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^1H NMR Study of High-Spin Ferric Natural Porphyrin Derivatives as Models of Methemoproteins

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Abstract: High-field ^1H NMR spectra have been recorded and analyzed for a series of five- and six-coordinate, high-spin ferric complexes of natural porphyrin derivatives. Protoporphyrins with α- and β-deuterated vinyl groups have been synthesized that reveal the location of the elusive vinyl H_α resonances in the models. Deuterium labeling of individual methyl groups reveals that the increasing spread of the methyl isotropic shifts as the porphyrin 2,4 substituents are made more electron withdrawing is similar to that observed in low-spin ferric complexes. This supports a direct influence of the substituents on the asymmetry of the ligand molecular orbital and argues against a role of the raising of the orbital degeneracy in the low-spin species. Strongly electron-withdrawing 2,4 substituents induce a methyl spread similar to that observed in metaquomyoglobins, suggesting that the in-plane asymmetry in proteins arises primarily from peripheral heme–apoprotein interactions. Comparison of pyrrole substituent shift patterns in the five- and six-coordinate models suggests that the pyrrole proton/methyl shift ratio may serve as a useful indicator of the state of occupation of the sixth site in high-spin hemoproteins.

^1H NMR studies over the past decade have established the value of the heme hyperfine or isotropic shifts as sensitive structural probes in paramagnetic hemoproteins.[2] The interpretation of the protein shifts has been facilitated by the analysis of the influence of controlled perturbations in selected model compounds.[3,4] Of particular utility are the four heme methyl groups, one on each pyrrole, whose peaks are prominent in the NMR spectra and which provide a direct index of the degree of similarity of the environments of the individual pyrroles. The ubiquitous vinyl groups have also been shown to serve as sensitive probes of protein–heme interactions.[4]

A qualitative, though highly characteristic difference between the NMR spectra of hemes in models and proteins is that the rhombic or in-plane asymmetry is always much larger in the protein environment[4] and appears to be highly characteristic of the type of protein.[5] Studies[6-8] on the more frequently studied low-spin (LS) ferric models and proteins using isotope labeling have indicated[6] that peripheral perturbations are the source of the protein-induced in-plane asymmetry, and that the asymmetry is sufficiently characteristic of a protein conformation so as to permit determination[9] of the heme orientation in a protein by NMR.

Although several high-spin (HS) ferric models and proteins have been investigated[2-4,10-13] by NMR, unambiguous assignments of all resonances have not been made, and the origin of the shifts in various models and their sensitivity to axial and peripheral perturbations remain unexplored. In order to interpret the protein spectra, it is necessary to know the physical/electronic properties reflected in the shift patterns[14] in models. In some preliminary work[15] on deuterium labeling of

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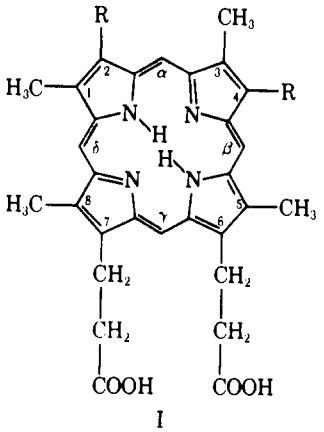
vinyl groups in myoglobins, the H_α peaks were located considerably downfield from where they have been proposed[11] to resonate in models. Hence, the vinyl assignments must be established unambiguously. A synthetic route following standard porphyrin chemistry to specifically deuterium-labeled vinyls has been proposed,[16] but such compounds have not yet been reported.

Although most HS ferric myoglobins and hemoglobins are six coordinate (axial water[17]), five-coordinate species have been found for insect hemoglobin[18] and proposed for horseradish peroxidase.[19] We wish to establish to what extent the heme isotropic shift pattern differences in five- and six-coordinate models can be used to determine if the sixth site is vacant in hemoproteins. Moreover, we wish to explore the influence of controlled rhombic perturbations on the heme methyl shift asymmetry and compare it with that observed in proteins and the analogous LS ferric models.

The previous ^1H NMR studies[10,11] of a few natural porphyrin complexes were carried out at 100 MHz, which prevented resolution of several critical resonances, and neither included a wide range of substituents nor benefitted from the availability of isotope-labeled hemes for specific peak assignments. The interpretation of the shifts and the quantitative characterization of the in-plane asymmetry require assignments of all functional groups, including the individual methyls.

The complexes of interest[6] can be considered as 2,4-disubstituted deuteroporphyrins, i.e., 2,4-R_2DP, depicted in I, where



I

R ranges from electron-donating (ethyl) to strongly electron-withdrawing (acetyl) groups. The halide complexes, as dimethyl esters, DME, 2,4-R_2DDMEFeCl,[10] and the recently characterized HS bis(dimethyl sulfoxide), Me_2SO complexes,[20,21] 2,4-R_2DPFe$(Me_2SO)_2^+$, serve as the five- and six-coordinate high-spin models, respectively.

Experimental Section

All porphyrin complexes and their methyl-deuterated analogues, in the form of the porphyrin iron(III) chloride or porphyrin dimethyl ester iron(III) chloride, are identical with those described[6] in detail previously, except protoporphyrin with deuterated vinyl groups, whose synthesis is described below.

2,4-Bis(2,2-dideuterioethenyl)deuteroporphyrin IX Dimethyl Ester, [2,4-(β-$^2H_2$)$_2$]-2,4-V_2DPDMEH$_2$. Method A. 2,4-A_2DPDME (200 mg) was stirred in the dark at room temperature with [^{1}H]methanol (7 mL) and [^{1}H]sulfuric acid (0.7 mL) for 24 h. The mixture was then diluted with dichloromethane (50 mL) and extracted from an aqueous sodium acetate solution (50 mL). The organic fraction was collected and washed once with water (50 mL), dried over sodium sulfate, evaporated to a solid residue, and crystallized from chloroform–methanol to give the deuterated 2,4-diacetyldeuteroporphyrin IX DME (A) in nearly quantitative yield. The absence of the acetyl-methyl resonances in the ^1H NMR spectrum indicated total deuteration of the acetyl residues.

A (200 mg) was dissolved in tetrahydrofuran (50 mL) and diluted with methanol (50 mL) containing sodium borohydride (0.5 g). This mixture was stirred at room temperature for 2 h before being diluted with dichloromethane (100 mL) and extracted from water (100 mL). The organic layer was washed once with water (100 mL) and dried over sodium sulfate, and the solvent was removed in vacuo. The brown residue was dissolved in dry benzene (200 mL) containing *p*-toluenesulfonic acid (1.5 g) and heated under reflux for 5 h. After cooling to room temperature, the solvent was removed under vacuum to give a black solid that was dissolved in dichloromethane (100 mL) and washed with water (3 × 100 mL), and the organic layer was dried over sodium sulfate before evaporation of the solvent. This gave a residue that was chromatographed on activity III neutral alumina eluting with chloroform. Collection of the first red band, removal of the solvent, and crystallization (chloroform/hexane) gave 40 mg of the title compound: mp 218–220 °C, lit.[22] 225 °C.; *m/e* 594 (M^+, 100%).

Method B. 2,4-V_2DPDMEH$_2$ (130 mg) was dissolved in a solution of *o*-dichlorobenzene (20 mL) containing *p*-[$^2H_3$]toluenesulfonic acid monohydrate (1.0 g) and deuterium oxide (0.5 g). The reaction mixture was stirred under N_2 at 95 °C for 44 h and then, after cooling to room temperature, was diluted with dichloromethane (75 mL) and extracted with water (3 × 50 mL). The organic layer was collected, the solvent removed, and the residue, dissolved in tetrahydrofuran, was treated with excess ethereal diazomethane. The solvent was removed and the purple residue was chromatographed on activity III neutral alumina eluting with dichloromethane. The major band was collected, the solvent evaporated, and the residue was crystallized (*n*-hexane/dichoromethane) to give 88 mg (68%) of the title compound. NMR spectroscopy showed ~90% deuteration of the vinyl CH_2 groups, as well as partial deuteration at the meso positions.

2,4-Bis(1-deuterioethenyl)deuteroporphyrin IX Dimethyl Ester, [2,4-(α-^2H)$_2$]-2,4-V_2DPDMEH$_2$. 2,4-A_2DPDME (200 mg), dissolved in tetrahydrofuran (15 mL), was treated with [$^2H_1$]methanol (1 mL) containing sodium borodeuteride (150 mg), and the solution was stirred at room temperature for 2 h. The reaction mixture was then diluted with dichloromethane (50 mL) and washed once with water (50 mL). The organic layer was collected and dried, and the solvent was evaporated to give a red residue that was dissolved in *o*-dichlorobenzene (30 mL) containing *p*-toluenesulfonic acid (0.75 g) and heated at 145 °C for 45 min with continuous nitrogen flow through the solution. The reaction mixture was cooled to room temperature, diluted with dichloromethane (50 mL), washed with water (3 × 50 mg), and dried over sodium sulfate, the solvent evaporated, and the residue was then dissolved in tetrahydrofuran and treated with excess ethereal diazomethane. After evaporation of the solvent, the purple residue was chromatographed on activity III neutral alumina, eluting with dichloromethane. The first major band was collected, the solvent removed, and the residue was crystallized (chloroform/methanol) to give 100 mg of the title compound.

The iron was inserted in the vinyl-deuterated porphyrins and the propionic side chains were deesterified according to published procedures. The ^1H NMR traces of the previously characterized biscyano complexes, 2,4-V_2DPDME$(CN)_2^-$, in [$^2H_4$]methanol (Figure 1), whose vinyl peaks have been unambiguously assigned by multiplet structure, verify the specificity and degree of vinyl deuteration.

For simplicity, we adopt the same system of abbreviation for all porphyrins based on 2,4-disubstituted deuteroporphyrin, 2,4-R_2DPH$_2$, or its dimethyl ester, 2,4-R_2DPDMEH$_2$, where the R range over ethyl = E, vinyl = V, proton = H, bromide = B, and acetyl = A. The extents of deuteration for the various starting materials for preparing the labeled compounds are: [1,3-$^2H_6$]-2,4-V_2DPH$_2$ (1-CH_3, ~60%; 3-CH_3, ~40%), [1,5-$^2H_6$]-2,4-V_2DPH$_2$ (1-CH_3, ~65%; 5-CH_3, ~90%), [2,4-(α-^2H)$_2$]-2,4-V_2DPH$_2$ (~90%), [2,4-(β-$^2H_2$)$_2$]-2,4-V_2DPH$_2$ (~90%).

The six-coordinate bis(dimethyl sulfoxide) complexes, 2,4-R_2DPFe$(Me_2SO)_2^+$, were prepared[11] by dissolving 2–3 mg of 2,4-R_2DPFeCl in [$^2H_6$]dimethyl sulfoxide, adding $AgNO_3$ to precipitate the chloride, and filtering the solution. The iron chloride complexes in [$^2H_6$]Me_2SO were prepared[11] by dissolving 2,4-R_2DPFeCl and adding excess LiCl. The five-coordinate complexes, 2,4-R_2DPDMEFeCl, were dissolved in [^{2}H]chloroform (0.3–4 mg/mL).

^1H NMR spectra were obtained on either Bruker WH-270 or Jeol PFT-100 Fourier transform (FT) NMR spectrometers operating at 270 and 100 MHz, respectively. The probe temperature was maintained at 25 °C except for the variable temperature data obtained on the Jeol PFT-100. The internal calibrant was tetramethylsilane

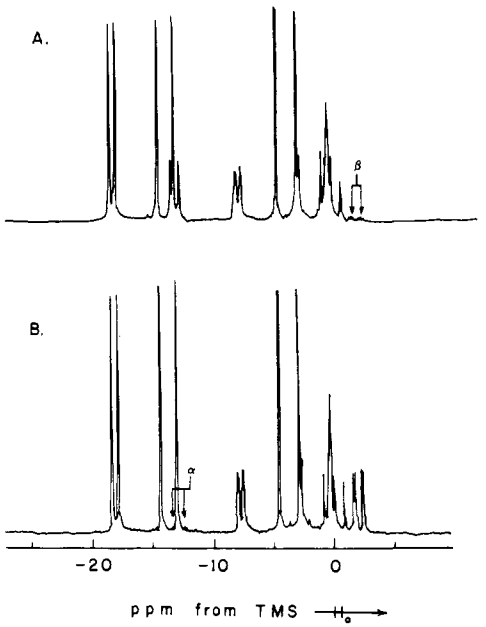


Figure 1. 100-MHz ^1H NMR spectra at 25 °C, in [$^2H_4$]methanol of low-spin biscyano(protoporphinato)iron(III), illustrating specificity and degree of deuteration of (A) vinyl H_βs, i.e., [2,4-(β-$^2H_2$)$_2$]-2,4-V_2DPFe(CN)$^-$, and (B) vinyl H_αs, i.e., [2,4-(α-$^2H_2$)$_2$]-2,4-V_2DPFe(CN)$_2^-$. The vinyl assignments, based on multiplet structure, as well as assignment of other peaks, are illustrated in ref 6.

(Me_4Si) in each case. Hyperfine or isotropic shifts are defined as the difference in shifts for a functional group in the iron(III) and a diamagnetic Zn(II) or Ru(II) prophyrin,[6] with upfield isotropic shifts defined as positive.

Results

The 270-MHz ^1H NMR spectra of the six-coordinate models, 2,4-R_2DPFe(Me_2SO)$_2^+$, in [$^2H_6$]dimethyl sulfoxide are illustrated in Figure 2. All methyls are resolved except for R = ethyl. The assignment of individual methyls and the vinyl H_α peaks in the protoporphyrin complex (R = V = vinyl) are shown in Figure 3. Conversion of the methyl-deuterated porphyrin to all other derivatives using standard procedures[6] resulted in all methyl assignments as listed in Table I. All shifts are independent of concentration. Also included in this table are the shifts for the proposed bis(dimethylformamide) complex (DMF), whose spectrum resembles the Me_2SO complex so closely that it is likely six coordinate.[20,21] Both the methyls and meso-Hs are essentially degenerate for 2,4-E_2DPFe-(Me_2SO)$_2^+$, with the shifts spreading out for both functional groups as R is made more electron withdrawing in the order ethyl < proton ~ vinyl < bromide < acetyl. Deuteration of the vinyl group proves that H_αs resonate some ~30–40 ppm downfield from Me_4Si, while the H_βs appear above Me_4Si.

The downfield portions of the 270-MHz proton traces for the five-coordinate complexes, 2,4-R_2DPDMEFeCl, in C^2HCl_3 are illustrated in Figure 4, and the individually assigned peaks are listed in Table II. Only very small shift changes (<0.2 ppm) are observed upon dilution by a factor of 10, indicating minimal aggregation.[25] Again the vinyl H_αs are found downfield, and the methyl shift spread increases as for the six-coordinate complexes. The meso-Hs yield a single peak at ~50 ppm upfield of Me_4Si (not shown) for all complexes.[10,11] Spectra essentially identical with those in Figure 4 are generated when an excess Cl^- in the form of LiCl is added[11] to 2,4-R_2DPFe(Me_2SO)$_2^+$ to form 2,4-R_2DPFeCl in [$^2H_6$]dimethyl sulfoxide (not shown).

Variable temperature studies of the pyrrole substituents in the six-coordinate complex of protoporphyrin (R = V = vinyl) yield the Curie plot[26] shown in Figure 5.

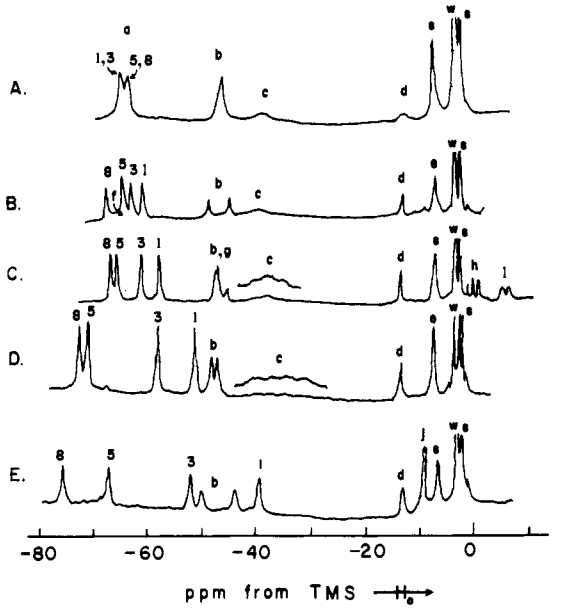


Figure 2. 270-MHz ^1H NMR traces at 25 °C, in [$^2H_6$]dimethyl sulfoxide, of 2,4-R_2DPFe(Me_2SO)$_2^+$ with (A) R = ethyl; (B) R = proton; (C) R = vinyl; (D) R = bromide; and (E) R = acetyl. Assignments of functional groups are as follows: (a) methyls; (b) α-CH_2; (c) meso-H; (d) COOH; (e) β-CH_2; (f) 2,4-H; (g) 2,4-H_α; (h) 2,4-H_β (cis); (i) 2,4-H_β (trans); (j) C(O)CH_3; (w) water; (s) solvent; and (x) impurity. Assignments of individual methyls are indicated by the appropriate position in structure I (see text).

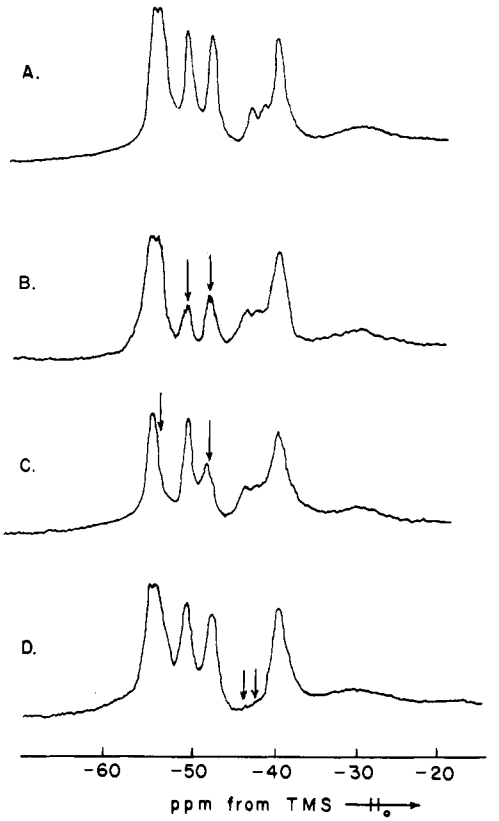


Figure 3. Low-field portion of the 100-MHz ^1H NMR traces at 97 °C, in [$^2H_6$]dimethyl sulfoxide, of (A) 2,4-V_2DPFe(Me_2SO)$_2^+$; (B) [1,3-$^2H_6$]-2,4-V_2DPFe(Me_2SO)$_2^+$; (C) [1,5-$^2H_6$]-2,4-V_2DPFe(Me_2SO)$_2^+$; and (D) [2,4-(α-$^2H_2$)$_2$]-2,4-V_2DPFe(Me_2SO)$_2^+$, which illustrate the assignment of the vinyl H_α and methyl resonances.

Discussion

Assignment of Vinyl Resonances. Most of the various functional groups in both the five- and six-coordinate com-

Table I. Proton Isotropic Shifts and Assignments for Six-Coordinate 2,4-R_2DPFe$(Me_2SO)_2^+$ Complexes[a]

	R =					
peaks	ethyl	H	vinyl	(vinyl)[b]	bromide	acetyl
CH_3s	−62.3 [1][c]	−64.3 [8]	−63.2 [8]	−64.4	−68.6	−72.4 [8]
	−62.3 [3]	−61.2 [5]	−62.2 [5]	−65.8	−67.3 [5]	−64.1 [5]
	−60.6 [5]	−59.8 [3]	−57.7 [3]	−62.6	−54.3	−48.7 [3]
	−60.6 [8]	−57.4 [1]	−54.4 [1]	−58.4	−47.3 [1]	−35.7 [1]
av	−65.0	−64.3	−63.0	−63.5	−63.0	−58.8
spread	1.7	6.9	8.8	8.7	21.3	36.7
α-CH_2	−42.9 (4)[d]	−43.7 (2)	−39.0, −38.0	−43.8 (2)	−43.7 (2)	−44.9 (2)
		−41.0 (2)	−35.7 (2)	−44.9 (2)	−42.8 (2)	−40.2 (2)
β-CH_2	−3.9 (4)	−3.8 (4)	−3.7 (4)	−3.8 (4)	−3.6 (4)	−3.7 (4)
meso-Hs	~−30 (4)	~−29 (4)	−50, −46		−42, −45	*e*
			−48 (2)		−48, −50	
2,4-R_2	−42.9 (4)[f]	−55 (2)[h]	−38 (2)[i]	−40 (2)[c]		−6.2[l]
	−3.9 (6)[g]		6.3, 7.4[j]	8.0, 9.5[j]		
			11.8, 12.4[k]	12.7, 14.3[k]		

[a] Shifts at 25 °C in parts per million referenced to position in diamagnetic complexes. [b] 2,4-R_2DPFe$(DMF)_2^+$ in dimethylformamide-d_7. [c] Assignment of individual methyls via deuteration given in square brackets. [d] Number of protons in a nonmethyl peak given in parentheses. [e] Not resolved owing to low solubility. [f] Ethyl α-CH_2. [g] Ethyl β-CH_2. [h] Under 5-CH_3 peak. [i] Vinyl H_α. [j] Vinyl H_β (cis). [k] Vinyl H_β (trans). [l] Acetyl methyls.

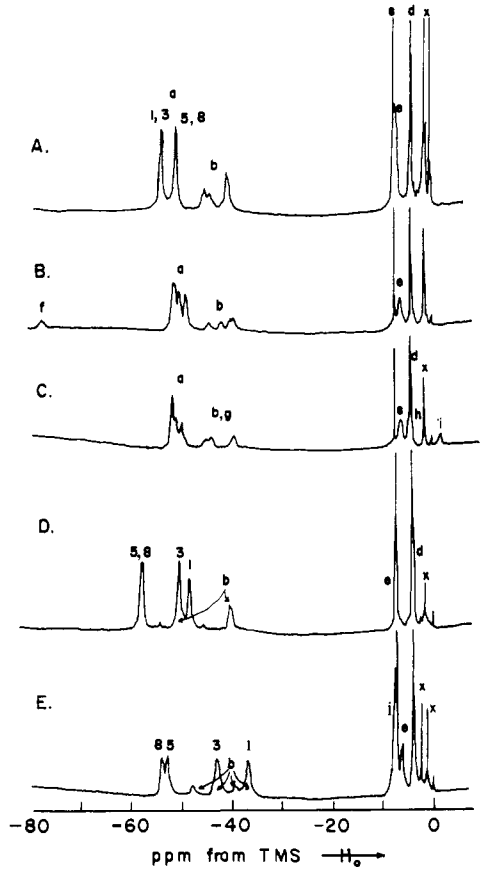


Figure 4. 270-MHz ^1H NMR traces at 25 °C, in [^2H]chloroform, of 2,4-R_2DPDMEFeCl with (A) R = ethyl; (B) R = proton; (C) R = vinyl; (D) R = bromide; and (E) R = acetyl. The upfield meso-H composite peaks located at ~40 ppm upfield of Me_4Si are not included. See caption for Figure 2 for assignments of resonances, except d = OCH_3.

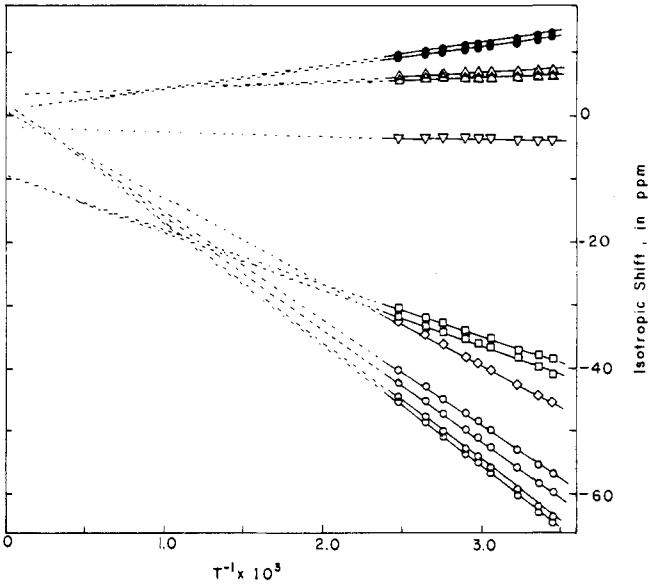


Figure 5. Curie plot for pyrrole substituent of 2,4-V_2DPFe$(Me_2SO)_2^+$, with (○) 1,3,5,8-CH_3s; (◇) 6,7-α-CH_2; (□) 2,4-H_α; (△) 2,4-H_β (cis); (●) 2,4-H_β (trans); and (▽) 2,4-β-CH_2. The intercepts for all but 2,4-H_α are within experimental error of zero at $T^{-1} = 0$.

plexes have been correctly assigned[11] based on the less-resolved 100-MHz spectra with the exception of the important vinyl groups. The H_αs resonate 30–40 ppm downfield from Me_4Si, in contrast to the earlier proposed[11] location near Me_4Si. The two pairs of signals near Me_4Si are the H_βs, with the upfield pair assigned to H_β (trans) based on their greater line width, which results from being closer to the iron than the H_β (cis). Thus, all three protons of a vinyl group resonate outside the diamagnetic region and can be expected to be resolved in a hemoprotein. The vinyl shifts in five-coordinate complexes are similar, although the smaller H_β shifts make them less likely to be resolved in a protein.[2,3,13] The facility and purity with which the vinyl groups in protoporphyrin can be specifically deuterated open up the possibility of not only unambiguously locating vinyl ^1H NMR resonances in hemoglobins, where their orientation has been proposed to be involved in allosteric control of O_2 binding,[27,28] but suggest that specific deuterium labeling of *individual* vinyls is also practical.

The Curie plot in Figure 5 indicates that, with the exception of the vinyl H_αs, all pyrrole substituents yield straight lines with essentially zero intercepts;[29] the vinyl H_α shifts decrease slower than T^{-1}. Similar, though substantially larger, deviations from Curie behavior have characterized[28] the vinyl H_α shifts in LS ferric models and proteins and have been shown to arise from temperature-dependent vinyl orientations. The much smaller deviations in the HS models probably are due to much smaller π vs. σ contact contributions[14] to the H_α isotropic shift (vide infra). Thus, the much smaller deviations from Curie behavior suggest that the vinyl H_α shift may not serve as useful a probe

Table II. Proton Isotropic Shifts and Assignments for Five-Coordinate 2,4-R_2DPDMEFeCl Complexes[a]

	R =				
peaks	ethyl	H	vinyl	bromide	acetyl
CH_3	−49.2 [1][b]	−47.2	−47.2	−53.5 [8][c]	−49.5 [8]
	−49.2 [3]	−47.0	−47.2	−53.2 [5]	−48.7 [5]
	−46.5 [5]	−46.0	−46.4	−46.2 [3]	−43.6 [3]
	−46.5 [8]	−44.7	−45.4	−44.2 [1]	−33.0 [1]
av	−47.9	−46.2	−44.3	−49.3	−43.7
spread	2.7	2.5	1.8	9.3	16.5
α-CH_2	−40.1 (2), 39.1[d]	−39.2, −36.7	−38.4 (2)	−45.3 (2)	−42.7, −37.9
	−38.7, −35.6 (4)	−35.1, −34.5	−34.5 (2)	−35.4 (2)	−34.5, −31.1
β-CH_2	~−3.3	−2.9	−3.0	~−3.8	−3.8
meso-H	~53	~47	~45	*e*	*e*
2,4-R_2	*f*	−66.2 (2)	−35.9, −34.3[g]		−5.0[j]
			1.4, 1.6[h]		
			7.7 (2)[i]		

[a] Shifts at 25 °C, in parts per million referenced to proton in diamagnetic complexes. [b] Assignment of individual methyls via deuteration given in square brackets. [c] Number of protons in a peak given in parentheses. [d] The α-CH_2s include both the propionic and ethyl groups. [e] Not resolved. [f] Ethyl resonances not resolved from propionic CH_2s. [g] Vinyl H_α. [h] Vinyl H_β (cis). [i] Vinyl H_β (trans). [j] Acetyl methyls.

of vinyl orientation/oscillatory mobility in HS ferric hemoproteins as previously found[28] in the LS analogues.

In-Plane Asymmetry. Since the HS models have orbitally nondegenerate ground states,[11] magnetic anisotropy is very small,[26] with contact interaction dominating the shifts, as discussed previously.[11] Analysis of synthetic HS models has shown[30] that the dipolar shifts contribute <10%. In the absence of significant magnetic anisotropy, the spread of the four heme methyl (and meso-H) shifts with increasing electron-withdrawing power of R must reflect differences in spin delocalization into individual pyrroles.[6,31] As shown in Tables I and II, and as also found previously for LS models,[6] the average methyl (or meso-H) isotropic shift is essentially independent of R, dictating that the *spread* in methyl shifts directly reflects a redistribution of unpaired spin smong the four pyrroles, with little net change in iron–porphyrin bonding.

Since the effect of 2,4-R_2 on in-plane asymmetry is qualitatively the same in HS and LS ferric models,[6] with the 1,3-methyls moving upfield and the 5,8-methyls moving downfield as R becomes more electron withdrawing, the same mechanism must be operative in both systems. The asymmetry in the LS mode had been previously proposed[32] to originate from the lifting of the orbital degeneracy by 2,4-R_2. However, since the HS systems do not possess orbital degeneracy, the methyl shift spread must reflect asymmetry in one of the ligand spin-containing molecular orbitals. Thus, the asymmetry in both systems must arise from perturbations directly on the ligand orbitals.

Although the trend in methyl shift asymmetry is the same in the five- and six-coordinate models, the shift spread is always larger in the latter models (vide infra). In the six-coordinate models, which are pertinent to metaquomyoglobin,[17] R = acetyl yields a spread of the heme methyls that is qualitatively similar to that found for the protein,[12,13] if one assumes that the protein peaks are correctly assigned to methyls. Quantitative comparison of the asymmetry must await some planned methyl deuteration studies. However, it is obvious that peripheral heme-apoprotein interactions could give rise to the in-plane asymmetry in HS proteins, as was proposed in LS proteins.[4,6,9] Although the "locked" proximal histidine is perhaps the more likely source of the rhombic perturbation, preliminary methyl deuteration[33] on a variety of LS myoglobins indicates that a second "locked" imidazole does not affect the methyl asymmetry. This observation suggests that neither proximal histidyl nor exogenous imidazole is the prime origin of the in-plane asymmetry.

Effect of Coordination Number. Since the isotropic shift patterns are the same in six-coordinate Me_2SO and DMF complexes, while the shift patterns for the chloride complexes are very similar to other five-coordinate complexes with diverse axial ligands,[10] the shift patterns in Tables I and II can be considered representative of the two coordination geometries. Although it would be highly desirable to include HS five- and six-coordinate imidazole complexes, the absence of such characterized systems forces us to base tentative conclusions on the present models. The most obvious difference between five- and six-coordinated complexes is that the meso-Hs resonate upfield in the former and downfield in the latter set of complexes.[10,11] The meso-Hs, however, are much broader than methyl peaks (1/3 intensity) and probably undetectable in large proteins,[12,13] making them doubtful probes of the occupation of the sixth site.

Table III. Comparison of Contact Shift Patterns in Ferric Porphyrin Complexes[a]

position	HS five coordinate[b]	HS six coordinate[c]	LS six coordinate[d]
pyrrole H	−66	−55	+22
pyrrole CH_3	−45	−63	−15
vinyl H_α	−35	−38	−6
vinyl H_β (cis)	+2	+7	+6
vinyl H_β (trans)	+8	+12	+7
contact shift origin	$\sim\sigma$	$\sigma + \pi$	π
Q^e for R = vinyl	0.04	0.14	0.41

[a] Shifts in parts per million at 25 °C. [b] From 2,4-V_2DPDMEFeCl (Table I). [c] From 2,4-V_2DPFe$(Me_2SO)_2^+$ (Table II). [d] From 2,4-V_2DPFe$(CN)_2^-$ (ref 6) corrected for dipolar shifts as discussed in footnote 34. [e] Asymmetry parameter Q = (spread of methyl shift)/(average methyl isotropic shift). (See text.)

The typical isotropic shifts for prominent and unambiguously assignable peaks in models and proteins for the model compounds are compared in Table III. As shown in this table and by a comparison of Figures 2 and 4, the pyrrole-H shift is ~20% smaller and the pyrrole-CH_3 shift some 25% larger in the five- than in the six-coordinate complexes. Moreover, the vinyl shifts, although similar in pattern, are larger (particularly for H_βs) in the six-coordinate models.[34] Therefore, the magnitude of the pyrrole-H vs. the pyrrole-CH_3 shift in deuteroporphyrin-reconstituted hemoproteins may be a useful indicator of the presence of a coordinated water. In a five-coordinate protein we would expect the 2,4-H to resonate considerably downfield from even the spread-out heme methyls. Such tests of hemoproteins are underway on myoglobin (six-coordinate[17]) and horseradish peroxidase (presumed five-coordinate[19]).

The characteristic difference in the pyrrole proton/methyl

and vinyl shift patterns must reflect a difference in spin transfer mechanisms.[14] As proposed originally by Kurland et al.,[11] the same directions of the proton and methyl shifts argue for dominant σ delocalization. This mechanism is predicted[14] to yield downfield proton and methyl shifts, with the methyl shifts smaller, as is found for the five-coordinate compounds (Tables II and III). Contact shifts arising from delocalized π spin density, on the other hand, yield upfield proton and downfield methyl shifts,[14] as exemplified[6] by LS iron(III) (also reproduced[35] in Table III). If both σ and π spin transfers take place, the added effect of the π mechanism on a dominant σ mechanism would be to decrease the downfield pyrrole-H and increase the downfield pyrrole-CH_3 shifts. This is precisely what differentiates the shift patterns in the five- and six-coordinate models, as shown in Table III. Moreover, the vinyl shift pattern[34] in both HS models resembles the π contact shift pattern found in LS ferric models[6,35] (Table III), except that these shifts are larger in the six- than the five-coordinate HS complexes. Thus, both the pyrrole-H/CH_3 shift ratio and the vinyl shift magnitudes support the conclusion that π spin transfer (porphyrin $\rightarrow$ Fe charge transfer) is more important in six- than in five-coordinate complexes.

The difference in the relative importance of π spin transfer also serves to explain the difference in sensitivity of the methyl shift asymmetry of the five- and six-coordinate models to the same rhombic perturbation in the form of 2,4-R_2, as measured by the ratio Q = (spread of methyl shift)/(average methyl shift), also included in Table III. Since the ligand π molecular orbitals are expected to be perturbed more than the σ orbitals by the peripheral substituents, it is expected that the asymmetry parameter Q is largest for dominant π spin transfer, as found[6] for LS iron(III), less for six-coordinate HS, and least for five-coordinate HS complexes with the same R (see Table III). We, therefore, expect that the heme methyl shifts will not be as sensitive probes of heme-apoprotein interactions in HS ferric as in LS ferric proteins.[2,4,9]

Current deuterium-labeling experiments in progress on HS ferric forms of myoglobin, horseradish peroxidase, and insect hemoglobin are expected to provide a quantitative basis for comparing the different models to proteins and assessing the influence of the protein environment on the electronic structure of the heme.

Acknowledgments. The authors are indebted to Professor R. Kosfeld, University of Aachen, for use of the Bruker WH-270 spectrometer, and to the National Institutes of Health for support of this research through Grants HL-16087 and HL-22252.

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