A Proton Nuclear Magnetic Resonance Study of Imidazole–Iron Bonding in Low-Spin Ferric Complexes with Synthetic Porphyrins

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Abstract: The proton NMR resonances for the coordinated axial imidazole ligand in low-spin ferric bisimidazole complexes with synthetic porphyrins have been resolved and assigned. The dominant dipolar shifts for certain porphyrin positions have allowed the quantitative separation of the observed imidazole shifts into their dipolar and contact contributions. The contact shifts are shown to arise from directly delocalized π spin density, which comparison with Hückel MO calculations suggests to arise primarily from iron $\rightarrow$ imidazole π^* charge transfer. The observation of Fe–Im π bonding suggests a possible role for axial histidyl imidazole in electron transfer in cytochromes.

The importance of the imidazole moiety as a ligand in metalloproteins is clearly illustrated by the confirmed or suspected presence of at least one histidyl imidazole as axial ligand in each of the various classes of characterized hemoproteins.[2-6] The ubiquitous nature of the imidazole ligand can be attributed to its characteristic properties[7] as a strong-field ligand (σ-bonding) or as its role as a π donor or acceptor. In the oxygen binding hemoproteins,[2,3] imidazole–iron π bonding has been frequently invoked, while, in the electron transport hemoproteins,[4-6] the imidazole π system has been suggested[8] to provide one of the paths for the electrons in the redox process.

Depending on the metal ion, its oxidation state, and the nature of the other ligands present,[7] imidazole can act solely as a σ base or also as a π base or acid. While iron–imidazole, Fe–Im, π bonding is likely in ferrous porphyrins, there exists little concrete evidence for the importance of π bonding in the ferric state such as that found in low-spin ferricytochromes.[4] Both spectroscopic[9] and proton NMR studies[10-13] of low-spin ferric porphyrin complexes with a variety of axial ligands concur on the dominance of P $\rightarrow$ Fe π change transfer in the porphyrin–iron, P–Fe, bond. The electron transfer for cytochromes has been shown to invoke two different paths for oxidation and reduction.[14] Although one path is consistent with electron transfer at the heme periphery[14] whose mechanism has been interpreted in terms of the delocalized spin distribution as determined by NMR,[15] the other pathway occurs via a channel behind the heme and is thought to involve the axial ligand. Similar dual pathways have been observed[16] in the reduction of cytochrome *c* by inorganic reducing agents. The existence of two coordinated histidyl imidazoles in the b_2- and b_5-type cytochromes[17] suggests that electron transfer through the coordinated imidazole moiety[8] by an outer-sphere mechanism cannot be ruled out at this time. It would be of interest, therefore, to learn whether Fe–Im π bonding occurs in model complexes and, if so, whether the imidazole acts as a π acceptor or π donor.

The success in characterizing the nature of the P–Fe π bonding in low-spin ferric porphyrin complexes by analyzing the porphyrin isotropic shifts[10-13] has prompted us to search for and characterize the resonances of the coordinated axial ligands of bisimidazole complexes of ferric porphyrins. Although several studies have reported proton NMR spectra of bisimidazole porphyrin complexes,[12,18-20] few have provided any information on the imidazole resonances. In one case,[19] the 1-CH_3 peak for coordinated 1-methylimidazole was located, though the peak was severely broadened by chemical exchange with free ligand; no other peaks were reported. The exchange broadening for the 1-CH_3 peak, however, suggests that all imidazole resonances may be located at appropriately lower temperatures where exchange broadening is suppressed.

In addition to providing data on the Fe–Im bond, the assignment of the coordinated imidazole peaks in these complexes may serve as a useful model for identifying the axial histidyl imidazole resonances in low-spin ferric hemoproteins. Such single-proton resonances have been reported in hemoprotein proton NMR spectra, and in some cases suggested to originate from some unspecified imidazole position.[21-23] The wealth of information on the environment near the heme surface obtained[13,21-24] from the identified peripheral heme methyl resonances suggests the utility of extending such studies to resonances arising from the axial ligand.

In order to enhance the chances for locating and identifying all imidazole resonances, synthetic porphyrins, *meso*-tetraphenylporphyrin,[25] TPP, and octaethylporphyrin,[26] OEP, will be used since their high symmetry yields simple spectra.[12] Natural porphyrin complexes yield upward of 14 resonances spread over a wide frequency range.[10,11,13] A more important reason for employing TPP rather than a natural porphyrin is that a detailed analysis of the phenyl shifts of this porphyrin in $TPPFe(Im)_2^+$ has provided[12] a quantitative measure of the magnetic anisotropy of the ferric ion. Since the isotropic shift[27] is the sum of the contact shift, which reflects the spin delocalization, and the dipolar shift, which is due to the magnetic anisotropy, i.e.,

$$\left(\frac{\Delta H}{H}\right)^{iso} = \left(\frac{\Delta H}{H}\right)^{con} + \left(\frac{\Delta H}{H}\right)^{dip} \quad (1)$$

the dipolar shift for each position must first be obtained before the contact shift can be analyzed. However, $(\Delta H/H)^{dip}$ for the phenyl resonances has been quantitatively determined.[12] Since the relative dipolar shifts for two protons in the complex are given by their relative geometric factors,[17] i.e.,

$$\left(\frac{\Delta H}{H}\right)_i^{dip} : \left(\frac{\Delta H}{H}\right)_j^{dip} = \langle(3\cos^2\theta - 1)r^{-3}\rangle_i : \langle(3\cos^2\theta - 1)r^{-3}\rangle_j \quad (2)$$

the dipolar contribution to the isotropic shift for all imidazole positions can be obtained directly from the relative computed geometric factors.

The resultant contact shift contributions for all imidazole positions can be interpreted in terms of the type of MO containing the delocalized spin.[28] Since spin density in σ and π MO's can be differentiated readily by comparing the shift for an aromatic proton and a methyl group at the same position, several methyl-substituted imidazoles will be investigated. In order to maintain consistency in numbering the imidazole positions for the various substituents, we will employ the fol-

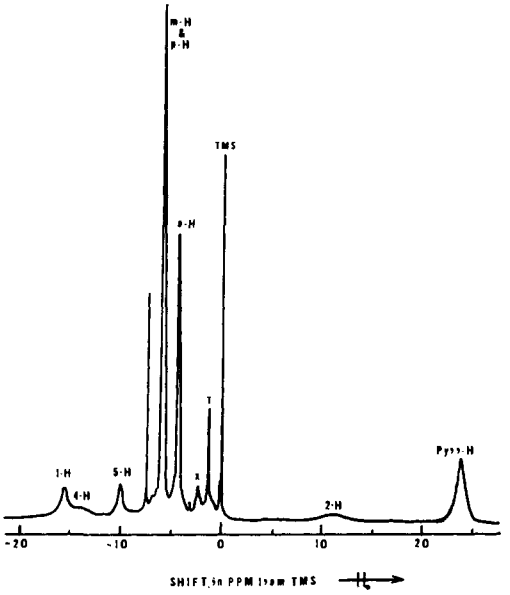
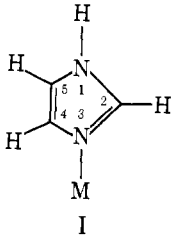


Figure 1. Proton NMR trace at −20 °C, for a $CDCl_3$ solution 0.020 M in TPPFeCl and 0.040 M in imidazole. All peaks present arise from the species $TPPFe(Im)_2^+Cl^-$.

lowing scheme, I, as depicted for the coordinated ligand, where



I

substituents are introduced at the 1-, 2- and 5-positions.

Experimental Section

meso-Tetraphenylporphyrin and its iron complex, TPPFeCl, were prepared by the methods of Adler et al.[25] The complex was chromatographed as a $CHCl_3$ solution on dry alumina (Baker 50–200 mesh) as its oxo-bridged dimer, which was cleaved with dry HCl gas prior to use. The purity of the complex was established by the optical spectrum and its proton NMR spectrum. Octaethylporphyrin was a gift from Professor H. H. Inhoffen. The metal complex was prepared in the same manner as the TPP complex.

Imidazole, Im (Sigma), and 2-methylimidazole, 2-CH_2-Im, were used as obtained commercially. Their NMR spectra reflected a very high (>99%) degree of purity. 2-D-Imidazole (2-D-Im) was prepared by standard methods.[29] 1-Methylimidazole, 1-CH_3-Im (Aldrich), was distilled at atmospheric pressure and the fraction boiling at 196–200 °C was collected; its proton NMR trace showed no detectable extraneous peaks. 1-Benzylimidazole, 1-$PhCH_2$-Im, (Aldrich) was sublimed onto a cold finger (0 °C) under a dynamic vacuum (<10 Torr). 5-Methylimidazole, 5-CH_3-Im, (Roc/Ric) was obtained as an impure, brown liquid, which was distilled in vacuo (<10 Torr) over a fractionating column. The fraction distilling at 100–105 °C was collected, and a proton NMR spectrum showed the compound to be >95% pure. 1-Methylbenzimidazole, 1-CH_3-BzIm, was prepared from benzimidazole (Eastman) by the procedure of Kimbrough.[30] The product was distilled under vacuum (<0.3 Torr) and the fraction at 102–106 °C collected. The cooled solid was recrystallized from pentane: mp ≃61–62 °C; lit. 61 °C.

Chloroform-*d* solutions were prepared ~0.02 M in porphyrin complex, to which were added increasing amounts of the axial base. For most bases, a molar ratio of 2:1 for base:complex yielded[31] exclusively the bisadduct at low temperatures. Higher ratios of base were needed for 2-CH_3-Im due to its much smaller equilibrium constant.[31] Me_4Si was added as internal calibrant.

Proton NMR spectra were run on a JEOL-PS100 FTNMR spectrometer operating at 99.5 MHz in the FT mode. Between 200 and

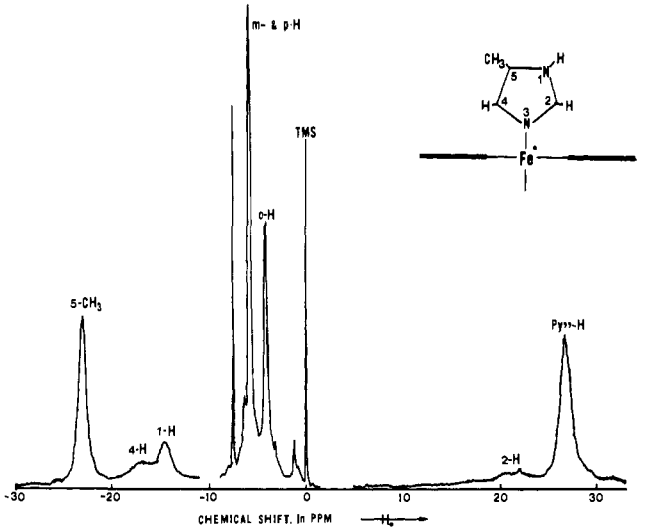


Figure 2. Proton NMR trace at −60° for a $CDCl_3$ solution 0.020 M in TPPFeCl and 0.040 M in 5-methylimidazole. All peaks arise from $TPPFe(5\text{-}CH_3\text{-}Im)_2^+Cl^-$. The low- and high-field portions of the trace have twice the vertical expansion of the central part.

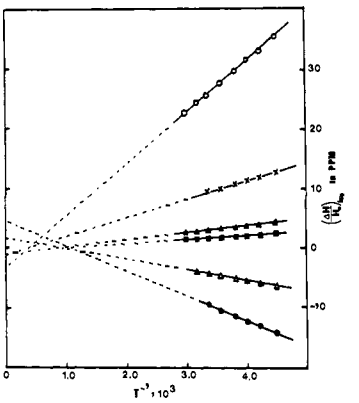


Figure 3. Curie plot for the proton resonances of $TPPFe(Im)_2^+Cl^-$ in $CDCl_3$: ○ = pyrr-H, ▲ = *o*-H, ■ = *m*-H, × = 2-H, ● = 4-H, △ = 5-H.

2000 transients were accumulated using 8K points over a bandwidth of 6250 Hz, with a ~20-μs 90° pulse. Variable temperature spectra were calibrated with a thermocouple both prior and after data accumulation.

Results

Figures 1 and 2 illustrate proton NMR traces where all resonances for both the porphyrin as well as the imidazole ligand are resolved. The chemical shifts relative to Me_4Si at 25° are listed in Table I, where we also include the isotropic shift. The isotropic shift is the difference between the observed shift in the low-spin ferric complex and the shift in a related, diamagnetic imidazole complex of a ruthenium porphyrin.[32] Figure 3 illustrates a Curie plot for the imidazole protons of $TPPFeIm_2^+$. Although straight lines are obtained, their intercept is generally not zero at $T^{-1} = 0$.[12]

Discussion

Assignment of Spectra. Although most of the coordinated imidazole resonances are too broad to detect at ambient temperatures, it was possible to resolve all peaks at low temperatures. Since some of the coordinated imidazole protons resonate in the same region of the spectrum as do some of the free imidazole protons, it was found advantageous to analyze spectra with the stoichiometric ratio for the 2:1 axial-base porphyrin adduct wherever possible. We have shown[31] elsewhere that the dominant species at low temperatures are solely the 2:1 adducts, and except in the case of 2-CH_3-Im and possibly 1-

Table I. Observed Shifts for Coordinated Imidazoles in $PFeL_2^+$ [a]

P	L		Ring position 1	2	4	5
TPP	Im	Me_4Si_{ref} [b]	−14.7	+8.6	−10.2	−8.5
		$(\Delta H/H)_{iso}$ [c]	~−2	+9.5	−9.7	−4.0
OEP	Im	Me_4Si_{ref}	−14.8	+6.5	−11.0	−9.2
		$(\Delta H/H)_{iso}$	~−2	+7.4	−10.5	−4.7
TPP	1-CH_3-Im	Me_4Si_{ref}	(−18.8) [d]	+7.7	~−7.3	~−7.3
		$(\Delta H/H)_{iso}$	(−17.2)	+8.5	~−6.9	~−3.2
TPP	5-CH_3-Im	Me_4Si_{ref}	~−11	+12.3	−11.0	(−15.2)
		$(\Delta H/H)_{iso}$	~0	+9.8	−11.6	(−15.5)
TPP	2-CH_3-Im	Me_4Si_{ref}	~−17	(~−10)	~−22	~−10
		$(\Delta H/H)_{iso}$	~−2	(~−12)	~−21	~−5.5
TPP	1-$PhCH_2$-Im	Me_4Si_{ref}	[−18.1] [e]	—	—	—
		$(\Delta H/H)_{iso}$	[−16]	——		—
TPP	BzIm	Me_4Si_{ref}	(−18.2)			
		$(\Delta H/H)_{iso}$	(~−16.6)			

[a] Shifts in ppm, in $CDCl_3$ at 25 °C; ~ indicates shifts are extrapolated from low temperature. [b] Me_4Si as internal reference. [c] Isotropic shift, with diamagnetic Ru(II) complex as reference from ref 23. [d] Methyl shifts in parentheses. [e] Methylene shifts in square brackets.

CH_3-BzIm, only the 2:1 adduct and the parent high-spin chloride exist in solution up to 25 °C.

At −20°, $TPPFe(Im)_2^+$ yields the expected four resonances which have intensities of 2 protons per porphyrin complex, as determined by the 1:4 ratio to the areas under the upfield pyrrole-H peak[12] (eight protons). In agreement with the relative positions of the four protons relative to the iron,[33] two broad and two narrow lines are observed, as shown in Figure 1. Addition of a small amount of methanol-d_4 to the solution at −60° has the effect of decreasing the intensity of the downfield imidazole resonance, leaving the remaining trace unaltered. This narrow peak can therefore be assigned to the exchangable 1-H peak. Employing imidazole deuterated at the 2-position eliminated the upfield imidazole signal, establishing its assignment as 2-H. The large line width for 2-H (~300–400 Hz) is due to its proximity to the metal.[33] This assignment is confirmed for the 2-CH_3-Im complex, where the upfield signal is missing and an additional downfield signal of intensity 6 is located. Contrary to reports which emphasize the tendency of 2-CH_3-Im to form only high-spin monoadducts,[34] the present complexes have been demonstrated to be the low-spin bis adducts at least in the temperature range −30 to −70 °C.

The 4-H and 5-H peaks in Figure 1 could be assigned on the basis of the expected similarity of line widths for 2-H and 4-H (150–200 Hz) as well as for 1-H (40–50 Hz) and 5-H (30–40 Hz). The relative line widths[33] reflect the approximate values of r^{-6}. The assignment of the broader peak to 4-H is confirmed in the trace for $TPPFe(5\text{-}CH_3\text{-}Im)_2^+$, illustrated in Figure 2, where the narrower of the two unassigned peaks is missing, and thus must arise from 5-H. The assignment of 1-H in Figure 2 was also confirmed by the addition of methanol-d_4 at −60 °C.

The observed shifts relative to Me_4Si for all positions in a number of substituted imidazoles are listed in Table I. Using the proton shifts for imidazoles coordinated to a diamagnetic ruthenium(II) mesoporphyrin dimethyl ester complex, MPDMERu(CO)L, the isotropic shifts were determined and are also found in Table I. It may be noted that, although two coordinated imidazole peaks resonate near their free ligand positions, these proton shifts still reflect substantial isotropic shifts since the ring currents of the diamagnetic porphyrin shift the imidazole peaks considerably upfield. The presence of substantial paramagnetic contributions to the shifts is demonstrated by their expected inverse temperature dependence,[12,27] as illustrated in Figure 3.

The data in Table I indicate that the shift pattern for imidazole protons is generally insensitive to the position of substitution on the imidazole ring. Only in the case of 2-CH_3-Im is there a difference in that the 4-H shift is approximately twice as large as for the other imidazoles. This probably originates from the steric effect produced by substitution at the 2-position. X-Ray studies have shown that the 2-CH_3 group causes[35] a "tilt" of the imidazole bond which places 4-H closer to the metal than in imidazole.[36,37]

In the cases of 1-$PhCH_2$-Im and 1-CH_3-BzIm only the 1-CH_2 and 1-CH_3 peak positions are given, since the larger number of resonances and the unavailability of substituents at all positions make unambiguous assignments impossible at this time.

In a heme protein,[2-6] the histidyl imidazole possesses a 5-CH_2-$CH(NH_2)CO_2H$ substituent, for which the 5-CH_2 shift may be expected to be very similar to the observed 5-CH_3 shift in Table I (i.e., compare 1-CH_3 in 1-CH_3-Im and 1-CH_2 in 1-$PhCH_2$-Im). Hence it may be expected that only the coordinated 2-H and 5-CH_2 protons can be resolved in the proton NMR spectra of low-spin ferric hemoproteins,[13,21-24] because only these two peaks occur outside the normal 0 to −10 ppm range which is obscured by the massive absorption of the protons from the bulk protein. The possible assignments of coordinated histidyl peaks on hemoproteins will be discussed elsewhere.[38]

Analysis of Shifts. Since the pattern of imidazole shifts is essentially unaltered with either substituent on the axial ligand or the porphyrins, an analysis of the shifts[12] for $TPPFe(Im)_2^+$ will provide the estimate of the dipolar shifts which will permit the separation of the dipolar and contact shift contributions for all ligands.

The detailed analysis of the porphyrin shifts in $TPPFe(Im)_2^+$ reported[12] earlier established that the mesophenyl shifts are totally dipolar in origin. This conclusion was based on the observation that the relative phenyl isotropic shifts were correctly predicted by the relative computed geometric factors according to eq 2. This parallel between relative shifts and relative geometric factors is reproduced in the first three lines of Table II. Having firmly established the sole origin of the phenyl shifts as dipolar, the dipolar contribution for any other proton, i, in the complex is given by the relation:

$$\left(\frac{\Delta H}{H}\right)_i^{dip} = \left(\frac{\Delta H}{H}\right)_{o\text{-}H}^{dip} \langle(3\cos^2\theta - 1)r^{-3}\rangle_i / \langle(3\cos^2\theta - 1)r^{-3}\rangle_{o\text{-}H} \quad (3)$$

Table II. Separation of Isotropic Shifts into Contact and Dipolar Contributions for Coordinated Imidazoles in $TPPFe(R\text{-}Im)_2^{+}$[a]

Position		$(\Delta H/H)_{iso}$	$\langle(3\cos^2\theta-1)/r^3\rangle$[c]	Relative $(\Delta H/H)_{dip}$	$(\Delta H/H)_{dip}$	$(\Delta H/H)_{con}$
TPP[b]	*o*-H	+3.09	−0.00363	+10.00	+3.09	0
	m-H	+1.49	−0.00165	+4.63	+1.43	~0
	p-H	+1.37	−0.00148	+4.10	+1.31	~0
R-Im	1-H	~−2	+0.0135	−37.5	−11.6	~+9.6
	1-CH_3	−17.2	+0.0080	−22.2	−6.9	−10.3
	2-H	+9.5	+0.0216	−60.0	−18.5	+28.0
	2-CH_3	~−12	<0.0002	~0	~0	~−12
	4-H	−9.7	+0.0209	−58.0	−17.9	+8.2
	5-H	−4.0	+0.0134	−37.4	−11.6	+7.6
	5-CH_3	−15.5	+0.0075	−21.4	−6.5	−9.0

[a] Shifts in ppm, in $CDCl_3$ at 25 °C. [b] Data on TPP shifts in $TPPFe(Im)_2^{+}$ taken from ref 12. [c] In units of $Å^{-3}$.

Using the structural data[37] from the x-ray study of $TPPFe(Im)_2^{+}$, the geometric factors for the imidazole protons have been computed, with the results listed in the third column of Table II. It may be noted that the geometric factors for the TPP and Im protons are of opposite sign due to the angular part.

If we make the reasonable assumption that the imidazole-iron geometry is essentially unaffected by imidazole substitution at any but the 2- and 4-position, the geometric factors for 1-CH_3 and 5-CH_3 can be calculated. In the case of 2-CH_3-Im, crystal studies of the related 1,2-$(CH_3)_2$-Im-Co(II) complex revealed[35] that the steric effect of the 2-CH_3 group is to "tilt" the imidazole skeleton in the M-N_1-C_2 plane so as to increase the M-N_1-C_2 angle. Using this increased Fe-N_1-C_2 angle, the geometric factor for 2-CH_3 was also computed. Although 2-CH_3 is considerably closer to the iron than all but 2-H and 4-H, its geometric factor is approximately zero since the methyl proton positions are, on the average, at the "magic angle".

The known value[12] for $(\Delta H/H)_{o\text{-}H}^{dip}$ in conjunction with the relative geometric factors given in the third column of Table II permit the determination of the dipolar contribution to all other positions via eq 3, as given in the fourth column of Table II. With the knowledge of both the isotropic and dipolar shifts, the contact shifts are obtained from eq 1, and are listed in the last column of Table II.

The validity of using the *o*-H dipolar shift for $TPPFe(Im)_2^{+}$ for all complexes is based on the fact that the *o*-H shift, as well as the *o*-H:*m*-H:*p*-H shift pattern, is essentially independent of L in $TPPFeL_2^{+}$. This is not surprising since ESR studies of these complexes as a function of L have indicated[34] that the magnetic anisotropy is rather insensitive to the nature of either the imidazole or porphyrin substituent. Although the *g* tensors for most of the complexes of interest are known,[34] these ESR data cannot be used directly to compute the magnitude of the dipolar shift, because the second-order Zeeman,[27] SOZ, effect has been shown to make substantial contributions to the dipolar shift in low-spin ferric hemes.[39] The presence of the SOZ effect is noticeable in the nonzero intercepts[12,27,39] for the Curie plot in Figure 3. Hence the accuracy of the above analysis of isotropic shifts is critically dependent on the availability[12] of the experimentally determined *o*-H dipolar shift in $TPPFe(Im)_2^{+}$.

Imidazole–Iron π Bonding. The contact shifts obtained in Table II are all upfield, with no obvious attenuation of shift magnitude with distance from the metal. Furthermore, for each position probed, (i.e., 1, 2, and 5), the contact shifts for the aromatic proton and methyl group are comparable in magnitude but opposite in direction. This lack of shift alternation and presence of proton and methyl shift sign differences[40] clearly establish[28] the origin of the delocalized spin density as a π-type molecular orbital.[41] Since all proton contact shifts are upfield, the metal spin must be transferred directly[28] into a ligand π MO. The dominance of direct π spin transfer is consistent with the spin configuration of low-spin ferric hemes, i.e., $(d_{xy})^2(d^{\pi}_{xz}, d^{\pi}_{yz})^3$. Hence the imidazole π system makes an important contribution to the total wave function for the impaired electron in the complex.

Table III. π Spin Distribution in Imidazole

Position	$(\Delta H/H)_{con}^{\pi}$[a]	A/h (Hz)	ρ_{HBO}[b]	ρ_{LAO}[c]
1	+12	4.0×10^5	0.01	0.19
2	+28	9.3×10^5	0.29	0.46
3	—	—	0.08	0.12
4	?	?	0.24	0.01
5	+7.6	2.5×10^5	0.38	0.22

[a] Contact shifts, in ppm at 25 °C, for positions for which π spin density can be shown to be dominant. [b] Predicted Hückel spin density for a whole spin in the highest bonding π MO; α(N-1) = $\alpha + 1.5\beta$, α(N-3) = $\alpha + 0.5\beta$, β(CN) = β. [c] Predicted Hückel spin density for one whole spin in the lowest antibonding π MO; parameters as in (*b*) above.

In order to gain some insight into which imidazole π MO is mixed significantly with d_{xz}, d_{yz}, and thereby establish whether it acts as a π donor or acceptor, a Hückel π molecular orbital calculation was performed. The reasonable parameters[42] of $\alpha_N = \alpha + 0.5\beta$, and $\alpha_N = \alpha + 1.5\beta$ for the pyridine-type (N-3) and pyrrole-type (N-1) nitrogens, respectively, and β(CN) = β, afforded the spin density distribution for the highest bonding orbital, HBO, and lowest antibonding orbital, LAO, as listed in Table III. As imidazole is nonalternant, no attempt was made to introduce spin correlation, since negative spin densities are not likely to appear.

First, it should be noted in Table III that the spin distribution for the HBO and LAO do not differ as markedly as in alternant molecules, so that a totally unambiguous differentiation cannot be made. The Hückel spin densities, however, do provide some clues as to the most likely spin-containing π MO. The HBO Hückel spin distribution differs from the contact shift pattern in two important areas: it predicts a negligible spin density at N-1, contrary to a large observed contact shift, and consistently predicts the largest spin density at C-5, while we observe the largest π contact shift at C-2. These conclusions are independent of a range of reasonable Hückel parameters.[42] The observed contact shift pattern, however, is generally in better agreement with the Hückel prediction for the LAO, in that a sizable N-1 spin density is predicted and the largest spin density is correctly predicted at C-2. The small ρ(C-4) cannot be confirmed since the unavailability of the 4-CH_3 contact shift leaves some question as to the dominant π origin of the 4-H shift. The predicted spin distributions for HBO and LAO do not differ sufficiently to rule out simultaneous L → M π and M → L π^* change transfer; if both are operative, then the M → L π^* mechanism appears to dominate.

Although the actual spin densities cannot be computed accurately from the McConnell relation,[40] $Q\rho = A$, due to the large SOZ contribution,[27,39] neglect of the SOZ yields spin densities which, when scaled to those for the LAO in Table II, indicate ~5% spin transfer onto each imidazole. The extent of π spin transfer onto the porphyrin has been estimated[10] at ~20%, or 5% per pyrrole. Hence the probability of finding the unpaired spin in an imidazole and a porphyrin pyrrole group is comparable.

Our analysis therefore suggests that imidazole-iron π bonding is important in low-spin ferric porphyrin complexes, and that the imidazole probably acts primarily as a π acceptor. Hence the imidazole π system[8] could conceivably play an important role in outer-sphere electron transfer in b_2- and b_5-type cytochromes.[17] The role of such π bonding in *c*-type cytochromes is less clear, since only one of the two axial ligands is a histidyl imidazole. In fact, preliminary experiments[38,43] with mixed axial ligand heme complexes have revealed that imidazole (but not porphyrin) isotropic shifts are altered significantly upon replacing one of the imidazoles with a cyanide ion. However, the many overlapping spectra due to the equilibrium mixture of the bisimidazole, biscyanide, and imidazole-cyanide complexes have precluded an assignment of the mixed ligand peaks.[43] Current studies in our laboratories should provide additional information on trans influences on imidazole-iron bonding.

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