Proton NMR Study of the Deprotonation of Axial Imidazole Ligands in Low-Spin Ferric Porphyrin Complexes

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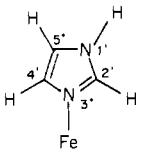
Abstract: The proton NMR spectra of a series of low-spin ferric bis(imidazolate) and mixed-ligand cyanide–imidazolate complexes of natural porphyrin derivatives have been recorded, assigned, and compared to those of the analogous complexes of neutral imidazole. Deprotonation of an axial imidazole leads to hyperfine shift changes for porphyrin substituents that reflect primarily a decrease in ligand → metal π charge transfer. The axial ligand hyperfine shifts are separated into their dipolar and contact contributions by using the magnetic anisotropy data derived from low-temperature ESR spectra of both the imidazole and imidazolate complexes. The imidazole 2′-H peak is shown to shift characteristically upfield upon deprotonating a coordinated imidazole, suggesting that this resonance may serve as a probe of the extent of imidazolate character of the proximal histidine in cytochrome b_5 and heme peroxidases.

The primary control of heme iron reactivity in hemoproteins is thought to involve either steric or electronic influence of the ubiquitous histidyl imidazole trans to the reaction site.[1,2] Considerable recent interest has focused on the role of hydrogen bonding of the axial imidazole N_1H, I, to a protein acceptor

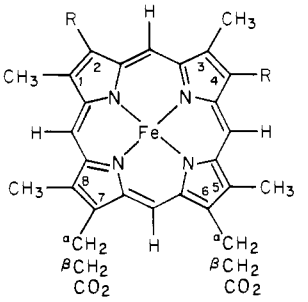


I

residue.[2-7] Such interactions have been proposed to range from only weak proton donation to a backbone carbonyl[3] to complete proton abstraction or imidazolate formation.[2,4-6] The presence of deprotonated imidazoles has been proposed for ferricytochrome b_5 on the basis of ESR data[2] and for horseradish peroxidases on the basis of optical[5] and resonance Raman data.[6] Imidazole N_1H hydrogen bonding has been suggested[7] to act as the allosteric control mechanism in the modulation of the oxygen affinity of hemoglobin. Studies on model systems have demonstrated.[8,9] that the thermodynamics and kinetics of ligand binding in ferrous porphyrins are significantly altered upon trans imidazole deprotonation, consistent with the imidazolate, Im^-, being a stronger σ donor than neutral imidazole, ImH. A variety of both monoimidazolate and bis(imidazolate) complexes have been characterized[2,10] by optical and electron spin resonance spectroscopy.

Proton NMR spectroscopy of paramagnetic hemoproteins has yielded a wealth of information on structural and dynamic properties relevant to their function.[11,12] The basis for interpreting the NMR spectral parameters is a comparison with model compounds.[12,13] The proposal that imidazole proton donation or complete deprotonation occurs in hemoproteins suggests a need for a direct NMR probe for the degree that the imidazole N_1H is associated with the axial ligand. Of interest are both an empirical probe of the state of protonation of the imidazole and, if possible, an understanding of the difference in axial bonding between imidazole and imidazolate.

We are interested here in model compounds for low-spin ferric hemoproteins, which are the most frequently studied form because of the efficient electron spin relaxation and resulting narrow proton NMR line widths.[11-13] It is for these protein forms that signals from the axial imidazole/imidazolate are most likely to be resolved.[14,15] Appropriate models for the prosthetic group of cytochromes b_5[16,17] and c_3[18] are the bis(imidazole) complexes of natural porphyrins, II; the proton NMR spectra for imidazole



II

R = vinyl, protohemin (PP)
R = H, deuterohemin (DP)

complexes have been reported[14,15,19] and analyzed.[14] The monoimidazole, monoimidazolate, and bis(imidazolate) complexes of ferric tetraphenylporphyrin have been characterized by optical and ESR spectroscopy.[10] Of more general interest are the mixed-ligand monocyano, monoimidazole compounds[20] of hemins,

(1) Perutz, M. F.; Ten Eyck, L. F. *Cold Spring Harbor Symp. Quant. Biol.* **1971**, *36*, 295-310.
(2) Peisach, J.; Blumberg, W. E.; Adler, A. *Ann. N.Y. Acad. Sci.* **1973**, *206*, 310-326.
(3) Valentine, J. S.; Sheridan, R. P.; Allen, L. C.; Kahn, P. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1009-1013.
(4) Nickolls, P. *Biochim. Biophys. Acta* **1962**, *60*, 217-228.
(5) Mincey, T.; Traylor, T. G. *J. Am. Chem. Soc.* **1979**, *101*, 765-766.
(6) Teroaka, J.; Kitagawa, T. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 674-700.
(7) Stein, P.; Mitchell, M.; Spiro, T. G. *J. Am. Chem. Soc.* **1980**, *102*, 7795-7797.
(8) Swartz, J. C.; Stanford, M. A.; Noy, J. N.; Hoffman, B. M.; Valentine, J. S. *J. Am. Chem. Soc.* **1979**, *101*, 3396-3398.
(9) Stanford, M. A.; Swartz, J. C.; Phillips, T. E.; Hoffman, B. M. *J. Am. Chem. Soc.* **1980**, *102*, 4492-4499.
(10) Quinn, R.; Nappa, M.; Valentine, J. S. *J. Am. Chem. Soc.* **1982**, *104*, 2588-2595.
(11) Morrow, J. S.; Gurd, F. R. N. *CRC Crit. Rev. Biochem.* **1975**, *3*, 221-287.
(12) La Mar, G. N. In "Biological Applications of Magnetic Resonance"; Shulman, R. G., Ed.; Academic Press: New York, 1979; pp 305-343.
(13) La Mar, G. N.; Walker, F. A. *Porphyrins* **1979**, *4*, 61-157.
(14) Satterlee, J. D.; La Mar, G. N. *J. Am. Chem. Soc.* **1976**, *98*, 2804-2808.
(15) La Mar, G. N.; Frye, J. S.; Satterlee, J. D. *Biochem. Biophys. Acta* **1976**, *428*, 78-90.
(16) Keller, R.; Groudinsky, O.; Wüthrich, K. *Biochim. Biophys. Acta* **1976**, *427*, 497-511.
(17) La Mar, G. N.; Burns, P. D.; Jackson, J. T.; Smith, K. M.; Langry, K. C. *J. Biol. Chem.* **1981**, *256*, 6075-6079.
(18) Dobson, C. M.; Hoyle, N. J.; Geraldes, C. F.; Wright, P. E.; Williams, R. J. P.; Bruschi, M.; Le Gall, J. *Nature (London)* **1974**, *249*, 425-429.
(19) Kurland, J. r.; Little, R. G.; Davis, D. G.; Ho, C. *Biochemistry* **1971**, *10*, 2237-2246.
(20) La Mar, G. N.; Del Gaudio, J.; Frye, J. S. *Biochim. Biophys. Acta* **1979**, *498*, 422-435.

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inasmuch as most histidyl imidazole-ligated hemoproteins can be converted to the low-spin ferric form by adding cyanide to the oxidized protein. We report here the proton NMR spectra of a variety of low-spin ferric porphyrin complexes possessing one or more ligated imidazolates and demonstrate that deprotonation of an axial imidazole yields a characteristic hyperfine shift change for nonexchangeable axial ligand protons which permits the resonances to serve as a probe for the degree of imidazolate character in hemoproteins.

Experimental Section

Materials and Methods. (Protoporphyrin IX)iron(III) chloride, PPFeCl, was obtained commercially (Man-Win Chemicals) and used without further purification. Deuterohemin was prepared by standard techniques.[21] Imidazole, ImH (Sigma), 2′-methylimidazole, 2′-CH_3ImH (Sigma), and 1′-methylimidzole, 1′-CH_3ImH (Aldrich), were used as obtained commercially. Their ^{1}H NMR spectra reflected a very high (>98%) degree of purity. [2′-^{2}H]Imidazole, [2′-^{2}H]ImH, was prepared by standard techniques.[22] [$^2H_4$]Imidazole, [$^2H_4$]ImH, was obtained commercially (Merck, Sharp and Dohme, Canada) and used without further purification. 5′-Methylimidazole, 5′-CH_3ImH (Ric/Roc), was obtained as an impure brown liquid, which was distilled under vacuum (<10 torr) on a fractionating column; the fraction distilling at 100–105 °C was collected; the ^{1}H NMR spectrum showed the compound to be >95% pure. AR grade potassium cyanide, KCN (Mallinkrodt), was dried under vacuum for 24 h. tetra-*n*-butylammonium imidazolate, [Bu_4N^+][Im^-] was prepared according to the procedure described by Landrum et al.[23]

Perdeuterated dimethyl sulfoxide, $(C^2H_3)_2SO$, was obtained from Aldrich and used as such, $(CH_3)_2SO$ was AR grade (Mallinkrodt) and used without further purification or drying. Base solutions used were ~0.2 M NaO^{2}H in ^2H_2O for ^{1}H NMR measurement or ~1 M NaOH in ^{2}H-depleted H_2O for ^{2}H NMR measurements.

NMR spectra were obtained on a Nicolet NT-360 FT NMR spectrometer operating in the quadrature mode (^{1}H frequency, 360 MHz). Between 100 and 1000 transients were accumulated over 8–12 kHz bandwidth with 16K data points and a 10-μs 90° pulse. Signal-to-noise ratio was improved by apodization of the free induction decay which introduced a negligible 1–3 Hz line broadening. Tetramethylsilane, Me_4Si, was used as internal reference. All measurements were done at 25 °C, unless otherwise noted. ESR measurements were done on a Bruker ER-200 X-band ESR spectrometer at 77 K, using 100-kHz field modulation.

Sample Preparation. The bis(imidazole) complex, A, and the dicyano complex, B, of ferric protoporphyrin and deuteroporphyrin were prepared by adding ~4 equiv of imidazole, ImH, and KCN, respectively, to a 0.001–0.010 M solution of the appropriate hemin chloride in $(C^2H_3)_2SO$. Excess ligands were used to ensure that only the bis complexes were present in solution.[19,24] The mixed-ligand complex,[20] monocyano, monoimidazole ferriporphyrin, C, was generated either by combining appropriate amounts of solutions of A and B, or by adding ~1.2 equiv of KCN and ~2 equiv of imidazole to a solution of hemin chloride in $(C^2H_3)_2SO$. Irrespective of the method of preparation, the mixed-ligand complex, C, cannot be prepared without at least small amounts of A and/or B. Attempts to prepare ferric tetraphenylporphyrin samples containing deprotonated imidazole in large enough concentrations to measure NMR spectra were unsuccessful, presumably due to polymerization.[10] However, such problems were not encountered with the present hemin samples up to ~0.010 M. The total amount of base added to a sample was generally kept equal to or less than the total imidazole concentration in order to avoid formation of the dimsyl anion.

Results

Monocyanoferriprotoporphyrin IX Monoimidazole. ^{1}H NMR spectra (360 MHz) of ferriprotoporphyrin IX bis(imidazole), $[PPFe(ImH)_2]^+$, A, and dicyanoferriprotoporphyrin IX, $[PPFe(CN)_2]^-$, B, are compared with that of the mixed-ligand complex, [PPFe(CN)(ImH)], C, in Figure 1. Unmarked low-intensity peaks in Figure 1c are due to a small amount of B coexistent with C in solution; the position of some of these peaks is slightly different from that in Figure 1b due to the effect of the free imidazole, which presumably causes deprotonation of the propionic acid side chains of B. In Figure 1c, peaks arising from the imidazole protons are indicated as such. The coordinated imidazole peaks in the bis(imidazole) complex have already been reported,[15] as have the porphyrin resonances in both complexes.[15,19,24] Comparison of integrated areas of the imidazole resonances with those of the porphyrin resonances indicates that there is only one imidazole molecule per hemin in C, as opposed to two in A, thus confirming the identity of the mixed-ligand complex.

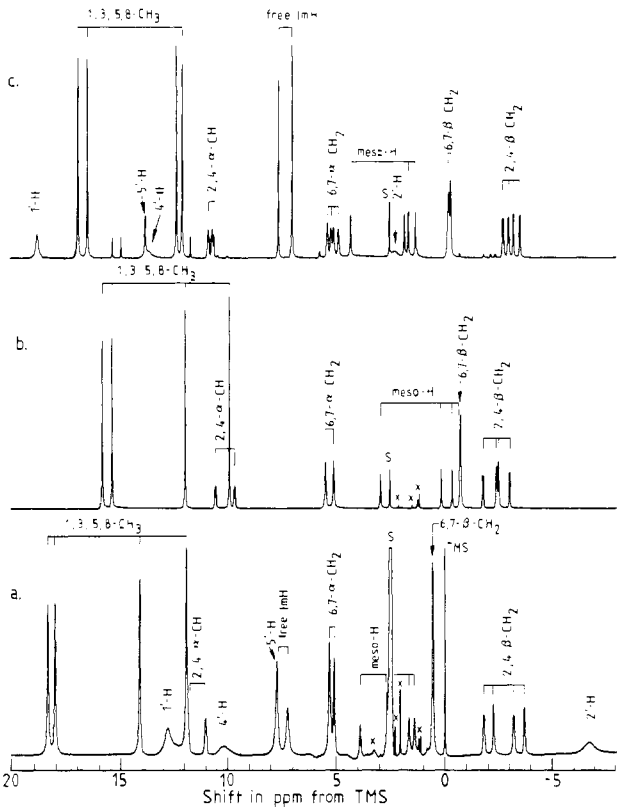

Figure 1. 360-MHz ^{1}H NMR spectra of (a) bis(imidazole)ferric protohemin complex, (b) dicyanoferric protohemin complex, and (c) monocyano, monoimidazoleferric protohemin complex. All spectra were obtained in $(C^2H_3)_2SO$ at 25 °C. Assignment of the various resonances are indicated (see text for details); s marks the residual solvent peak; x indicates impurity peaks. Low-intensity unmarked peaks in c are from a small amount of complex B present in solution.

Assignments of the imidazole resonances in the mixed-ligand complex were achieved the following way: Imidazole resonances 1′-H and 2′-H disappeared when [1′-^{2}H]imidazole and [2′-^{2}H]-imidazole, respectively, were used. The broader of the two imidazole peaks at ~13.7 ppm is assigned to H-4′ on account of the similarity of its line width to that of 2′-H (70 ± 5 Hz for both). The relative line widths[13,25] reflect the approximate values of r^{-6}. By the same reasoning assignment of the sharp peak (line width 16 ± 2 Hz) at ~13.7 ppm to 5′-H is obvious. These assignments were also verified by using 1′-methylimidazole, 2′-methylimidazole, and 5′-methylimidazole and observing the accompanying changes in the spectra.

Deprotonation of $[PPFe(ImH)_2]^+$. ^{1}H NMR spectral changes accompanying the titration of NaO^{2}H/^2H_2O into a solution of $[PPFe(ImH)_2]^+$, A, in $(C^2H_3)_2SO$ are illustrated in Figure 2. Upon addition of NaO^{2}H to A, peaks due to A are seen to broaden considerably; the free imidazole peaks also show noticeable line broadening, indicating chemical exchange. A new set of peaks marked A′ appear. On continuing titration, peaks A and A′ disappear with the appearance of another set of peaks, A″.

(21) Caughey, W. S.; Alben, J. D.; Fujimoto, W. F.; York, J. L. *J. Org. Chem.* **1966**, *31*, 2631–2640.
(22) Vaughan, J. D.; Mughrabi, Z.; Wu, E. C. *J. Org. Chem.* **1970**, *35*, 1141–1145.
(23) Landrum, J. T.; Hatano, K.; Scheidt, W. R.; Reed, C. A. *J. Am. Chem. Soc.* **1980**, *102*, 6729–6735.
(24) Wang, J. T.; Yeh, H. F. C.; Johnson, D. F. *J. Am. Chem. Soc.* **1978**, *100*, 2400–2405.
(25) Swift, T. J. In "NMR of Paramagnetic Molecules"; La Mar, G. N., Horrocks, W. D., Jr., Holm, R. H. Eds.; Academic Press: New York, 1973; pp 32–83.

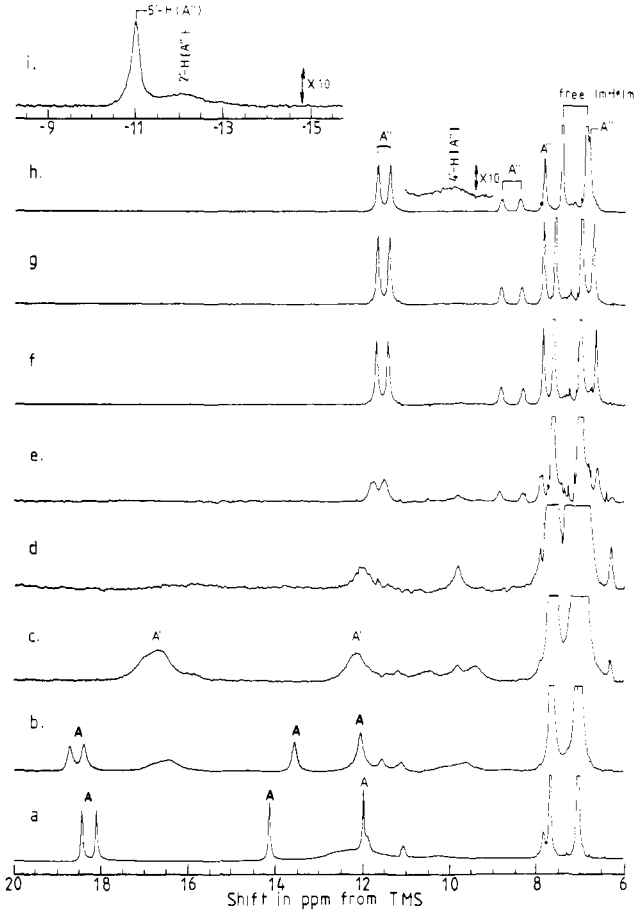

Figure 2. Portions of the 360-MHz ^1H NMR spectra illustrating the formation of the monoimidazole, monoimidazolate complex, A′, and bis(imidazolate) complex, A″, by the addition of $NaO^2H/^2H_2O$ to a ~0.002 M solution of the bis(imidazole) complex, A, in $(C^2H_3)_2SO$ at 25 °C. (a) No base, (b–h) increasing amount of 0.2 M $NaO^2H/^2H_2O$ added at each stage, and (i) upfield portion of the spectrum in h: the total ImH concentration is ~0.006 M.

Concomitant with the spectral changes illustrated for the low-field part in Figure 2a–h, two new peaks emerge at −11 to −13 ppm. These peaks for the spectrum in Figure 2h are illustrated in Figure 2i.

The ^1H NMR spectrum of A″ (Figure 2h,i) is characteristic of a low-spin ferric hemin complex.[13-15,19,24] All the porphyrin resonances can easily be located and identified from their known spectral features. There are three peaks (two of them very broad) which do not belong to the porphyrin. Therefore, they must arise from the axial imidazole ligands. Comparison of integrated areas indicate that each of these three peaks has two-proton intensity, indicating that the species A″ has two coordinated axial ligands per hemin. When [2′-^{2}H]ImH is used instead of ImH, the peak at ~−12 ppm disappears, indicating that 2′-H of imidazole is responsible for this signal. The broad peak at ~10 ppm is assigned to 4′-H on the basis of its comparable line width[14] to that of 2′-H (line width ~500 Hz for both). By elimination and on the basis of its narrow line width (~70 Hz), the imidazole resonance at ~−11 ppm is assigned to 5′-H.

When the base addition was carried out on a solution of A containing 1′-CH_3ImH instead of ImH, no noticeable changes were observed until large excess of base was added, when a set of very broad lines in the region 45–35 ppm appear, presumably due to hydroxide-coordinated ferriprotoporphyrin complex, similar to the observation by La Mar et al.[26] in aqueous solutions of dicyanoferriprotoporphyrin. Thus A″ is not a hydroxide-coordinated hemin complex. Upon addition of increasing amounts of base to the bis(imidazole) complex, A, the final product is exclusively A″, even when excess base is added, A′ being present only when less than 1 equiv of base (with respect to the total imidazole present in solution) has been added. Thus A″ is concluded to be the bis(imidazolate) complex of hemin. This is further supported by the observation that A″ cannot be formed if 2′-CHImH is used instead of ImH, presumably due to steric hindrance from the bulky methyl group at position 2. If, on the other hand, 5′-CH_3ImH is used, A″ is readily formed, indicating also that A″ is a monomeric hemin complex, as also indicated by optical data.[10] If A″ were dimeric or polymeric, then it should not be possible to generate it with 5′-CH_3ImH, for the same reason that A″ does not form with 2′-CH_3ImH.

The intermediate species, A′, observed in the base titration of A, is thought to be the product of deprotonation of only one of the imidazole ligands in the bis(imidazole) complex. This species has been characterized more thoroughly by optical spectroscopy.[10] Chemical exchange between free and coordinated ligand and/or proton exchange between imidazole and imidazolate ligands is the most likely reason for the broadening of the lines when A′ is present; free imidazole peaks also broaden considerably (see Figure 2b–d). Coordinated imidazole peaks due to A′ cannot be recognized at all, probably due to extreme broadening caused by exchange.

Additional proof for the identification of A″ as the bis(imidazolate) complex comes from the following experiment. When a 10-fold excess of tetrabutylammonium imidazolate, $[Bu_4N^+][Im^-]$, in $(C_2H_3)_2SO$ was added to a dilute (~0.001 M) solution of hemin chloride in the same solvent, the ^1H NMR spectrum is essentially identical with that illustrated in Figure 2h,i. When more hemin chloride is added to this solution, a new set of broad peaks similar to A′ in Figure 2b resulted. This is most likely due to partial protonation of the imidazolate ligand in solution, caused by the addition of hemin (a weak acid). This is similar to the formation of A′ from A″ by the addition of ^2HCl in ^2H_2O. Lastly, the ESR spectrum[27] of A″ is essentially the same as that reported[2] previously for a KOH melt of $[PPFe(ImH)_2]^+$, proposed to be $[PPFe(Im)_2]^-$. All these observations confirm the identification of A′ as the monoimidazole, monoimidazolate complex and A″ as the bis(imidazolate) complex of hemin.

Deprotonation of PPFe(CN)(ImH). ^1H NMR spectral changes accompanying base titration of a $(C^2H_3)_2SO$ solution containing complexes B and C and a 2-fold excess free imidazole are illustrated in Figure 3. For small amounts of base, the resonances due to C are seen to broaden, as also those of the free imidazole peaks, indicating moderatley fast exchange, most likely involving deprotonated and neutral imidazole and the corresponding complexes. After approximately a molar equivalent (with respect to the total amount of imidazole present) of base has been added, a new set of peaks, marked C′ in Figure 3, appear and continue to grow with further addition of base. On continuation of the base addition, another set of peaks, similar to A″ obtained in the titration of A (vide supra), also appear. Concomitant with the appearance of C′ and A″, new peaks are seen in the upfield region. The upfield portion of the spectrum in Figure 3f is shown in Figure 3g. All of the resonances due to A″ can be easily identified by comparison with A″ generated from A (see Figure 2h,i).

The only noticeable effect on the dicyano complex, B, peaks upon addition of base are small. These small changes correlate well with the changes in the nature of the solvent[20,28] from pure $(C^2H_3)_2SO$ to a mixture of $(C^2H_3)_2SO$ and water (the latter being added upon base titration).

(26) La Mar, G. N., Minch, M. J.; Frye, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 5383–5388.

(27) ESR measurements at 77 K yielded the following g values for the various complexes: $[PPFe(ImH)_2]^+$ (A) 2.92, 2.25, 1.55; $[PPFe(Im)_2]^-$ (A″) 2.74, 2.27, 1.76; [PPFe(CN)(ImH)] (C) very broad signal with g value extrema of ~4 and ~1.1; $[PPFe(CN)(Im)]^-$ (C′) 3.1, 2.2, 1.4. The g values for A and A″ are in substantial agreement with those reported by Peisach et al.[2] for frozen melts and those reported by Valentine and co-workers[10] for ferric tetraphenylporphyrin complexes. As in the case of the bis(imidazole) complex, deprotonation leads to decreased magnetic anisotropy in the mixed-ligand complex as well, indicating the stronger axial ligand field produced by the imidazolate ligand.

(28) Frye, J. S.; La Mar, G. N. *J. Am. Chem. Soc.* **1975**, *97*, 3561–3562.

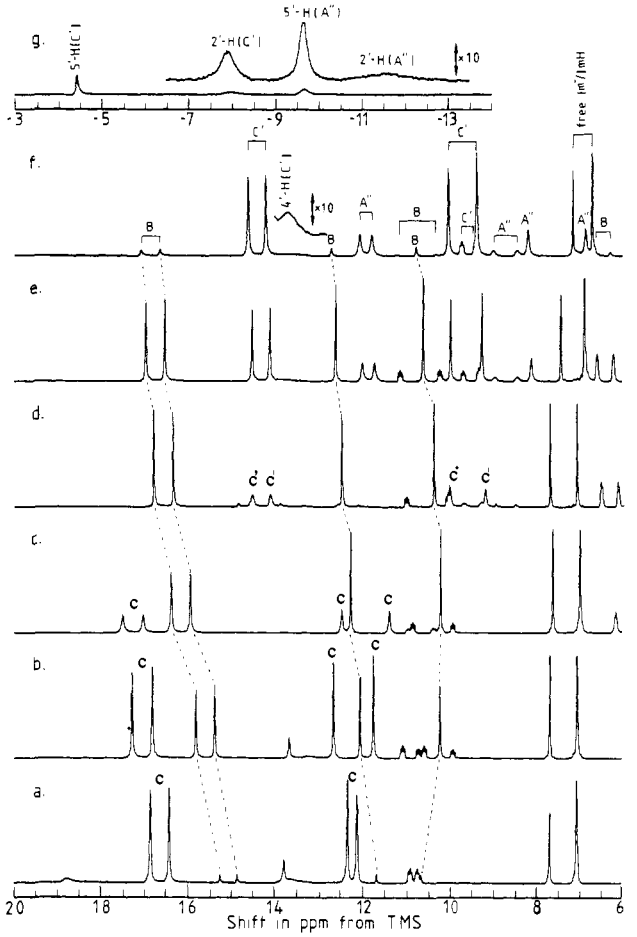

Figure 3. Portions of the 360-MHz ^1H NMR spectra illustrating the formation of the monocyano, monoimidazolate complex, C′, and the bis(imidazolate) complex, A″, by the addition of $NaO^2H/^2H_2O$ to a solution containing 0.010 M PPFeCl, 0.012 M KCN, and 0.020 M imidazole in $(C_2H_3)_2SO$ at 25 °C. (a) No base, (b–f) increasing amounts of $NaO^2H/^2H_2O$ added at each stage, and (g) upfield portion of the spectrum in f. Peaks for the various species are indicated in f and g: methyl peaks for each species are labeled by the letter designation for that species. Dotted lines connect the methyl peaks of the dicyano complex, B.

All of the porphyrin resonances of C′ are readily located and identified by their characteristic spectral features. As in the case of A′, there are three resonances which do not belong to any of the porphyrin protons and must therefore arise from the coordinated axial ligand. Integration indicates that each of these signals has one-proton intensity in C′, dictating that only one heterocyclic ligand per hemin is present. These resonances are assigned to the various imidazole positions with the techniques outlined above the A″ and are indicated in Figure 3f,g. By analogy with the identification of A″ as the bis(imidazolate) hemin complex, C′ can be identified as monocyano,monoimidazolate complex. This is confirmed by the following observations. If the dicyano complex, B, alone is treated with base, the hydroxide-coordinated complex is formed when large excess of base is added[26] and not C′. If 1′-CH_3ImH instead of ImH is used, C′ cannot be formed, indicating the need for an ionizable proton on the imidazole for the formation of C′. Upon addition of a pinch of KCN to a solution of A″, generated from A or from $[Bu_4N^+](Im^-)$, C′ is readily formed. These observations clearly indicate that C′ is monocyano, monoimidazolate complex of hemin.

In an attempt to verify the assignment of the imidazole resonances in C, C′, and A″, ^2H NMR spectra[29] were measured in $(CH_3)_2SO$ using $[^2H_4]$ImH. In C, only one ^2H signal could be clearly resolved at ~14 ppm. This corresponds to the two resolved peaks (4′-H and 5′-H) in the ^1H spectrum. From the observed separation (~40 Hz at 360 MHz) of the two peaks in the ^1H spectrum, a separation of ~6 Hz is expected in the ^2H spectrum (55.3 MHz) and the peaks are not resolved. While electron-nuclear dipolar relaxation dominates the ^1H line widths,[18,25] leading to differential broadening of 5′-H and 4′-H, nuclear quadrupolar relaxation is the dominant line-broadening mechanism[29] in the ^2H spectrum and leads to more similar line widths for these two ^2H nuclei.[30] The 2′-H signal appears at 2.32 ppm in the ^1H spectrum of C. Resolution of this signal from the solvent signal at 2.52 ppm was not possible. 1′-^2H signal of C was not observed in the ^2H spectrum because it was exchanged with protons from the small amount of H_2O present. All of the coordinated imidazole peaks are well resolved in the ^2H spectrum of C′ and A″. Their positions match those observed for the respective protons in the ^1H spectrum. The ^2H spectra are illustrated in Figure 4.

Table I. Chemical Shifts of Some Porphyrin Resonances in Various Complexes[a]

complex	1,3,5,8-methyls	2,4-substituents	
		2,4-α-CH	2,4-β-CH_2
PPFe			
A	18.29, 17.97, 14.06, 11.94 (15.6)[b]	(11.5)[b]	(−2.6)[b]
B	15.69, 15.24, 11.88, 9.88 (13.2)	(10.1)	(−2.5)
C	16.89, 16.46, 12.34, 12.08 (14.4)	(10.8)	(−3.1)
C′	14.61, 14.21, 10.02, 9.36 (12.1)	(9.6)	(−1.6)
A″	12.05, 11.78, 8.18, 6.84 (9.7)	(8.7)	(−0.9)
		2,4-H	
DPFe			
A	18.89, 16.79, 14.46, 12.58 (15.7)[b]	−15.80, −19.50 (−17.7)[b]	
B	16.10, 13.84, 12.93, 10.95 (13.5)	−17.84, −18.24 (−18.0)	
C	17.69, 15.35, 13.52, 12.35 (14.7)	−18.79, −19.56 (−19.2)	
C′	14.71, 12.98, 10.76, 10.02 (12.1)	−15.77, −16.54 (−16.2)	
A″	12.01, 11.00, 8.65, 7.31 (9.7)	−10.66, −12.91 (−11.8)	

[a] Shifts in ppm in $(C^2H_3)_2SO$ at 25 °C with Me_4Si as internal reference. [b] Average shifts are given in parentheses.

The chemical shifts of selected hemin resonances for the various complexes formed from ferriprotoporphyrin, PP, and ferrideuteroporphyrin, DP, are presented in Table I. Spectral data obtained for ferriprotoporphyrin with various substituted imidazoles are listed in Table II.

Discussion

The influence of deprotonation of axial imidazole is clearly observed by the resolution of a set of distinct heme signals for each species, $[PPFe(ImH)_2]^+$, A, PPFe(ImH)(Im), A′, and $[PPFe(Im)_2]^-$, A″. While exchange involving ligands and/or protons contributes to the line widths for A′, obscuring the signals for the axial ligands, resolved methyl peaks dictate lifetimes ≥ 1 ms. Similarly, well-resolved spectra for both PPFe(ImH)(CN) and $[PPFe(Im)(CN)]^-$ are observed. While it is known that the magnetic anisotropy decreases[2,27] on going from $[PPFe(ImH)_2]^+$ to $[PPFe(Im)_2]^-$, the dominant hyperfine shift changes observed for the porphyrin resonances must involve the contact interaction.[13,14] It is seen from Figure 2 as well as Table I that conversion from $[PPFe(ImH)_2]^+$ to $[PPFe(Im)_2]^-$ or from PPFe(ImH)(CN)

(29) Mantsch, H. H.; Saito, H.; Smith, C. *Nucl. Magn. Reson. Prog. Spectrosc.* **1977**, *11*, 211–272.

(30) In such large molecules, internal rotation of the imidazole about the Fe–N bond is likely to control the relaxation rate, which is dependent on the relationship between the ^2H quadrupole axis and the axis of rotation.[29] Qualitative considerations dictate that such internal motions yield broader 5′-^2H than 2′-^2H or 4′-^2H lines.

(31) Faller, J. W.; Chen, C. C.; Malerich, J. C. *J. Inorg. Biochem.* **1979**, *11*, 151–170. The shifts, in ppm, for the diamagnetic ruthenium complex are as follows: 0.95 (2′-H), 0.48 (4′-H), 4.43 (5′-H), −2.56 (2′-CH_3), 0.41 (5′-CH_3). The exchangeable 1′-H peak was not reported; however, its diamagnetic coordinated shift can be estimated by adding a ring current contribution to the free imidazole 1′-H shift of the same magnitude as observed for 5′-H.

Table II. Observed Shifts of Axial Ligand Resonances in Ferriprotoporphyrin Complexes

		ring position							
		1'		2'		4'		5'	
ligand	complex	$(\Delta H/H)^a$	$(\Delta H/H)_{hf}{}^b$	$(\Delta H/H)^a$	$(\Delta H/H)_{hf}{}^b$	$(\Delta H/H)^a$	$(\Delta H/H)_{hf}{}^b$	$(\Delta H/H)^a$	$(\Delta H/H)_{hf}{}^b$
ImH/Im$^-$	A	12.76	~0	−6.64	−7.59	10.21	9.73	7.78	3.35
	A''			−11.55	−12.50	10.10	9.62	−9.69	−14.12
	C	18.88	6.18	2.32	1.37	13.67	13.19	13.78	9.35
	C'			−7.92	−8.87	13.74	13.26	−4.46	−8.89
5'-CH_3ImH/5'-CH_3Im$^-$	A	*c*		−10.44	−11.39	11.80	11.30	13.84	13.43
	A''			−18.20	−19.15	*c*		29.67	29.26
	C	17.90	5.20	*c*		14.67	14.19	12.28	11.87
	C'			−15.80	−16.75	16.54	16.06	31.85	31.46
2'-CH_3ImH[d]/2'-CH_3Im$^-$	C	18.40	5.70	15.73	18.29	18.16	17.68	10.64	6.21
	C'			30.21	32.77	22.51	22.03	−8.38	−12.81

[a] Chemical shift in ppm at 25 °C with Me_4Si as internal reference. [b] Hyperfine shift with diamagnetic Ru(II) complex as reference.[31] [c] Not resolved. [d] A'' not formed with 2'-CH_3ImH and A formed only at very low temperatures.[14]

Table III. Separation of the Hyperfine Shift into Contact and Dipolar Contributions for Axial Ligands in $[PPFe(ImH)_2]^+$ and $[PPFe(Im)_2]^-$

	$[PPFe(ImH)_2]^+$			$[PPFe(Im)_2]^-$		
position	$(\Delta H/H)_{hf}$	$(\Delta H/H)_{dip}{}^a$	$(\Delta H/H)_{con}$	$(\Delta H/H)_{hf}$	$(\Delta H/H)_{dip}{}^b$	$(\Delta H/H)_{con}$
H-1'	~0	11.0	−11.0			
H-2'	−7.6	18.5	−26.1	−12.5	13.1	−25.6
H-4'	9.7	17.9	−8.2	9.6	12.7	−3.1
H-5'	3.4	11.6	−8.2	−9.7	8.2	−17.9
5'-CH_3	13.8	6.5	7.3	29.7	4.6	25.1

[a] Taken from ref 14. [b] Calculated by adjusting the geometric factor[13,14,27] for A by the term $(g_\parallel^2 - g_\perp^2)_{A''}/(g_\parallel^2 - g_\perp^2)_A$.

to $[PPFe(Im)(CN)]^-$ yields an upfield bias for the 1,3,5,8-CH_3 shifts and a downfield bias for the 2,4-H porphyrin hyperfine shifts of the analogous deuterohemin complex, II. Changes primarily in dipolar shifts require that both methyl and 2,4-H shifts vary in the same direction inasmuch as they possess essentially the same geometric factor. Thus, the decrease in porphyrin substituent hyperfine shifts upon deprotonating an axial imidazole are indicative of a decrease in the porphyrin → iron π spin delocalization,[13-15,19] as would be expected if Im$^-$ acted as a stronger ligand than ImH. The intermediate positions of the methyl shifts for PPFe(ImH)(Im) between those of $[PPFe(ImH)_2]^+$ and $[PPFe(Im)_2]^-$ indicate that the effect of multiple deprotonation is approximately additive.

The influence of deprotonation of the axial ligand on the axial bonding is most readily analyzed for the bis(imidazole)/imidazolate complexes because the hyperfine shifts for the former species have been quantitatively separated.[13,14] The known geometric factors, which can be assumed to be very similar for the Im$^-$ complexes, and the availability of the ESR data on both complexes[2,27] permit an estimation of the dipolar shifts for all positions in $[PPFe(Im)_2]^-$ by scaling the previously derived[14] $[PPFe(ImH)_2]^+$ dipolar shift by $(g_\parallel^2 - g_\perp^2)_{A''}/(g_\parallel^2 - g_\perp^2)_A$. This factor of ~0.7 yields the Im$^-$ dipolar shifts listed in Table III. The contact shift is determined from the relation $(\Delta H/H)_{hf} = (\Delta H/H)_{con} + (\Delta H/H)_{dip}$, as also listed in Table III. Comparison of the data for coordinated ImH and Im$^-$ in complexes A and A'' reveals very similar contact shift patterns with the sign differences for proton and methyl group at position 5' clearly establishing the dominance of π delocalization.[13,14] The larger contact shifts of position 5' for Im$^-$ than ImH favors the interpretation that spin transfer in both species involves ligand → iron π charge transfer, inasmuch as this mechanism would be enhanced by deprotonation. This reverses our earlier suggestion[14] of iron → ligand π charge transfer based on approximate Hückel spin densities. More recent INDO calculations also indicate[32] that the highest bonding orbital of an axial imidazole is involved in the spin delocalization in ferric porphyrins. Similar conclusions have been reached on the basis of the effect of an axial imidazole on the in-plane asymmetry in natural porphyrin complexes.[33]

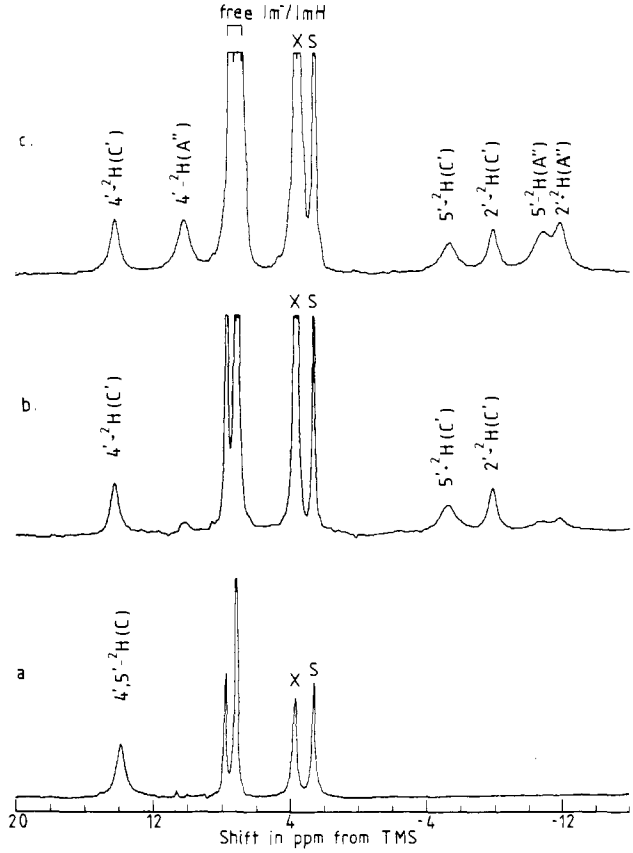

Figure 4. 55.27-MHz ^2H NMR spectra of (a) $(CH_3)_2SO$ solution containing 0.005 M PPFeCl, 0.006 M KCN, and 0.010 M[$^2H_4$]imidazole, (b) after adding $NaOH/H_2O$, and (c) after adding excess [$^2H_4$]imidazole and more $NaOH/H_2O$; s marks the natural abundance ^2H peak of $(CH_3)_2SO$; x is due to water. (For an explanation of ^2H linewidths in these spectra, see footnote 30.)

While separation of dipolar and contact shifts for the mixed-ligand complexes is not possible on the same quantitative basis, the similarity in the pattern of shifts in mixed-ligand complexes and the bis(imidazole) complexes and the similar shift changes

(32) Migita, C., private communication.
(33) Traylor, R. G.; Berzinis, A. P. *J. Am. Chem. Soc.* **1980**, *102*, 2844-2846.

induced upon deprotonation suggest that the same spin transfer mechanisms apply to the former complexes. If we assume a similar 30% decrease in dipolar shifts upon deprotonating PPFe(ImH)(CN), the Im^- contact shifts are also determined to be larger than the ImH contact shifts, with both the 2′- and 5′-positions exhibiting the opposite sign for proton and methyl group contact shifts.

Potential Protein Probes for the State of the Imidazole N_1H. The average heme methyl and 2,4-H shifts could, in principle, be used as a probe for determining the state of protonation of the axial histidyl imidazole(s) in low-spin ferric forms of hemoproteins. For most ferric cyano hemoproteins, only two or three of the needed resonances are resolved, making such analysis difficult. For ferricytochrome b_5, all four heme methyls are observed for the major component,[16,17] with the hyperfine shifts: 5-CH_3,21.8; 3-CH_3,14.4; 1-$CH_3$11.7; 8-CH_3,3.8 ppm; and 2-H, −28.2 and 4-H, −4.8 ppm for the deuterohemin reconstituted protein. The average shifts are as follows: CH_3, 13.2 ppm; 2,4-H, −16.5 ppm. These values compare better with 15.6 and −17.7 ppm for $[PPFe(ImH)_2]^+$, A, than with 9.7 and −11.8 ppm for $[PPFe(Im)_2]^-$, A″, indicating that in the major component of ferricytochrome b_5, the axial ligands most likely are neutral histidyl imidazoles, contrary to an earlier suggestion based on ESR data.[2]

From consideration of the chemical shifts of the axial imidazole protons in the present complexes, it seems reasonable to expect that the 2′-H of the axial histidyl imidazole ligand(s) in low-spin ferric hemoproteins should resonate upfield of the diamagnetic region irrespective of the state of protonation. In [PPFe(ImH)(CN)], C, 2′-H is observed at ~2 ppm, whereas in the deprotonated form, $[PPFe(Im)(CN)]^-$, C′, it appears considerably upfield, particularly with 5′-CH_3Im^-. Therefore, the large shift change of 2′-H can be used as a probe of the state of protonation of the histidyl imidazole ligand.

For met-cyanomyoglobin, broad single-proton resonances have been detected[34] at ~19 and ~4 ppm which can be safely attributed to 4′-H and 2′-H of a neutral histidyl imidazole. In the cyanide complexes of horseradish peroxidase, HRP–CN, and cytochrome *c* peroxidase, CcP–CN, however, similar single-proton resonances are found[34] at approximately 16 ppm and −16 to −31 ppm. While proton NMR spectroscopy in H_2O solution has demonstrated[35] that there is an exchangeable proton associated in some manner with the axial imidazole, the large upfield bias for 2′-H has been interpreted[34] in terms of appreciable imidazolate character for the axial ligand, probably the result of strong hydrogen bonding of the N_1H to a glutamine side chain.[36] In contrast, the appearance[17] of 2′-H and 4′-H axial imidazole peaks in cytochrome b_5 at the positions expected for neutral ImH rather than Im^- argues against deprotonation of the axial ligand(s) in that protein. Although the magnitude of the 2′-H shift is not yet quantitatively interpretable in terms of the exact extent of hydrogen bonding, it appears that location and assignment of the 2′-H and 4′-H histidyl imidazole resonances in a variety of low-spin ferric cyano proteins by methods outlined elsewhere,[34] together with resonance Raman data on the iron–imidazole stretching frequency,[6,37,38] should lead to a more accurate description of the degree of hydrogen bonding in hemoproteins.

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Registry No. DPFeCl, 21007-21-6; PPFeCl, 16009-13-5; deuterium, 7782-39-0; $[PPFe(ImH)_2]^+$, 25875-11-0; PPFe(Im)(ImH), 83603-96-7; $[PPFe(Im)_2]^-$, 83603-98-9; $[PPFe(CN)_2]^-$, 41127-52-0; PPFe(CN)(ImH), 33773-02-3; $[PPFe(CN)(Im)]^-$, 83603-97-8; $[DPFe(ImH)_2]^+$, 83603-99-0; $[DPFe(CN)_2]^-$, 52674-62-1; DPFe(CN)(ImH), 83604-00-6; $[DPFe(CN)(Im)]^-$, 83604-01-7; $[DPFe(Im)_2]^-$, 83604-02-8; $[PPFe(5'\text{-}CH_3ImH)_2]^+$, 83604-03-9; $[PPFe(5'\text{-}CH_3Im)_2]^-$, 83604-04-0; PPFe(CN)(5′-CH_3ImH), 83604-05-1; $[PPFe(CN)(5'\text{-}CH_3Im)]^-$, 83604-06-2; PPFe(CN)(2′-CH_3ImH), 83604-07-3; $[PPFe(CN)(2'\text{-}CH_3Im)^-$, 83604-08-4; 2′-$CH_3$ImH, 693-98-1; ImH, 288-32-4; KCN, 151-50-8.

(34) La Mar, G. N.; de Ropp, J. S.; Chacko, V. P.; Satterlee, J. D.; Erman, J. E. *Biochim. Biophys. Acta*, in press.
(35) de Ropp, J. S. Ph.D. Thesis, University of California, Davis, 1981.
(36) Poulos, T. L.; Kraut, J. *J. Biol. Chem.* **1980**, *255*, 8199–8205.
(37) La Mar, G. N.; de Ropp, J. S. *J. Am. Chem. Soc.* **1982**, *104*, 5203–5206.
(38) Teroaka, J.; Kitagawa, T. *J. Biol. Chem.* **1981**, *256*, 3969–3977.

Electronic States of the Quadruply Bonded $Re_2Cl_8^{2-}$ Species: An ab Initio Theoretical Study

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Abstract: The electronic structure of the "quadruply bonded" $Re_2Cl_8^{2-}$ species has been studied by using ab initio wave functions and relativistic effective core potentials. The metal–metal bonding in the ground state is discussed, and the rich spectrum of electronic states below 6 eV (50 000 cm^{-1}) is treated in detail, including correlation effects and spin–orbit coupling. Highly correlated multiconfiguration wave functions are needed to describe the electrons in the weak δ bond, particularly in the ground $^1A_{1g}$ state and the excited $^1A_{2u}$ (calculated 2.8 eV, experimental 1.8 eV), $^3A_{2u}$ (calculated 0.4 eV), and $2\,^1A_{1g}$ (calculated 3.2 eV) states. These excited states involving δ–δ* excitations all undergo torsional distortions to D_{4d} geometries. The nature and relative intensities of weaker transitions involving excitations among the ten 5d orbitals as well as the strong transitions involving the Cl 3p–Re 5d charge-transfer excitations are also discussed.

The discovery of molecules with multiple metal–metal[1,2] bonds has stimulated numerous experimental and theoretical studies aimed toward understanding their electronic structure and spectral properties.[3–5] Calculations on such species have been carried out at the extended Huckel,[6,7] Xα scattered wave,[8,9] and ab initio levels.[10–14] The calculations to date have focused primarily on

(1) F. A. Cotton et al., *Science (Washington, D.C.)*, **145**, 1305 (1964).
(2) F. A. Cotton, *Inorg. Chem.*, **4**, 334 (1965).
(3) F. A. Cotton, *Acc. Chem. Res.*, **11**, 225 (1978).
(4) W. C. Trogler and H. B. Gray, *Acc. Chem. Res.*, **11**, 232 (1978).
(5) J. L. Templeton, *Prog. Inorg. Chem.*, **26**, 211 (1979).
(6) F. A. Cotton and C. B. Harris, *Inorg. Chem.*, **6**, 924 (1967).
(7) S. Shaik and R. Hoffmann, *J. Am. Chem. Soc.*, **102**, 1194 (1980).
(8) A. P. Mortola, J. W. Moskowitz, N. Rosch, C. D. Cowman, and H. B. Gray, *Chem. Phys. Lett.* **32**, 283 (1975).
(9) J. G. Norman and H. J. Kolari, *J. Am. Chem. Soc.*, **97**, 33 (1975).

0002-7863/82/1504-7007$01.25/0