$ complexes in Tables I and II is supported by the invariance of the β_2 with TPPFeCl concentration (7–50 mM) with 1-CH_3Im and 4-CH_3py (see Table I). Furthermore, comparison of the β_2 values in Table II for the R-TPPFeCl and 1-CH_3Im systems with similar data obtained by the more conventional optical titrations[20] reveals a surprising degree of consistency in view of the divergent concentrations employed. In each of the four cases in Table II, the β_2's determined by NMR and those obtained by optical spectroscopy[20] are within experimental error. This suggests that the degree of aggregation of R-TPPFeCl in $CDCl_3$ is not significant at 20 mM and ambient temperatures. For the case of OEPFeCl and 1-CH_3Im, our value for β_2 (Table II) is found to be a factor of 10 smaller than that determined optically.[20] However, OEP complexes are known to aggregate significantly, indicating that our β_2 is not a valid equilibrium constant and hence will not be considered further.

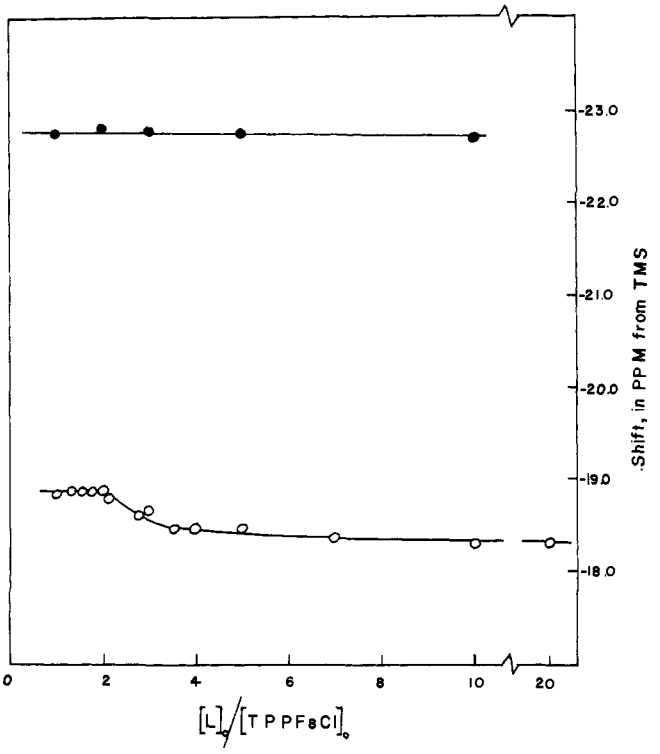


Figure 5. Plot of 1-CH_3 shift (●) of coordinated 1-CH_3Im and 5-CH_3 shift (○) of coordinated 5-CH_3Im in $TPPFeL_2^+Cl^-$ as a function of the ratio of added ligand to total porphyrin concentration. The titration was performed in $CDCl_3$ at −20 °C.

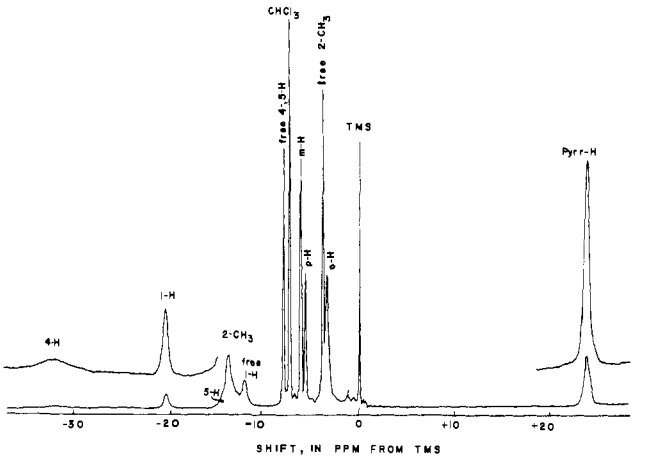


Figure 6. Proton NMR trace of $TPPFe(2\text{-}CH_3Im)_2^+Cl^-$ in $CDCl_3$ at −60 °C. The 1:4 ratios of the 4-H or 1-H to pyrrole-H peaks clearly establishes the 2:1 stoichiometry. [TPPFeCl] = 0.020 M, $[2\text{-}CH_3Im]_o$ = 0.080.

Although 1-CH_3Bzim is a sterically hindered imidazole, it was found that only the TPPFeX and $TPPFe(1\text{-}CH_3\text{-}Bzim)_2^+X^-$ species are detectable in the temperature range −25 to −60 °C, so that β_2's were determined; β_2 at −45 °C is included in Table I. This value of β_2 permits an assessment of steric and halide effects on β_2 (vide infra).

On the basis of the comparison of β_2 for the complexes in Table II (except OEPFeCl) with optical data,[20] we conclude that the NMR technique provides a useful alternate method for determining equilibrium constants for the reaction in eq 5. The method has the advantage that the stoichiometry can be simultaneously determined if the rate of axial ligand exchange[12,22] is sufficiently slow. The range of β_2 values which can be accurately determined by this NMR technique, however, is much more restricted than for optical titrations.[4,5,7-11,13] The upper limit for β_2 obtainable is $\sim 10^5$ due to the high porphyrin concentrations needed for NMR sensitivity. At $\beta_2 \sim 10^5$, the ~5% uncertainty in integration of the low-spin pyrrole-H peak always yields a value for $[PFeL_2^+X^-]$ within experimental error of $\frac{1}{2}[L]_o$. Although this could be circumvented by careful direct integration of the small free L peak, the short lifetime of this minor component broadens the peak to the point of nondetectability.[22] Lower limits of $\beta_2 \gtrsim 10$ are dictated by the uncertainties in the integrations due to the ill-defined baseline which results from the large $[L_o]$ needed to form a significant amount of the low-spin complex. Within the range $50 \gtrsim \beta_2 \gtrsim 3 \times 10^4$, however, precise determinations of β_2 are obtainable. The error limits in Tables I and II represent the ~5% uncertainties in the integrals. In comparison of a series of R-TPPFeX + L complexes, where L is constant and β_2 varies with R, differences of as little as 10% in β_2 can be readily detected by monitoring the [LS]/[HS] ratio in cases where $[PFeX]_o$ and $[L]_o$ remain invariant.

The results of the temperature dependence of the equilibrium constants were less satisfying in that only in a few cases was it possible to obtain data over a wide enough temperature range to yield useful thermodynamic parameters. The major problems were that the β_2 was too large to obtain accurately at lower temperatures, while at higher temperatures, ligand exchange broadening and the dominant free L peaks interfered with the determination of peak intensities. The most carefully studied system is TPPFeCl and 1-CH_3Im, for which the plot of β_2 vs. T^{-1} is shown in Figure 4. The value of $\Delta H \sim -26.1 \pm 1.2$ kcal is some 3 kcal larger than that reported for TPPFeCl plus Im by optical titration;[8] $\Delta S \sim -37 \pm 3$ eu is slightly smaller than that for Im,[8] but may arise from the presence of the NH group in Im (vide infra). The significance of the difference in ΔH is not understood at this time and our data must await analysis until these parameters are checked by proven methods. One possible additional source of error is that the extent of aggregation of TPPFeCl is not negligible at low temperature. The cited uncertainty is determined completely by the variation in ΔH permitted by the errors in the calculated β_2's. Although several other porphyrins were studied, the β_2's over a more restricted temperature range yielded ΔH's very close to 26 kcal and with much larger errors. Hence, we were unable to clearly distinguish ΔH differences for the R-TPPFeCl + 1-CH_3Im system, although the differences in β_2 most likely arise from enthalpy effects.

The other system for which data could be obtained, which yielded a significantly different ΔH, is TPPFeCl and 1-CH_3Bzim, for which the data are also included in Figure 4. Although the data have much more scatter due to the smaller β_2's, the values of $\Delta H = -15 \pm 3$ kcal and $\Delta S \sim -30 \pm 10$ eu indicate that at least the enthalpy is significantly different from that for 1-CH_3Im.

Effect of Porphyrin on β_2. The value of β_2 in the series of complexes R-TPPFeCl and 1-CH_3Im listed in Table II increase in the order R = p-Cl < p-H < p-CH_3 < p-CH_3O; a plot of β_2 vs. the Hammett σ^b parameter yields a slope of −0.41. Both the trend in β_2's with R and the slope of β_2 vs. σ^b agree with the data on these compounds obtained by optical titrations by Walker et al.[20]

Thus coordination of L is facilitated by electron-releasing substituents, which is opposite to that found for the coordination of bases to porphyrins of divalent metal ions.[35,36] The reverse trend for the present ferric system has been interpreted as arising from the stabilization of the positive charge on the ferric ion by electron-releasing groups, as discussed in more detail by Walker et al.[20]

Effect of Halide Ion on β_2. Although it was not possible to obtain β_2 for p-CH_3TPPFeX with X = Br and I, the lower limits for $\beta_2 \gtrsim 10^6$ for these halide ions indicate that the trend in β_2 is Cl ≪ Br and I. This is clearly demonstrated by the β_2

values in Table II for 1-CH_3Bzim with TPPFeCl and *p*-CH_3TPPFeBr. The difference in para substituent is small (as shown in Table II) compared to the $\sim 10^2$ increase due to changing Cl to Br. However, caution must be exercised in interpreting the changes in β_2 with X. We have shown[37] elsewhere that, although R-TPPFeCl is essentially monomeric in $CDCl_3$ solution up to ~30 mM, the analogous bromide complexes dimerize slightly and the iodide complexes aggregate significantly. This aggregation, however, is expected to decrease the apparent β_2, as noted above for OEPFeCl. Thus it is possible that the differences in β_2 with X are even larger than given in Table II. Another complication is that TPPFeX dissociates slightly in chloroform solution[38] for X = Br and I.

It should be noted that sizable increases in β_2 in the order F < Cl < Br < I can be expected on the basis of the enthalpy of Fe–X bond formation in the reactant. The enthalpy of the product, $PFeL_2^+X^-$, is likely to be essentially independent of X, since the halide interaction is only electrostatic for L = 1-CH_3Im, and ion pairing[39] is nearly complete at the high concentrations and in a solvent of such dielectric constant ($CDCl_3$). The enthalpy of formation of PFeX, however, is expected to increase by about 10 kcal for each halide ion,[40] with $\Delta H_f°$ most negative for X = F. We can therefore anticipate sizable increases in β_2 in the order F < Cl < Br < I, if entropy contributions are comparable. Indeed, it is observed that TPPFeF does not form a low-spin complex with 1-CH_3Im (it does, however, with 5-CH_3Im[22]).

Effect of Base on β_2. The values of β_2 for the reaction of TPPFeCl with a variety of substituted imidazoles (Table I) clearly show that there is no overall correlation with base strength as measured by pK_a's.[41,42] There is a characteristic difference dependent on substitution at 1-N, with ligands with N–H exhibiting much larger β_2's than ligands with N–R. A similar difference based on N-substituents, derived from a wider variety of ligands, was also identified by Walker et al.[20]

Within the group of N-substituted imidazoles, β_2 increases with basicity, as witnessed by comparing 1-CH_3Im (1.4×10^3) and 1-BzIm (3.5×10^2), or 1-CH_3Im and 1-CH_3-5-ClIm (~34), which probably reflects primarily electronic effects. On the other hand, the decrease in β_2 for 1-CH_3Bzim (~25 at −45 °C) relative to that of 1-CH_3Im most likely reflects steric influences, since molecular models[43] reveal that there is a strong interaction between 4-H of the ligand the the porphyrin ring for the optimum overlap of the iron and nitrogen orbitals. The steric effect causes a ~10 kcal decrease in ΔH, as shown by the data in Figure 4.

Although equilibrium constants were too large to be determined by NMR for imidazoles possessing a N–H, the $\gtrsim 10^3$ increase for Im relative to 1-CH_3Im suggests a special role for their unique proton. It has been suggested[20] that the N–H bond can impart enhanced stability to the bis complexes by delocalizing the positive charge through the increased polarity of the N–H bond. The interaction of this polar N–H bond with the halide ion would also stabilize the bis complex; such interactions have been confirmed[44] for the fluoride ion using ESR. This type of interaction is also supported by our NMR data in that, although 1-CH_3Im fails to yield a low-spin complex, bis complexes of Im and 5-CH_3Im are readily formed.[22] The increased N–H bond polarity will also increase its hydrogen bonding strength. The facts that β_2 values are dependent on imidazole concentration in certain solvents[7] and that the reaction in eq 5 suggests that four imidazoles are required in benzene[20] have been alternatively suggested to arise from either a planar stacking of the extra two imidazoles over the porphyrin π system[13] or from strong hydrogen bonding of the coordinated imidazole to free ligand.[20]

Although the NMR experiment fails to provide definitive values of β_2 for N–H containing imidazoles, it does provide data which allow us to assess the role of the free imidazole in modulating β_2 and suggests a basis for interpreting the apparent 4:1 stoichiometry[20] for the reaction in eq 5 in benzene. If the effect of excess imidazole involves hydrogen bonding to the coordinated ligand, then perturbations on the shifts for the low-spin complex $PFeL_2^+X^-$ upon addition of excess L should be localized on the axial ligand and be observable only if a N–H is present. On the other hand, if the additional L stack over the porphyrin, not only should the effect be comparable for Im and 1-CH_3Im,[45] but the excess free ligands should exhibit upfield ring-current shifts[46] and the effect on the low-spin complex should be localized on the porphyrin.

The graph in Figure 5 gives the shift from Me_4Si of the 1-CH_3 and 5-CH_3 peaks of the coordinated L in $TPPFeL_2^+Cl^-$ upon addition of excess L. At −20 °C, where these titrations were performed, $\beta_2 > 10^6$ for both ligands, so that when $[TPPFeCl]_o$ = 20 mM and $[L]_o$ = 40 mM, ≥95% of the porphyrin is present as $TPPFeL_2^+Cl^-$, and hence less than 5% as free L. As shown in Figure 5, addition of excess 1-CH_3Im has no detectable effect on the coordinated 1-CH_3Im peak; the free 1-CH_3Im peaks were found at the same positions as for the same concentration of 1-CH_3Im in the absence of the porphyrin.

In the case of 5-CH_3Im, however, Figure 5 clearly shows that there is a characteristic decrease in the coordinated 5-CH_3 shift with excess ligand, which is indicative of a labile equilibrium involving interaction of free and coordinated 5-CH_3Im. The absence of any comparable shifts for the porphyrin pyrrole-H indicates that the effect is localized on the axial ligand. The free 5-CH_3Im shifts experience no upfield shift, confirming the absence of planar π stacking. We have observed[47] similar effects for all N–H containing imidazoles, and, more importantly, we have found these effects absent in any N–R imidazole. We conclude, therefore, that the changes in coordinated 5-CH_3 peaks arise from perturbation due to hydrogen bonding of the neighboring 1-H to a free 5-CH_3Im. Such hydrogen bonding interactions could be even more important in benzene[20] and could account for the apparent 4:1 stoichiometry. Further studies of the hydrogen bonding of coordinated imidazole and its influence on thermodynamic and kinetic parameters are in progress and will be reported elsewhere.[47]

It is important to note that, although we can demonstrate hydrogen bonding to a free imidazole which should stabilize the bis complex, this type of hydrogen bonding is not responsible for the $\sim 10^3$ increase in β_2 for Im relative to 1-CH_3Im. This is demonstrated clearly by the fact that the 20 mM solution of TPPFeCl containing a 2:1 ratio of Im at 25 °C yields a ~95% conversion to the low-spin bis complex (i.e., $\beta_2 \geq 10^6$) even though there remains in solution an insignificant amount of free Im for hydrogen bonding. The stabilizing factor is probably due to the interaction of N–H group with the halide ion, as suggested by the formation of a bis complex of TPPFeF with a N–H but not with a N–CH_3 containing imidazole.[22]

Sterically Hindered Ligands. Although molecular models[43] of Bzim (and 1-CH_3Bzim) indicate a significant steric interaction between the 4-H and the porphyrin skeleton, previous Mössbauer studies[16] had suggested a low-spin state for the adduct with TPPFeCl. Hence our observations of the 2:1 adduct is not unexpected, though it is somewhat surprising that there is no direct evidence for a monoadduct below −25 °C. The solution susceptibility data in Table III confirm the low-spin configuration below −25 °C. Thus the steric effect, although it does lower the enthalpy for reaction 5, is not large enough to destabilize the six-coordinate, low-spin relative to some five- or six-coordinate high-spin form.

In the case of 2-CH_3Im, however, both ESR[14] and Mössbauer[16] investigations in frozen glasses have reported the absence of detectable low-spin species, thereby concluding that a 2-methyl substituted base precludes formation of the bis

adduct due to the steric influence of the methyl group. In fact, these conclusions,[14,16] together with some optical titrations with ferrous porphyrins,[10,11] have led to the generally accepted hypothesis[24] that 2-CH_3Im is incapable of yielding bis complexes of metalloporphyrins, and hence stabilizes the high-spin, five-coordinate state for iron. Although primarily the five-coordinate species have been observed optically for ferrous porphyrins,[10,11,24] it is by no means clear that solely steric forces prevail.

Thus solutions of 2-CH_3Im with TPPFeCl in $CDCl_3$ at low temperatures yield primarily the 2:1 low-spin adduct, as confirmed by the proton trace in Figure 6 and the susceptibility data in Table III. The bis stoichiometry, TPPFe(2-CH_3Im)$_2^+Cl^-$, can be confirmed in Figure 6 on the basis that the unambiguously identified coordinated 1-H peak (it disappears upon addition of methanol-*d* to the solution[25]) has the correct 1:4 peak intensity relative to pyrrole-H. The 5-H peak is assigned[25] on the basis of the same intensity as 1-H and its increased width. The narrower 5-H signal is under the methyl peak and can be partially resolved at higher temperatures. Below −20 °C, the magnetic moment indicates solely low-spin species with a 10:1 excess of 2-CH_3Im for a 20 mM TPPFeCl solution. Optical studies by Walker et al.[20] confirm a sizable β_2 ($\sim 3 \times 10^3$), although the spin state was not determined. The characteristic low-spin pyrrole-H peak was resolvable even at 25 °C, although at considerably reduced intensity. No attempt was made at extracting an equilibrium constant, since the inability to resolve the TPPFeCl peaks under circumstances where not all of the pyrrole-H peak's intensity resided in the low-spin peak indicated the presence of a third species. Either the third species is present in sizable amounts, but does not yield a resolvable NMR trace, or a rapid exchange between TPPFeCl and some monoadduct severely broadens the peaks for the former species.[22] Clear evidence for the third species is obtainable from kinetic analysis of the line widths and will be discussed elsewhere.[22]

In order to further substantiate the inordinate solution stability of the bis low-spin configuration, both the low-temperature proton trace (not shown) and the solution susceptibility (Table III) for the 1,2-$(CH_3)_2$Im adduct of TPPFeCl were obtained. This ligand provides even more steric hindrance to coordination, since bending of the C_2-CH_3 bond away from the porphyrin plane is resisted by the 1-methyl group. Again, the integration of the coordinated 5-H and pyrrole-H peaks[48] verifies the dominant 2:1 stoichiometry and $\mu \sim 1.8\ \mu_B$ (Table III) confirms the low-spin state in solutions.

In order to test the utility of frozen glass ESR and Mössbauer studies as indicators of solution properties of the 2-CH_3Im and 1,2-$(CH_3)_2$Im complexes, we froze the solutions on which our NMR measurements were performed into glasses and confirmed the previous report[14] that at 77 °C, exclusively high-spin ESR spectra were observed. Since the interpretation of the frozen-glass ESR data in terms of spin state is unambiguous for ferric complexes,[49,50] these data indicate that freezing the solutions changes drastically the relative stability of the five- and six-coordinate form of the complexes. We therefore recommend that such physical measurements on frozen glasses[14,16] be interpreted with considerable caution.

Since we have just demonstrated that the low-spin 2:1 adducts of 2-CH_3Im and 1,2-$(CH_3)_2$Im with ferric porphyrins are not only formed but are the dominant species at low temperature, the hypothesis[10,11,14,16,24] that 2-methyl substitution sterically precludes the bis configuration must be reexamined. Although 2-CH_3Im has yielded mainly high-spin monoadducts with ferrous complexes,[10,11,24] the absence of the bis form is unlikely to be due primarily to steric effects, since there is no reason why the bis form should be sterically destabilized significantly more for the ferrous than the ferric complexes.

In fact, the slightly larger radius of LS Fe(II) relative to LS Fe(III) suggests that steric effects should be *less* important in the former oxidation state. We therefore suggest that the exclusive population of the monoadduct for the ferrous complexes arise from electronic stabilization of this geometry. The enhanced stability of the monoadduct of Fe(II) porphyrin could arise either from the greater importance of Fe → Im π back-bonding[51] in the reduced state or from the fact that the HS Fe(II) is displaced[52] from the porphyrin plane more than HS Fe(III), permitting a stronger bond to the crowded nitrogen of the base. It is clear that further work is required before a definite conclusion can be reached on this.

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Reactivity of Coordinated Nucleophiles. A Comparison of Metal Bound Imidazolate and Hydroxide Ions as Models for Carbonic Anhydrase

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Abstract: The cleavage of 4-nitrophenyl acetate by the simple metal complexes $(NH_3)_5CoOH^{2+}$ and $(NH_3)_5CoIm^{2+}$ (Im = N-deprotonated imidazole) has been studied in water and dimethyl sulfoxide (Me_2SO) solvents. In both solvents for both complexes the reactions are exclusively nucleophilic, as demonstrated by the detection of the acetylated reactants, $(NH_3)_5CoO_2CCH_3^{2+}$ and $(NH_3)_5CoImCOCH_3^{3+}$. The pK_a determined titrimetrically for $(NH_3)_5CoImH^{3+}$ in water (25 °C, μ = 1.0, $NaClO_4$) is 10.0 and the large difference in nucleophilic capacity towards 4-nitrophenyl acetate between $(NH_3)_5CoIm^{2+}$ (k_N = 9 M^{-1} s^{-1}, 25 °C, μ = 1.0, $NaClO_4$) and $(NH_3)_5CoCH^{2+}$ ($k_N = 1.5 \times 10^{-3}$ M^{-1} s^{-1}) is closely parallel to the difference in basicity (pK_a $(NH_3)_5CoOH_2^{3+}$ = 6.4, 25 °C, μ = 1.0, $NaClO_4$). In Me_2SO the complexes are of similar activity towards the ester (k_{Im} = 30 M^{-1} s^{-1}, k_{OH} = 0.72 M^{-1} s^{-1}, 25 °C) and it is found that this may be largely attributed to a marked increase in the basicity of $(NH_3)_5CoOH^{2+}$ relative to that of $(NH_3)_5CoIm^{2+}$ in this dipolar, aprotic solvent. Similar trends for dimethylformamide are indicated and mechanistic and kinetic aspects of this study are discussed in relation to the esterase properties of the zinc metalloenzyme, carbonic anhydrase.

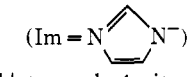
One of the most thoroughly investigated metalloenzymes is carbonic anhydrase,[1] a highly efficient catalyst for the equilibrium

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

and a control for the CO_2, HCO_3^-, and H^+ levels in biological systems. The hydration–dehydration reaction is exceptionally fast ($k_{cat} \sim 10^7$ M^{-1} s^{-1} at 25 °C), but at much slower rates the enzyme has been shown to catalyze several analogous reactions, notably the hydrolysis of esters and sultones and the hydration of carbonyl groups.[2]

X-Ray crystallographic analyses of several enzyme forms[1,3,4] indicate that the Zn^{2+} ion is bound by three histidine imidazole residues. Two bonded through N(3′) and one through N(1′), Figure 1. A water molecule or an hydroxide ion appears to be coordinated as a fourth ligand to give overall a distorted tetrahedral geometry of the bonded atoms about the metal ion. Numerous publications[1,4-12] have described enzyme mechanisms and several elementary models for the active site have been advanced.[13-17] The so-called "zinc-hydroxide" mechanism (Figure 1) (N denotes the N donor atom of a histidine imidazole ring) has usually been the object of assessment in these model studies, and both positive attributes and deficiencies have been noted. Recently, detailed proton magnetic resonance studies of the enzyme[18,19] have provided support for a different mechanism involving deprotonation not of water but of histidine imidazole coordinated to Zn(II), Figure 2.

While the indicated general base function for the metal-coordinated nucleophile has been preferred by Pesando et al.[18a,c] little justification presently exists for exclusion of the direct analogue of the "zinc–hydroxide" mechanism in which the "zinc–imidazolate" nucleophile captures CO_2,[20] Figure 3. As the facile generation of coordinated imidazolate anions has been described several times,[21] a number of systems are available for testing as models for carbonic anhydrase. The present work reports an attempt to establish both the nucleophilic and general base capacities of the simple complexes imidazolatopentaamminecobalt(III) ion, $[(NH_3)_5CoIm]^{2+}$



and $[(NH_3)_5CoOH]^+$ towards 4-nitrophenyl acetate. These ions are kinetically robust and the parent ligands do not leave the metal center in the lifetime of the reactions to be discussed. With such models the kinetic results are not complicated by equilibria involving dissociation of the ligands from the metal center.

Experimental Section

Instrumentation. Spectrophotometric measurements were made on Cary 16K and 118C and Gilford 2100 instruments. Rates up to a half-life of ~1 s were followed by use of a simple rapid mixing device. pH-stat titrations were made under nitrogen by use of a Radiometer Model 26 pH meter and SBR2 automatic burette with a G202B glass electrode and a saturated calomel reference electrode (connected to reaction mixtures via an $NaNO_3$ salt bridge). The glass electrode was standardized with phosphate (pH 6.86, 25 °C) and borate (pH 9.18, 25 °C) buffers and checked in the range 9.5–12.0 pH with various carbonate and phosphate buffers. Final pH readings on all reaction media were also taken under nitrogen on the same equipment. Proton magnetic resonance spectra were recorded at 100 MHz on a JEOL MH-100 "Minimar" spectrometer.