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Six-Coordinated High-Spin Models for Ferric Hemoproteins: NMR and ESR Study of the Diaquo(protoporphyrinato IX)iron(III) Cation and Aquohydroxo(protoporphyrinato IX)iron(III) Intercalated in Aqueous Detergent Micelles

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Received October 7, 1987

Temperature-dependent ^1H NMR and ESR studies are reported for six-coordinated monomeric iron(III) protoporphyrin complexes of the type $[Fe(PPIX)(H_2O)L]^+$ (L = H_2O or OH) in aqueous solutions of detergent micelle SDS. The studies establish that the compounds are high-spin and that the ring methyl isotropic proton shift is almost wholly contact in origin. A large change in the ring methyl isotropic shift is observed between the diaquo and the aquo hydroxo complexes. The significance of the studies as the model hemes in the hydrophobic environment of the micelles is discussed. This is the first report of the proton NMR studies on the hemes in micelles.

Introduction

The ferric ion in the high-spin iron(III) hemoproteins is generally six-coordinated. On the other hand, the majority of the high-spin ferric porphyrins are known to be five-coordinated. In order to find better models for the high-spin hemoproteins, there has been continuing effort in stabilizing and characterizing six-coordinated high-spin iron(III) porphyrins.[1-10] In recent years several six-coordinated high-spin ferric porphyrins have been characterized and studied, the diaquo- and bis(dimethyl sulfoxide)(tetraphenylporphyrnato)iron(III) perchlorates being the best known among them.[2,3] It has been shown that the large size of the high-spin ferric ion can be accommodated inside the N_4 porphyrin core through its radial expansion, which arises from the population of the $d_{x^2-y^2}$ orbital, and a significant nonbonding repulsion of the axial ligand by the porphyrin core.[3,7,10]

The six-coordinated high-spin ferric porphyrins that have so far been investigated generally refer to studies in nonaqueous solvents and studies of mostly synthetic porphyrins. The studies and characterization of analogous natural porphyrin complexes are very limited, and those in aqueous medium are almost totally lacking. This is mainly due to solubility restriction and aggregation,[11] which make the study of monomeric natural porphyrin complexes difficult. However, Simplicio et al.,[12-14] in a series of papers, have shown that protoporphyrin ferric complexes can be solubilized and stabilized as monomers when intercalated in aqueous detergent micelles. Monomeric complexes such as $[Fe(PPIX)(H_2O)L]^+$, where L = H_2O or OH, have been found[12-14] to be stable in aqueous micellar solutions over a range of pH and temperature. NMR and magnetic characterizations of these complexes in monomeric form have not as yet been reported.

We report here magnetic resonance studies on monomeric six-coordinated high-spin iron(III) complexes of protoporphyrin in aqueous solutions of detergent micelles. To our knowledge this is the first such study reported so far. The hydrophobic interactions within the micelles monodisperse the iron(III) porphyrins so well that the results obtained here are free from aggregation.

Experimental Section

Protoporphyrin ferric chloride (hemin chloride) and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemicals. The samples were prepared by following the method of Simplicio,[12] mixing an alkaline solution of hemin chloride with freshly prepared 5% SDS aqueous micelles. The pH was measured accurately to ±0.05 unit. The pH meter electrode was incubated with 5% SDS solution for 1 h before use, and the pH of the solutions was adjusted with dilute HNO_3 and dilute NaOH (NaOD for NMR samples). The hemin chloride in aqueous micellar solutions at the adjusted pH was allowed to equilibrate for about 2 h at 40–50 °C, and the final pH was measured before each experiment. The pink complex obtained in the micelles at pH 2.6 shows (Figure 1) the following λ_{max} values (nm) and molar extinction coefficients ($\times 10^5$ M^{-1} cm^{-1}): 392, 0.9; 500, 0.09; 532 (sh), 0.08; 630, 0.03. This complex was identified as $[Fe(PPIX)(H_2O)_2]^+$, while the green complex $[Fe(PPIX)(H_2O)(OH)]^+$ was obtained at pH 12.5 with the following λ_{max} values (nm) and molar extinction coefficients ($\times 10^5$ M^{-1} cm^{-1}): 400, 0.82; 490, 0.10; 523 (sh), 0.07; 570 (sh), 0.06; 600, 0.07. These values and the spectra in Figure 1 match closely with the earlier reported spectra for the diaquo and aquo hydroxo complexes in SDS micelles.

It is also known that the pink diaquo complex is in equilibrium, over a wide range of pH, with the green aquo hydroxo complex:

$$Fe(PPIX)(H_2O)_2\text{·micelle} \rightleftharpoons Fe(PPIX)(H_2O)(OH)\text{·micelle}$$

Since the pK of this equilibrium has been determined[14,15] to be 5.5, all measurements on the diaquo complex were made at pH 2.6 and those on the aquo hydroxo complex at pH 12.5 to ensure their exclusive presence.

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 proton signals and the the water signals needed to be saturated. A microprogram for multiple-frequency irradiation was used to irradiate all the micelle peaks and the water signal. About 5000 transients were acquired over a spectral width of ~45 kHz with 8K data points. NMR experiments were done over a hemin concentration range of 0.1–1.5 mM, and no concentration-dependent line width or shift of heme methyl signals was observed, which further confirmed the existence of monomeric species in solution. The temperatures of the NMR samples were measured accurately to ±0.5 °C. The isotropic shifts are with respect to TMS as external standard, and downfield shifts are taken as positive. The ESR spectra of the samples in the glassy state were recorded at 4.2 K on an X-band variant ESR spectrometer working at 9.32 GHz, fitted with an Oxford variable-temperture liquid-helium-flow cryostat.

Results and Discussion

Figure 2 shows the ESR spectra recorded at 4.2 K for the diaquo and aquo hydroxo complexes intercalated in SDS micelles. The spectra are typically characteristic of high-spin ferric por-

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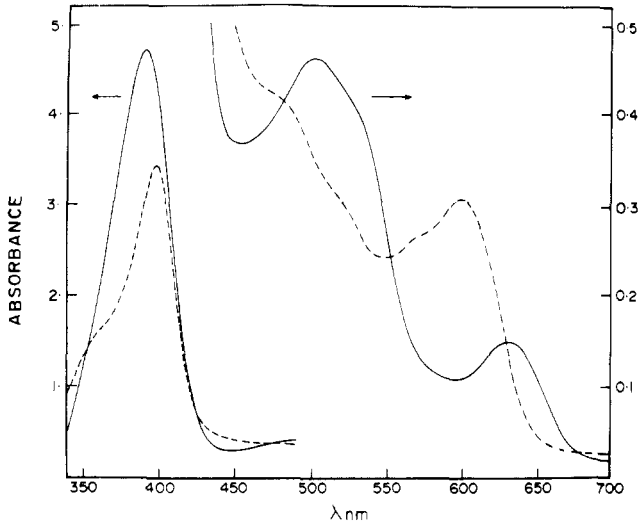

Figure 1. Electronic spectra of 5×10^{-5} M solutions of (a) $[Fe(PPIX)(H_2O)_2]^+$ (—) and (b) $Fe(PPIX)(H_2O)(OH)$ (---) in 5% aqueous sodium dodecyl sulfate micelles at 26 °C.

phyrins with a zero-field-split $^6A_1$ ground state and $M_s = \pm^1/_2$ lying lowest. For the diaquo complex the spectrum is axially symmetric ($g_\parallel = 2.05$, $g_\perp = 6.0$), while for the aquo hydroxo complex a small rhombic component is clearly seen ($g_1 = 6.1$, $g_2 = 5.6$, g_3 ($g_\parallel$) = 2.0). The rhombic g values observed in the aquo hydroxo complex are perhaps a consequence of the asymmetric nature of the axial ligands, which lowers the symmetry of the ligand field at the iron site. ESR data for a high-spin ferric heme complex intercalated in micelles have previously been reported at 77 K by Smith et al.,[16] who observed a characteristic $g = 6$ resonance.

The traces of the ^1H NMR spectra of the two complexes are shown in Figure 3. The assignment of the methyl resonances is based on reported spectra of similar high-spin ferric porphyrins.[6,17] The methyl isotropic proton shift (IPS) of the diaquo complex lies in the same region as that of the high-spin bis(dimethyl sulfoxide)iron(III) prophyrin complex,[4,6,18] while that of the aquo mono (hydroxo) complex lies in the region similar to that of hemoproteins in alkaline-pH solutions.[17] The broadness of the ring methyl resonances may arise from the hindered rotational tumbling motion of the heme inside the micelles. It is rather interesting that the positions and line widths of methyl resonances in these complexes are very similar to those observed in the aquo and hydroxo hemoproteins.[17] The temperature dependence of the IPS (corrected for diamagnetism by using the corresponding values for zinc(II) protoporphyrin) for the two complexes is shown in Figures 4 and 5.

The IPS of the high-spin ferric porphyrins is expected to vary with temperature as[19,20]

$$(\Delta H/H)_{IPS} = -\frac{35g\beta A}{12(\gamma_N/2\pi)kT} + \frac{28g^2\beta^2}{3k^2T^2}\left(\frac{3\cos^2\theta - 1}{r^3}\right)D \quad (1)$$

Here A is the hyperfine coupling constant and D the zero-field-splitting parameter. Other symbols have their usual meaning.[20,21] The first and second terms in the above equation are the contact and dipolar contributions to the IPS. Figures 4 and 5 show that

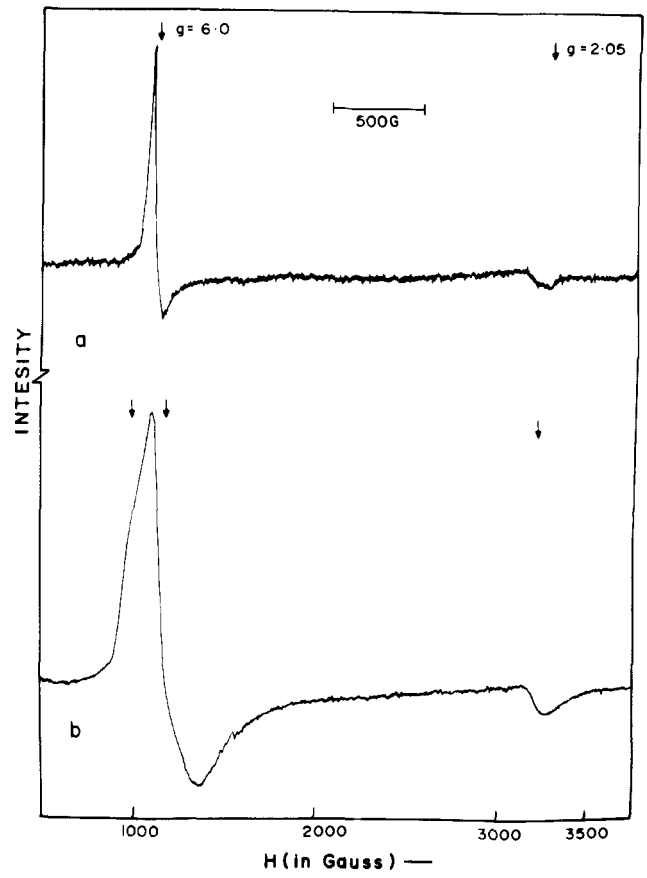

Figure 2. ESR spectra at 4.2 K of frozen solutions in 5% aqueous sodium dodecyl sulfate micelles: (a) $[Fe(PPIX)(H_2O)_2]^+$ (5 mM); (b) $Fe(PPIX)(H_2O)(OH)$ (1 mM).

the experimental IPS values in both compounds obey closely the Curie law of temperature dependence. A more rigorous test[9] of the same is shown as insets in Figures 4 and 5, where the plots of the experimental IPS $\times$ T vs $1/T$ give straight lines parallel to the temperature axis. This suggests that the contribution of the dipolar term to the IPS is negligibly small and that the IPS is predominantly contact in origin. The values of A for the methyl protons can then be obtained from the extrapolated intercepts of the IPS $\times$ T vs $1/T$ plots, which give the following values. Diaquo: 0.224, 0.218, 0.215, 0.199 MHz. Aquo hydroxo: 0.119, 0.142, 0.117, 0.117 MHz. These values agree quite well with those reported for high-spin six-coordinated ferric protoporphyrin complexes.[9,18] The unpaired spin density at the proton site in the diaquo complex is nearly double that in the aquo hydroxo complex. This is a reasonable result, since the positive charge on the iron is decreased substantially in the latter complex with the increased ligand basicity, giving rise to porphyrin–metal charge-transfer at higher energy (Figure 1). Similar correlations of ligand basicity with paramagnetic shifts were reported previously for low-spin ferric porphyrin complexes.[22]

It is easy to see that the data in Figures 4 and 5 are not sufficiently sensitive for the determination of the zero-field-splitting parameter (D), since the second term in eq 1 makes little contribution to the IPS. Equation 1 has, in the past, been used to determine D in high-spin ferric porphyrins,[20,23] but in those porphyrin complexes the contribution of the dipolar term is significant especially for some protons. Further, in the present measurements, the temperature range is very limited (290–340 K) because of the micellar system. Evidently, the data in this temperature range are not sufficiently sensitive for accurate calculation of the zero-field-splitting parameter.

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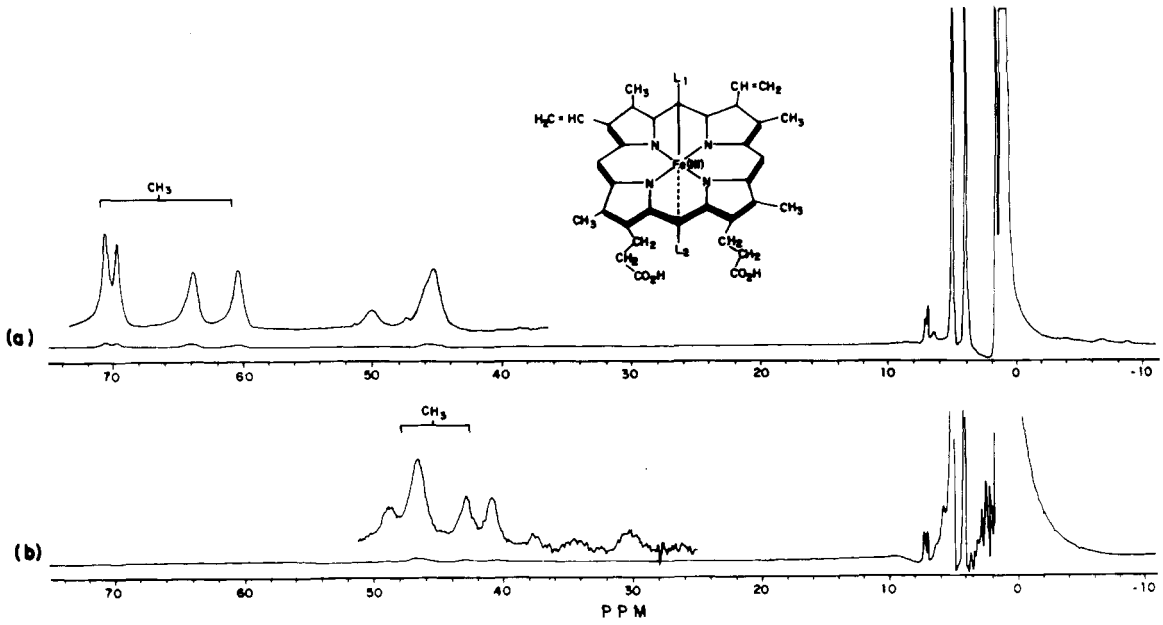

Figure 3. Proton NMR spectra (500 MHz) at 299 K of (a) $[Fe(PPIX)(H_2O)_2]^+$ (1 mM; at pH 2.6) and (b) $Fe(PPIX)(H_2O)(OH)$ (1 mM; at pH 12.0) in 5% aqueous sodium dodecyl sulfate micelles.

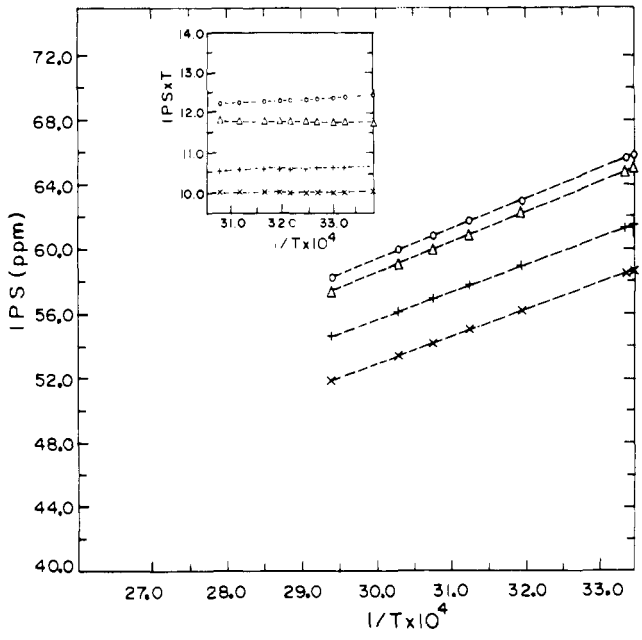

Figure 4. Temperature variation of the IPS of ring methyl proton resonances of the diaquo hemin complex in 5% aqueous SDS micelles (the IPS × T vs $1/T$ variations are shown in the inset).

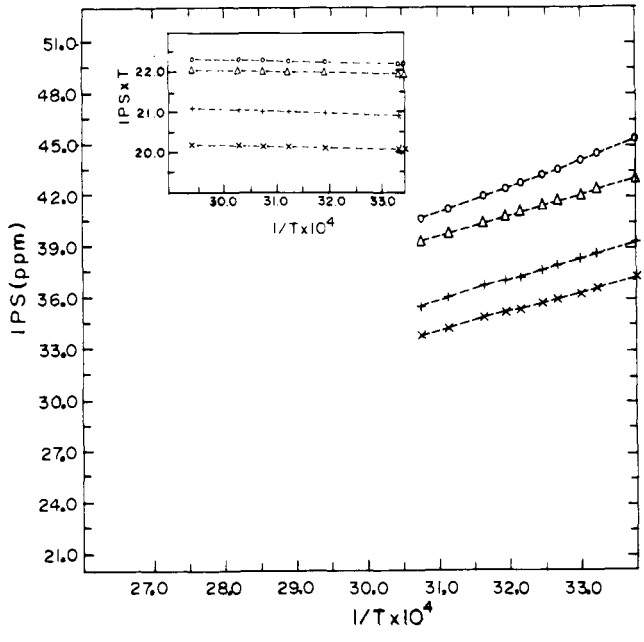

Figure 5. Temperature variation of the IPS of ring methyl proton resonances of the aquo hydroxo hemin complex in 5% aqueous SDS micelles (the IPS × T vs T variations are shown in the inset).

The electronic structure of iron(III) in the complexes discussed above is best described by a high-spin $^6A_1$ ground state with axial (in diaquo) and small rhombic (in aquo hydroxo) perturbations. Proton NMR studies have indicated that the dipolar shifts are very small for the porphyrin complexes intercalated in the micelles and that the paramagnetic shifts are mainly contact in origin.

Studies here clearly demonstrate that in a model compound small changes, such as deprotonation of an axial ligand, may greatly influence the electronic structure of iron in the porphyrin. Replacement of one of the axial water molecule by a hydroxide ion leads to a paramagnetic shift of 25 ppm upfield, the like of which is seen only in a protein environment.[17] The replacement of H_2O by OH^- in the hemoproteins is also accompanied by a change in their ground spin state, while no such change is observed in the micellar systems and other hydroxo ferric porphyrins.[17,24-26] Thus, though the effect of the micellar environment may not be as substantial as that of the protein, the hemes in aqueous micellar solutions seem to provide a possible model for the hemoproteins.

Acknowledgment. The NMR work reported was done at the 500-MHz FT NMR National Facility. We thank the staff members for their cooperation and Dr. Shedbalkar for help in the ESR work. O.K.M. also thanks TIFR for financial support and Professor P. K. Sharma (Gauhati University) for encouragement.

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