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Proton NMR and Optical Spectra and Magnetic Properties of Four-Coordinated Intermediate-Spin, Five-Coordinated High-Spin, and Six-Coordinated Low-Spin Iron(II) Hemes Encapsulated in Aqueous Detergent Micelles: Model for Hemoproteins

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Protoporphyrin IX and deuteroporphyrin IX complexes of iron(II) have been studied in aqueous cetyltrimethylammonium bromide (CTAB) micelles by ^{1}H NMR and electronic spectra and solution magnetic moment measurements. The four-coordinated complexes (S = 1) without any axial ligand, five-coordinated complexes (S = 2) with 2-methylimidazole as the axial ligand, and six-coordinated complexes (S = 0) with two pyridines as axial ligands have been unambiguously characterized in the micelle. The proton NMR spectra of the S = 1 and S = 2 hemes in the aqueous CTAB micelle show much broader resonances with a larger spread of the heme methyl proton resonances than those observed in organic solvents. The isotropic shifts of the heme protons, their temperature dependence, and magnetic moments are all characteristic of the assigned spin state. The isotropic shift in the five-coordinated S = 2 complexes is predominantly contact in origin and reflects σ spin transfer. In the S = 1 four-coordinated complexes the shift has an overall downfield bias and is dominated by dipolar contribution. The present work is the first ^{1}H NMR study of iron(II) heme in micellar aqueous solution. The significance of this research in heme model studies is discussed.

Introduction

The studies of iron(II) porphyrins in various stereochemistries that are stable under physiological conditions are of interest toward understanding structure–function relationships in heme proteins.[1,2] The correlation between oxidation state, spin state, and molecular structure is the principal aim of such studies.[3,4] The proton NMR study of iron porphyrin has proved very useful for such purposes,[5,6] as well as for being an aid in the assignment and understanding of complex protein spectra.[7,8] If such studies are carried out in an environment similar to that in heme proteins, then their significance as a model is further enhanced.

Previous NMR investigations of iron(II) porphyrins have generally been done in organic solvents.[5,6,9–12] The high-spin (S = 2) complexes have been generated in organic solvents by binding sterically hindered imidazoles, such as 2-methylimidazole (2-MeIm), as the lone axial ligand.[13–16] Solution magnetic susceptibility[17] and ^{1}H NMR studies have been employed to characterize the spin state and metal–ligand bonding in these complexes. The molecular structure of such porphyrin complexes where iron(II) is in an out-of-plane position with a vacant sixth site indicates its relevance as a model for deoxymyoglobin.[4] The intermediate-spin state (S = 1) has been suspected in iron(II) heme proteins,[18] but its existence has not been unambiguously established. However, the occurrence of this spin state has been definitely confirmed in several four-coordinated iron(II) porphyrins.[13,19–22] Detailed magnetic susceptibility[21] and ^{1}H NMR[10,11,20] investigations have proved useful in understanding the electronic structure of these intermediate-spin iron(II) porphyrins. Brault and Rougee[23] have observed that four-coordinated iron(II) porphyrins in benzene solution show splitting of the Soret band into several components. This is a useful criterion and has been verified in structurally characterized four-coordinated iron(II) porphyrins.[17]

Studies of iron(II) complexes of natural porphyrins in aqueous solutions face several difficulties. First, the hemes are oxidized quite rapidly in air if unprotected from dimerization.[24] Second, the hemes are ordinarily difficult to solubilize in aqueous medium under conditions of pH and ionic strength as in heme proteins. Third, the hemes are known to undergo extensive aggregation in aqueous solution.[25] As a result, studies of iron(II) complexes of natural porphyrins in aqueous solutions have not attracted sufficient attention.

Some of these difficulties can be overcome by generating iron(II) porphyrin inside aqueous detergent micelles. It is known from the studies of Simplicio et al.[26,27] that iron(III) protoporphyrins can be readily solubilized and stabilized as monomers when intercalated in aqueous detergent micelles. We have recently generated, by this method, stable monomeric diaquo- and aquohydroxoiron(III) protoporphyrin complexes inside micelles and studied their ^{1}H NMR and magnetic properties.[28] Iron(II)

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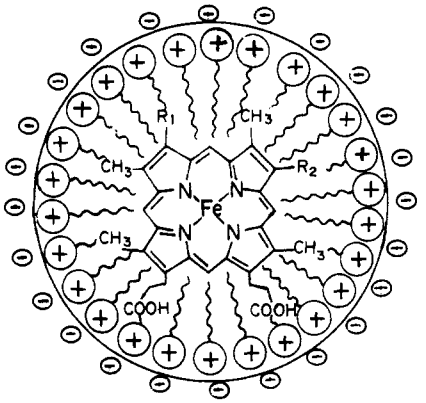

Figure 1. Heme inside micelle. For PP, $R_1 = R_2$ = vinyl; for DP, $R_1 = R_2$ = H.

porphyrins can be readily produced and stabilized inside micelles by reducing the micelle-encapsulated ferric porphyrins with sodium dithionite. This procedure offers the possibility of studying iron(II) porphyrins in the monomeric state in an aqueous surrounding. The study of iron(II) porphyrins inside micelles is also significant in view of the hydrophobic nature of micellar interaction, which simulates the biological environment of globular proteins.[29]

We report here ^{1}H NMR and optical spectra and magnetic moments of four-coordinated ($S = 1$), five-coordinated ($S = 2$), and six-coordinated ($S = 0$) iron(II) complexes of protoporphyrin IX (PP) and deuteroporphyrin IX (DP) encapsulated in aqueous cetyltrimethylammonium bromide (CTAB) micelles at pH $\approx$ 10. The porphyrin and micelle structure is shown in Figure 1. The aggregation number of CTAB is 61, and the average radius of the micelle is estimated to be 10–15 Å,[30,43] which favors one heme per micelle as observed by Simplicio and others[28,31] (see also later). Several UV–vis and other spectroscopic studies[32,33] have been carried out on hemes in micelles, particularly with regard to their reactivity in the hydrophobic micellar cavity. The present work is, however, the first ^{1}H NMR study of the iron(II) hemes in aqueous micellar solution.

Experimental Section

Iron(III) protoporphyrin IX chloride (hemin chloride, Fe(PP)Cl) and deuteroporphyrin IX ligand (DP) were obtained from Sigma Chemicals. Iron(III) deuteroporphyrin IX chloride (Fe(DP)Cl) was prepared from the ligand by a known procedure.[34] CTAB was purchased from E. Mark Co. Pyridine was purchased from BDH Chemicals; deuterated pyridine (used for NMR spectroscopy) and 2-methylimidazole were obtained from Sigma Chemicals Co. Ferric porphyrins in 5% aqueous CTAB micellar solution at pH $\approx$ 10 were prepared by following the method of Simplicio et al.[26,27] An alkaline solution of Fe(PP)Cl (or Fe(DP)Cl) was mixed with a freshly prepared, thoroughly deoxygenated 5% CTAB aqueous micellar solution. The pH of the solution was adjusted with dilute H_2SO_4 (or D_2SO_4) and dilute NaOH (or NaOD). The aqueous micellar solutions of the ferric porphyrins at adjusted pH (=10) were allowed to equilibrate for about 2 h at 40–50 °C.

For the synthesis of ferrous porphyrins all operations were carried out under an inert atmosphere and all reagents (including water/D_2O) were thoroughly deoxygenated. The minimum amount of a saturated aqueous solution of sodium dithionite ($Na_2S_2O_4$) was used to reduce the ferric to the ferrous complex. A ligand-binding study was carried out by injecting a concentrated aqueous solution of the ligand into the iron(II) porphyrin solution under a nitrogen atmosphere. The extent of reduction was estimated by the pyridine hemochrome method.[34] The pyridine hemochrome and 2-methylimidazole adducts were also synthesized by dithionite reduction of the corresponding ferric complexes encapsulated in aqueous micelles. For visible spectral studies the Fe(III) porphyrin ($\sim 10^{-5}$ M) was taken into a quartz cuvette fitted with a rubber septum, and the reduction was carried out by injecting the dithionite solution followed by gentle shaking of the solution. NMR samples were prepared by reducing 0.2–1 mM solutions of Fe(III) porphyrins with dithionite inside the NMR tube fitted with a rubber septum. Oxygen-free nitrogen was passed into the cuvette and into the NMR tubes before the solutions were prepared inside them. Samples prepared in this way were found to be spectroscopically stable even for 2–3 days. Degassing of a few samples was carried out by the freeze–thaw method in a vacuum line but with no apparent advantage compared to the above method.

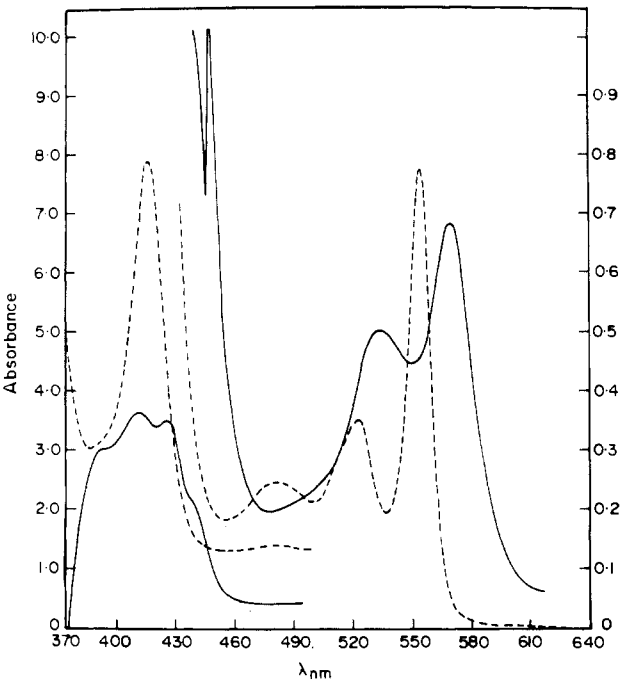

Figure 2. Electronic spectra of 5×10^{-5} M solutions of [Fe^{II}(PP)] (—) and [Fe^{II}(PP)(py)$_2$] (---) in 5% aqueous CTAB micellar solution at pH = 10 (26 °C).

Optical spectra were recorded on a Cary 17D UV–visible spectrophotometer, and the pH was measured with a digital pH meter. The phenomenological pH was not corrected for isotope effect. The magnetic moments in the micellar solutions were measured by the Evans method adapted for proteins.[35]

The proton NMR experiments were done on a 500-MHz Bruker FT-NMR spectrometer. In order to observe the paramagnetically shifted porphyrin protons, the micelle and water proton signals needed to be saturated. A microprogam for multiple-frequency irradiation was used to irradiate all the micelle peaks and the water signal. About 4000–5000 transients were acquired over a spectral width of $\sim$45 kHz with 8K data points to get a good signal-to-noise ratio. NMR experiments in each case were done over a range of the porphyrin concentration, and no concentration dependence of the line width or the shift of the heme proton resonances was observed. This further confirmed the existence of monomeric ferrous species in the micelle. The temperature of the NMR samples was measured accurately to $\pm$0.5 °C. The chemical shifts reported are with respect to TMS as an external standard, and downfield shifts are taken positive. The diamagnetic corrections for calculating isotropic shifts were taken from reported values.[9,10]

Results and Discussion

Four-Coordinated Iron(II) Heme. As mentioned above, dithionite reduction of the micelle-encapsulated aquohydroxoiron(III) porphyrins at pH $\approx$ 10 (under an inert atmosphere) gave the iron(II) porphyrin whose optical spectrum is shown in Figure 2. The estimation of the amount of Fe(II) by the pyridine hemochrome method[34] showed that the reduction was 99–100% complete. The optical spectrum of the Fe(II) complex bears a clear signature of four-coordinated Fe(II) porphyrins and is very similar to that reported for such complexes in benzene solution.[22,23] The two well-defined visible bands at 524 and 567 nm and a Soret

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Table I. Proton Chemical Shifts for Iron(II) Hemes with Different Spin States

complex	spin state (S)	chem shift, ppm: ring Me	2-4-H	vinyl CH	meso H	6,7-CH_2	ref
Fe(PP)(2-MeIm) [in CTAB]	2	22.1, 21.47, 19.4, 6.1		12–13		13.7	this work
Fe(PP)(2-MeIm) [in C_6D_6]	2	12.8, 12.1, 11.6, 6.9		15.5, 11.7 (trans) 10.3, 9.0 (cis)		10.7, 10.3	9
Fe(DP)(2-MeIm) [in CTAB]	2	21.53, 20.25, 14.24	46.2, 44.0			13.5	this work
Fe(DP)(2-MeIm) [in C_6D_6]	2	12.6, 12.3, 12, 7.6	53.55, 49.05			10.4, 10.1	9
deoxyMb^{II} [at pH = 8.0]	2	16, 11[a]					41
deoxyHb [at pH = 7]	2	~22.5 (β), ~16, ~12					41, 47
Fe(PP) [in CTAB]	1	47.9, 47.1, 40, 37.2		38	64.5, 62.1	34.3, 33.8	this work
Fe(PP) [in C_6D_6]	1	46.9, 46.1 40.6, 40.4		41.2 39.6	72.6, 69.5 68.4, 64.8	32.2, 314	10
Fe(DP) [in CTAB]	1	48.0, 45.05, 43.83, 34.75			67.8, 66	34, 32	this work
Fe(DP) [in C_6D_6]	1	47.6, 46.1, 45.5, 43.9	5.3		74.6, 71.7, 69.9, 69.6	32.3, 30.9	10
Fe(TPP)(2-MeIm) [in C_6D_6]	2		52.15		6.55 (*o*-H) 6.59 (*m*-H) 6.68 (*p*-H)		9
Fe(TPP) [in C_6D_6]	1		12.85		20.8 (*o*-H) 12.5 (*m*-H) 12.5 (*p*-H)		10
Fe(PP)$(py)_2$ [in CTAB]	0	2.6–3.1[b]			9–10[b]		this work
Fe(PPDME) [in $CDCl_3$ with N_2D_4]	0	3.5, 3.6			9.4, 9.56 9.65, 9.7	3.25	48

[a] Exact values of the shift not available. [b] Assignment of CH_3 and other protons difficult because of masking by resonances from solvent and micelle protons.

band, which is split into four bands (Figure 2), are typical of the four-coordinated iron(II) porphyrins. The magnetic moment measurements carried out on several samples in the micellar solution gave a reproducible constant value of $\mu = 3.8 \pm 0.2\ \mu_B$ between 290 and 330 K. The value is similar to that reported for four-coordinated $S = 1$ iron(II) porphyrins and phthalocyanine.[21,36,37] The large orbital contribution ($\mu_{so} = 2.83\ \mu_B$ for $S = 1$) observed in this iron(II) porphyrin complex is a common feature of all $S = 1$ four-coordinated iron(II) porphyrins and similar systems.[36]

The proton NMR spectra of Fe^{II}(DP) and of Fe^{II}(PP) in aqueous CTAB micelles are shown in Figure 3. The line width and the isotropic shifts were found to be nearly independent of concentration in the range 0.1–1 mM, indicating that the aggregation is minimal in the range of the present study. It is important to emphasize that, in the absence of the micelles, such spectra cannot be obtained in aqueous solution. The general features in the spectra and the isotropic shift agree well with those reported for four-coordinated $S = 1$ iron(II) porphyrins in benzene.[10,20] The assignments of the porphyrin proton resonances are based here on observed relative intensities and multiplet structure as well as a comparison with the earlier reported assignments in benzene.[10,20] Further assignments were made possible by comparing the spectrum of the PP complex with that of the DP complex. Representative chemical shift data for some $S = 1$ iron(II) porphyrins are included in Table I.

The temperature dependence of the isotropic shift of ring methyl protons for the two complexes is shown in Figure 4. The insets in the figure indicate that the isotropic shift obeys closely the Curie law in the temperature range of the study. This is consistent with the $S = 1$ ground electronic state. Previous magnetic[21,36] and proton NMR studies[9,11] on the planar iron(II) porphyrins have shown that the ground state is $^3A_2$, which is extensively mixed with low-lying ^3E and $^3B_1$ states. This mixing causes the commonly observed large orbital contribution to the magnetic moment in the $S = 1$ iron(II) hemes, including those under discussion. The present limited magnetic and NMR data are not adequate for any further probe into the spin mixing, beyond confirming that these planar iron(II) porphyrins encapsulated in the micelles belong to the $S = 1$ state.

The observed shifts in Figure 3 for all porphyrin ring protons appear in the downfield region of the spectra. The overall bias

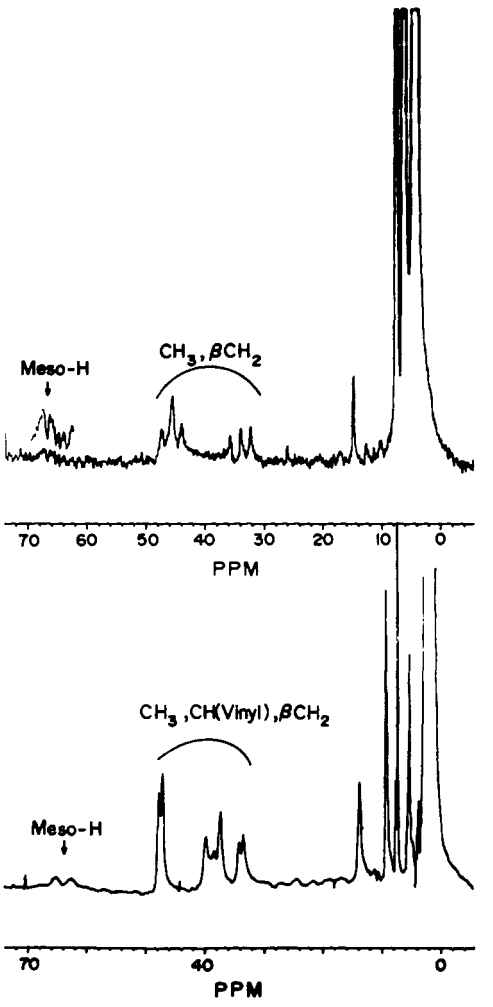

Figure 3. Proton NMR spectra (500 MHz) at 298 K of the four-coordinated $S = 1$ (top) [Fe^{II}(DP)] and (bottom) [Fe^{II}(PP)] in 5% CTAB solution (pH = 10; concentration 0.5 mM).

of the shifts in one direction indicates[10] that at least a major portion of the isotropic shift originates from the dipolar interaction. The isotropic shift is given by[38,39]

$$\frac{\Delta H}{H} = \left(\frac{\Delta H}{H}\right)_{CS} + \left(\frac{\Delta H}{H}\right)_{DS}$$

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where the contact shift, $(\Delta H/H)_{CS}$, is given as

$$\left(\frac{\Delta H}{H}\right)_{CS} = \frac{Ag\beta S(S+1)}{3\gamma\hbar kT}$$

the dipolar term, $(\Delta H/H)_{DS}$, in axial symmetry is

$$\left(\frac{\Delta H}{H}\right)_{DS} = -\frac{1}{3N}(K_{\parallel} - K_{\perp})\left(\frac{3\cos^2\theta - 1}{r^3}\right)$$

and the symbols have their usual meaning.[38,39] Relative values of the axial geometric factors, $(3\cos^2\theta - 1)/r^3$, for nonequivalent protons in the molecule determine the relative magnitude of the dipolar shifts. It is known from measurements of magnetic anisotropy on the single crystals of structurally characterized, four-coordinated, $S = 1$ Fe^{II}(TPP) that these planar molecules are highly anisotropic.[21] The large orbital contribution to the magnetic moments of these $S = 1$ iron(II) complexes is consistent with their anisotropic character.[21] It is thus expected that the dipolar contribution would be significant and form a major portion of the isotropic shifts for the most protons, though its relative magnitude will vary for different proton sites. Since the magnetic anisotropy of these systems is known[21,37] to vary nearly as $1/T$ in the temperature range under discussion, and since the contact term is also expected to vary as $1/T$, the observed Curie behavior of the isotropic shift is consistent with the $S = 1$ spin-state formulation.

The ^1H NMR spectra of the Fe(II) porphyrins in the aqueous micelle are, however, characterized by two important differences from those in benzene. The porphyrin proton resonances are much broader in aqueous micelles and are similar to those observed in heme proteins.[41] The broadening of the heme proton resonances is mainly due to an increase in rotational correlation time of the heme inside the micelle. The line width of a paramagnetic complex is given as

$$\sigma_{obs} = 1/(\pi T_2) = K_1 S(S+1) f(\tau_c) + K_2 S(S+1) f(\tau_e)$$

where T_2 is the spin–spin relaxation time, K_1 and K_2 are constants given in the Solomon–Bloembergen equation ($K_1 = 2\gamma^2 g^2\beta^2/15r^6$; $K_2 = A^2/3\hbar^2$), A is the hyperfine coupling constant for a given proton, $f(\tau_C)$ and $f(\tau_e)$ are functions of the correlation times ($f(\tau_C) = 4\tau_C + 3\tau_C/(1+\omega_r^2\tau_C^2) + 13\tau_C/(1+\omega_s^2\tau_C^2)$ and $f(\tau_e) = \tau_e + \tau_e/(1+\omega_s^2\tau_e^2)$ with $1/\tau_C = 1/\tau_s + 1/\tau_R$ and $1/\tau_e = 1/\tau_s$), ω_s and ω_I are the electronic and nuclear Larmor precessional frequencies, γ is the magnetogyric ratio, τ_s is the electron relaxation time τ_R is the rotational correlation time, and τ_C is the total correlation time. The above expression indicates that the line width of a given signal increases as the total correlation time τ_C increases. The typical value of τ_S for paramagnetic iron(II) (i.e., $S = 1$ or $S = 2$) porphyrins[5] is $\sim 10^{-13}$ s. The rotational correlation time (τ_R) of the heme in simple solution is ca. 10^{-11} s,[42] while τ_R for the micelle protons is ca. 10^{-9}–10^{-10} s.[43] This increase in τ_R in micelles increases the τ_C value, and thus the line widths of the heme protons in micelles are larger than those in simple nonaqueous solutions. In heme proteins a similar mechanism is responsible for a major contribution to the increase in line width. Apart from this increase in line width, the position and spread of the heme methyl resonances are also found to be somewhat larger (see Table I) in the micellar solutions than those in the organic solvent. The spread of the heme methyl signals has been a subject of extensive study in both ferric as well as ferrous hemes and hemoproteins.[5,6,8] It has been proposed that the spread of the heme methyl protons increases as the in-plane asymmetry of the porphyrin ring increases. In the present case a possible explanation for the increase in spread in methyl signals may be due to an increase in asymmetry in the porphyrin ring inside the micellar cavity because of a difference in the hydrophobic interaction of the micelles with the vinyl end of the ring compared to that with the propionic acid group side of the heme (see later).

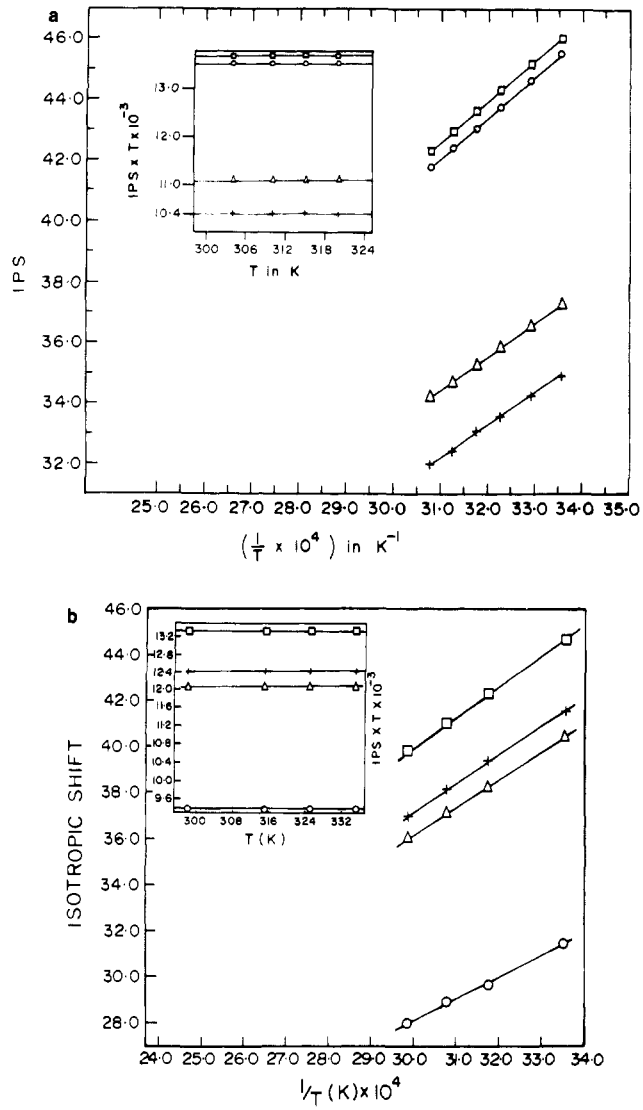

Figure 4. Temperature dependence of the isotropic proton shift (IPS) of ring methyl proton resonances of (a) [Fe^{II}(PP)] and (b) [Fe^{II}(DP)] in 5% aqueous CTAB micellar solution. The (IPS)T vs T variations are shown as insets.

Five-Coordinated $S = 2$ Hemes. Addition of a large excess (~50 times in moles) of 2-methylimidazole to an aqueous CTAB solution of the four-coordinated heme under a nitrogen atmosphere gave a monoligated adduct, [Fe(Por)(2-MeIm)], in the micelle. It was also obtained by dithionite reduction of an aqueous solution of the ferric 2-methylimidazole complex in CTAB solution.[40] Both of the methods gave identical optical spectra, shown in Figure 5. The similarity of the spectrum in the visible region to that of deoxymyoglobin (in water) is clear, suggesting that the complex in the CTAB micelle is a five-coordinated mono(imidazole) complex with the sixth position vacant. [Fe^{II}(Por)(2-MeIm)] in CTAB thus mimics closely the active-site coordination of deoxymyoglobin. The magnetic moment of the complex in the micellar solution was measured to be $\mu = 4.9$ μ_B at room temperature. The value is close to that observed for high-spin deoxymyoglobin,[41] deoxyhemoglobin,[47] and five-coordinated iron(II) porphyrins.[13,44]

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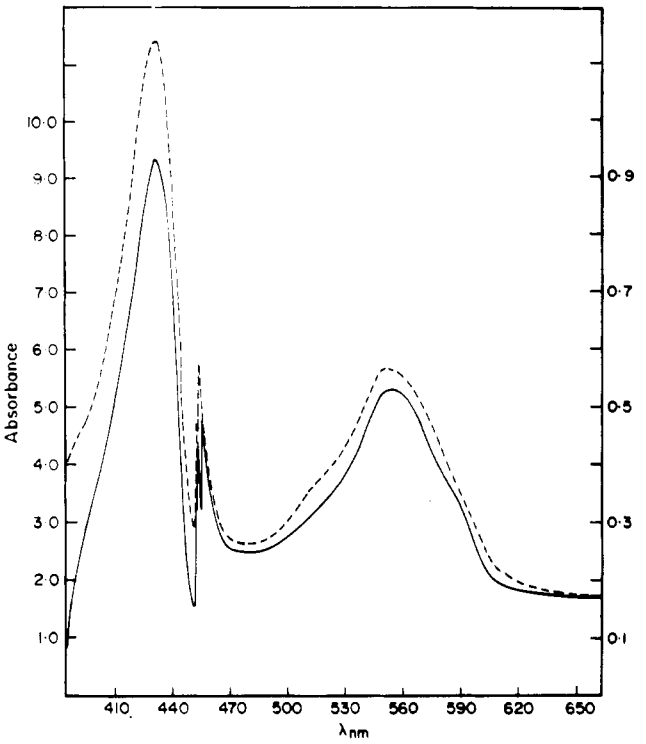

Figure 5. Electronic spectra of [Fe^{II}(PP)(2-MeIm)] (0.5 mM) in 5% aqueous CTAB micellar solution (—) and deoxymyoglobin in water (---) at pH = 10 (26 °C).

The proton NMR spectra of [Fe(PP)(2-MeIm)] and [Fe(DP)(2-MeIm)] in aqueous CTAB micellar solution are shown in Figure 6. As before, the effect of any aggregation was not observed in the NMR spectra of these complexes as well. The ^{1}H NMR spectra as in Figure 6 could not be obtained in aqueous solution in the absence of micelles. The spectra agree well with that reported for deoxymyoglobin.[41] Assignments of different resonances were made on the basis of relative intensity and multiplet structures and by comparison with the spectra of the deuteroporphyrin analogue. Further assignments were made on the basis of the assignments reported for high-spin Fe(II) porphyrins in benzene.[9]

The isotropic shift (IPS) of the methyl protons for the two complexes shows a linear temperature dependence in the range of study (Figure 7), indicating that the shift obeys strictly the Curie law. A more rigorous test of the same is shown as insets in the figure, where the plots of (IPS)T vs T give straight lines parallel to the temperature axis. Such behavior indicates that one spin state is populated in the temperature range of study. Table I lists some typical values of the chemical shifts for the high-spin Fe(II) porphyrins along with those for deoxymyoglobin. Table I shows similarity in the isotropic shift patterns for the high-spin Fe(II) porphyrins in aqueous and in benzene solutions. It may therefore be assumed that both contact and dipolar contributions to the isotropic shift are very similar in the two solvents. As in benzene solutions,[9] the isotropic shift for most porphyrin protons of the Fe(II) complex in the aqueous micelles may also be predominantly contact in origin. This implies that the dipolar contribution to the isotropic shift (and the magnetic anisotropy of the molecules) is not large. Since the magnetic moment of these high-spin complexes is very close to the spin-only value, it is expected that the complexes will not be very anisotropic in the present temperature range. Assuming therefore that the isotropic shift for the complex is dominantly contact type, we note that the small downfield CH_3 shifts (~21 ppm) and large downfield pyrrole H shift (45.8 and 43.7 ppm) in the DP complex indicate primarily a σ spin transfer mechanism.[9] There is the possibility of a small π delocalization to the meso position, but the meso-H resonances were very broad in our study, appearing between −2.4 and −3.6 ppm and could not clearly be resolved and assigned. The dominance of σ spin transfer is quite reasonable in view of an unpaired

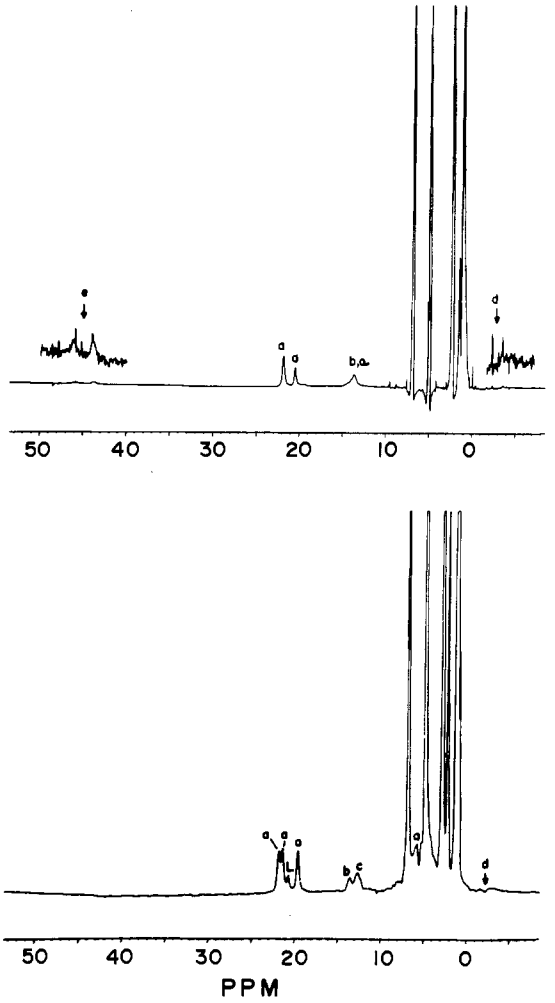

Figure 6. Proton NMR spectra (500 MHz) at 298 K of (top) [Fe^{II}(DP)(2-MeIm)] and (bottom) [Fe^{II}(PP)(2-MeIm)] (0.5 mM, pH = 10) in 5% aqueous CTAB solution: (a) ring CH_3; (b) β-CH_2; (c) vinyl H; (d) meso H.

electron in the $d_{x^2-y^2}$ orbital which is strongly antibonding type.

Although there is a similarity in the patterns of the isotropic shifts of the high-spin iron(II) porphyrins in aqueous micellar solution and in benzene solution, there are some differences as well. First, the porphyrin resonances in the micelle are much broader than in benzene and resemble more those reported for deoxymyoglobin.[41] The possible reason for the broadening has been discussed earlier. Second, the downfield shift of the methyl resonances in the micelle (appearing at ca. 19 ppm) is much larger than that observed in benzene solution but is similar again to that observed in deoxymyoglobin. The close similarity of ^{1}H NMR and optical spectra (Figure 5) of the high-spin [Fe(PP)(2-MeIm)] complex in the micelle and those of deoxymyoglobin suggests a similarity in their electronic structures. As we have noticed in the case of the four-coordinated iron(II) heme in micelles, the spread of the four methyl protons in these five-coordinated complexes is also found to be larger in micellar solution (~10 ppm) as compared to that in the benzene solution (~6 ppm). In the case of deoxymyoglobin[41] this spread of heme methyl resonances is even larger. There are two possible explanations of the increase in spread of ring methyl proton signals in five-coordinated hemoproteins.[41] First, the increase in the heme in-plane asymmetry due to interaction between heme peripheral substituents with the nearby protein moiety may cause this spread. This has been discussed in the earlier section. The second reason for an increase in the spread of heme methyl resonances may be the decrease in symmetry due to restricted orientation of the axial ligand (histidine). However, in the temperature range of the present study, the axial 2-methylimidazole inside the micellar cavity is presumably free enough to rotate about the Fe–N bond. Thus, the

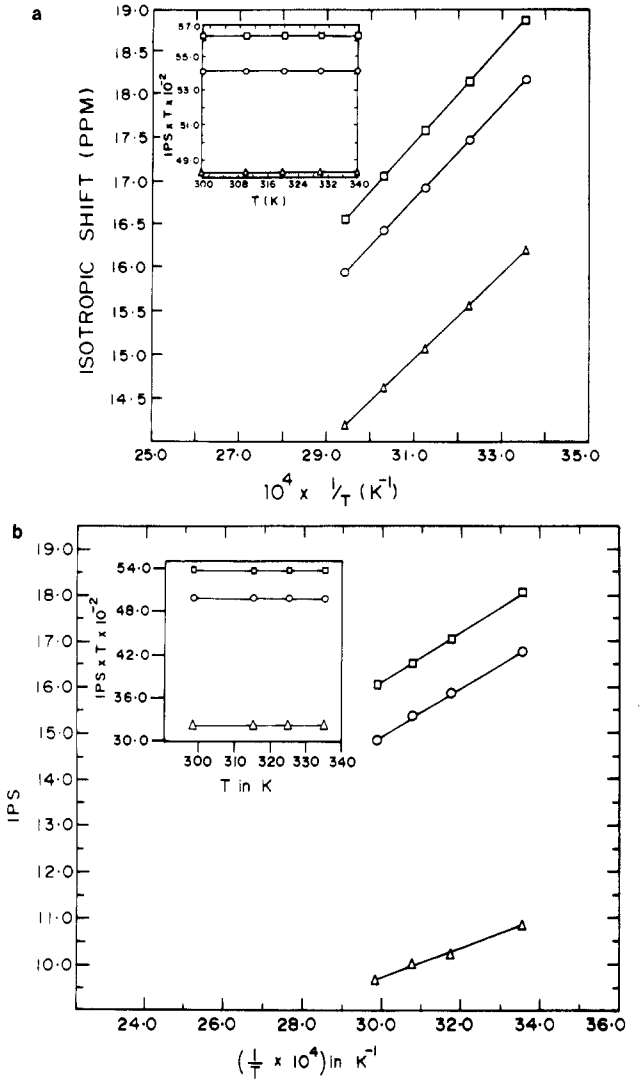

Figure 7. Temperature dependence of the isotropic proton shift (IPS) of ring methyl proton resonances of (a) [Fe^{II}(PP)(2-MeIm)] and (b) [Fe^{II}(DP)(2-MeIm)] in 5% aqueous CTAB micellar solution. The (IPS)T vs T variations are shown as insets.

increase in the spread of the heme methyl protons in high-spin Fe(II) hemes is mainly due to the porphyrin–micelle interaction as proposed in the case of the four-coordinated Fe(II) hemes.

Six-Coordinated Low-Spin Iron(II) Hemes. The micelle-encapsulated bis(pyridine)iron(II) porphyrins were prepared by reducing aquo(pyridinato)iron(III) porphyrin[45,46] in 5% CTAB by aqueous sodium dithionite. The optical spectrum of [Fe^{II}(PP)$(py)_2$] in micellar solution (Figure 2) is identical with the one reported earlier for a six-coordinated bis(pyridine)iron(II) porphyrin complex.[34] The magnetic moment measurement in solution proved that the complexes are, as expected, diamagnetic. The ^{1}H NMR spectra showed characteristic low-spin iron(II) resonances (S = 0) with shifts lying in the diamagnetic region. The relevant chemical shifts are listed in Table I. The nature of the spectra and the resonance positions did not change with a change in temperature.

A comparison of the chemical shifts of the three types of spin systems discussed above is now worth considering. The comparison is clearly shown in Table I. It is interesting that both the meso and the methyl proton shifts have a large upfield bias in going from intermediate-spin (S = 1) complexes to high-spin (S = 2) complexes; the magnitude of the upfield bias in the latter is ~65 ppm for meso H and ~30 ppm for the methyl protons at room temperature. However, the pyrrole H resonances (2,4-H of DP) show a downfield bias of ~40 ppm in going from the intermediate-spin state to the high-spin state. These trends in the shift are related to the changes in the electronic structure, spin delocalization,[9] and magnetic anisotropy associated with the changes in spin states. It is also observed that the meso and pyrrole H resonances are more sensitive to spin state than the methyl proton resonances. Unfortunately these resonances are too broad and ill-resolved in the aqueous micellar solution for obtaining such information.

Concluding Remarks

The results presented in this paper on the monomeric iron(II) complexes of natural porphyrins in aqueous micellar solution have brought out several features of general interest. The iron(II) porphyrins in different spin states and stereochemistries can be stabilized in aqueous detergent micelles. The micellar cavity appears to protect the iron(II) porphyrins, to some extent, against the aerial oxidation and makes their study easier. This, together with the hydrophobic nature of the CTAB micellar cavity, seems to suggest the present system as an attractive model for iron(II) heme proteins. The general similarity in the optical spectra and in the nature and resonance positions in the ^{1}H NMR spectra with those of the heme proteins favors the above suggestion.

CTAB micelles with an aggregation number of 61 and a molecular weight of ~60 000 are similar in size to a heme protein. The quality of the ^{1}H NMR spectra of the heme protons in the micelle is therefore encouraging. We have recently obtained[28] similar-quality ^{1}H NMR spectra for iron(III) hemes inside aqueous SDS detergent micelles. These observations indicate that ^{1}H NMR spectroscopy of hemes in the micelles can be conveniently studied in different oxidation and spin states.

It is interesting that an iron(II) porphyrin with intermediate spin state can be stabilized inside the hydrophobic cavity of the CTAB micelle. As noted earlier, this spin state is still not clearly established in iron(II) heme proteins, though its existence for iron(II) porphyrins in the solid state and organic solvents is well established. The existence of these "missing" heme spin states in the micellar cavity is therefore interesting and encourages a detailed reassessment of the magnetic properties of some "anomalous" iron(II) proteins, which may be suspected to belong to this spin state.[18,36,44]

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