Thus, it seems that when excess substrate is present, the mesoxalato-bridged complex predominates, as indicated by its characteristic absorption spectrum. The fact that not all of the excess substrate in the solution is converted to carbon dioxide suggests that some of the mesoxalate may also decompose through another, nonredox process, before it has time to react with the regenerated dinuclear cobalt-dioxygen complex. We are now investigating the possibility that the substrate is undergoing simultaneous metal ion catalyzed decarboxylation to glyoxylic acid, a reaction type that has been observed in other systems with analogous substrates.

The rapid equilibration of the oxygen of dissolved CO_2 (as $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- \rightleftharpoons CO_3^{2-}$) with the solvent water molecules prevents the use of tracers from distinguishing between the two reaction sequences for oxygen atom transfer in the redox process. The ^{18}O label may go directly to water, and then to CO_2, or simultaneously to both water and CO_2, as

$$^{18}O_2 + {}^-OOC{-}\overset{\overset{O}{\|}}{C}{-}COO^- + 2H^+ \longrightarrow 2CO_2 + {}^{18}O{=}C{=}O + H_2O^{18}$$

or

$$H_2O + {}^{18}O_2 + {}^-OOC{-}\overset{\overset{O}{\|}}{C}{-}COO^- + 2H^+ \longrightarrow 3CO_2 + 2H_2O^{18}$$

$$CO_2 + H_2O^{18} \rightleftharpoons {}^{18}OCO + H_2O$$

While the tracer experiment does not distinguish between these two pathways, it does demonstrate that the oxidation products, CO_2 and H_2O, are derived from molecular oxygen through dioxygen complex formation and formation of the mixed-bridged (tribridged) intermediate, **3**.

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Stability and Characterization of Iron(III) and Iron(II) Heme Peptides Encapsulated in Aqueous Detergent Micelles: ^1H NMR and UV-Vis Spectroscopic Studies

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Proton NMR and optical spectral studies on a heme undecapeptide in aqueous SDS micelles at different pHs are reported. In a micellar solution the monodispersed heme peptide is found to be encapsulated inside the hydrophobic micellar cavity. pH dependence of the ferric heme peptide inside the SDS micelle shows equilibrium conversion from the diaqua ferric heme at low pH (~2) to the monoaqua monohistidine complex at pH ~6, which on further increase in pH undergoes deprotonation to give the monohistidine monohydroxo heme peptide complex. pH titration by optical spectroscopy shows a pK of 7.2, corresponding to the aqua (high-spin) ⇌ hydroxo (low-spin) transition. The cyano complex of the heme peptide in micellar solution shows a large spread of the heme methyl signals compared to the signals in the cyano protohemin complex, and its spectrum is similar to those of cyano heme proteins. Ferrous heme peptide complexes have also been stabilized inside the SDS micelles. Optical as well as NMR spectra of the ferrous complex suggest a five-coordination geometry. The line widths of the heme methyl resonances for these heme peptide complexes are much broader compared to those of the corresponding protoheme complexes but are similar to those found in the spectra of heme proteins.

1. Introduction

Heme peptides are obtained by enzymatic degradation of cytochrome *c*.[1,2] Pepsin degradation of cytochrome *c* gives a heme undecapeptide, in which the heme is bound to an eleven amino acid peptide chain, while tripsin digestion of cytochrome *c* gives a heme octapeptide, in which the heme is bound to an eight amino acid peptide chain of known sequence (Figure 1). Both the heme peptides contain an iron(III) porphyrin complex covalently linked to the peptide through two thioether linkages involving cysteine residues of the peptide and the α-carbons of the saturated vinyl groups on two pyrrole positions of the porphyrin ring (Figure 1). The N-terminal valine, lysine, and glutamic acid residues on the heme undecapeptide are absent in the heme octapeptide. The effects of the protein environment on the electronic structure and properties of the heme prosthetic group in heme proteins have been investigated by different techniques in substituted porphyrin model compounds.[3-5] Heme peptide complexes in aqueous solutions have therefore been studied by ^1H NMR,[6,7] circular dichroism,[8-10] and UV-visible[1,2,11] spectroscopy as models for cytochromes and other heme proteins. Further interest in the study of these heme peptides arises from the knowledge that they show peroxidatic activity[1,12-15] similar to that of heme peroxidases.

Previous studies[6,7] in aqueous solutions show that the spectroscopic properties of the heme peptides have general similarity to those of the cytochrome *c* complexes, but some subtle differences do exist. In the case of low-spin cyano as well as pyridine complexes of heme octapeptide, the NMR resonances of the heme methyl protons are quite sharp and their spread is smaller compared to the spectra of the corresponding cytochrome *c* complexes.[7] Moreover, NMR spectra of heme peptides in aqueous solutions show significant pH dependence,[6] associated with a spin-state transition similar to that observed in metmyoglobins. Tempera-

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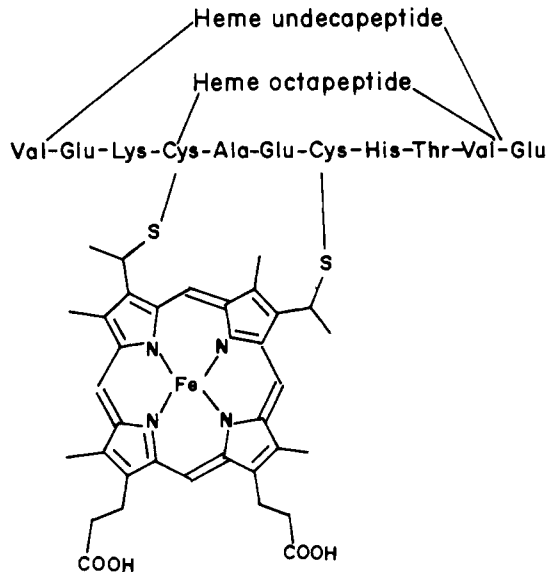

Figure 1. Schematic diagram of the heme peptide.

ture-dependent magnetic moment measurements[6] of heme octapeptide at different pHs suggest the presence of a spin-state equilibrium at both pH 7.4 and pH 12. pH-dependent magnetic moment measurements of the heme undecapeptide suggested that at very low pH the heme undecapeptide is a high-spin species and the spin transition is associated with a pK of 6.3 in 2% sodium dodecyl sulfate solution.[16]

The heme peptides have a strong tendency to form aggregates in simple aqueous solutions,[1,2,6-9,16] which makes the study of these species as models for heme proteins in simple aqueous solutions difficult. Circular dichroism studies show[8,9] that while in the heme undecapeptide the ϵ-amino group of lysine is involved in a head-to-tail type aggregation with ca. 16 Å separation between two heme centers in an aggregate, the aggregates in heme octapeptides are formed by a stacked conformation with a heme–heme distance of ~7 Å. It has been proposed[2,16] that the lysine residue in heme undecapeptide forms intermolecular metal–ligand bonds between two peptide molecules. Because of this strong tendency to aggregate, the heme peptides no longer behave as models for the heme-active sites in proteins, which essentially have one heme monomeric unit in the protein cavity.

It has been shown by earlier UV–vis[17-19] and NMR[20-22] studies that several free-base porphyrins and iron(III) porphyrin complexes can be solubilized and stabilized in the monomeric state inside the hydrophobic cavity of aqueous detergent micelles. These studies[17-22] also show that the hydrophobic environment of the micellar cavity mimics closely the protein environment in the heme cavity. It would therefore be interesting to investigate the properties of monomeric heme peptide complexes in a protein-like hydrophobic environment. The solubilization of these peptides in an aqueous detergent micellar solution may promote formation of monomeric species in the hydrophobic micellar cavity. Moreover, studies in aqueous micellar solutions can avoid the precipitation of the heme peptide at its isoelectric point.[16]

The present paper reports a detailed NMR and UV–visible spectral study of heme undecapeptide and heme octapeptide complexes (Figure 1) inside aqueous sodium dodecyl sulfate micelles at different oxidation and spin states with different axial ligands. The results have been used to determine the electronic properties of the heme peptides in micellar solutions in the monomeric state.

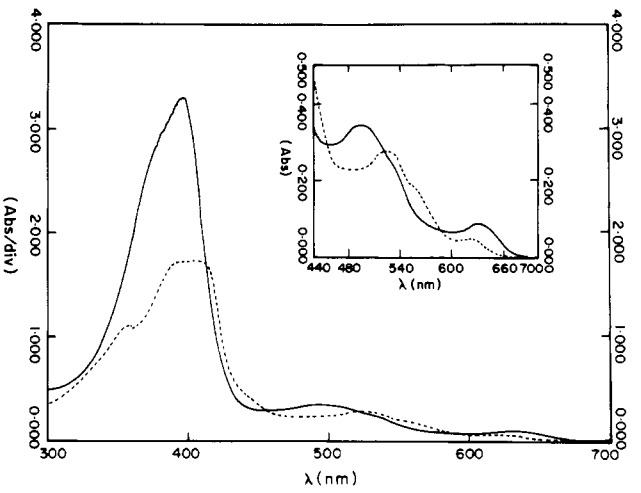

Figure 2. UV–visible spectra of 1.5×10^{-6} M heme undecapeptide in simple aqueous solution (---) and in 5% aqueous SDS micellar solution at ambient pH (—).

2. Experimental Section

The heme undecapeptide (microperoxidase-11 or MP11), the heme octapeptide (microperoxidase-8 or MP8), and sodium dodecyl sulfate (SDS) detergent were obtained from Sigma Chemical Co. The heme peptides were purified by passing through a gel filtration column (Sephadex G-50)[12,23] equilibrated with 1.0 M aqueous ammonia followed by dialyzing at neutral pH against water. The purified peptides were lyophilized and stored desiccated in the dark at ~0 °C. Micellar solutions were prepared by warming at 50 °C a 5% solution of the SDS detergent containing 0.1 N TMAB (tetra-*N*-methylammonium bromide) in aqueous solution. The heme peptide complexes were incorporated inside the micelles by adding an aliquot of the aqueous solution of the purified peptide in the aqueous micelles, and the resulting mixture was equilibrated at 50 °C for 1 h. The solution prepared in this way followed Beer's law over a range of $\sim 10^{-8}$ to 1×10^{-3} M, while the heme undecapeptide in aqueous solution in the absence of micelles did not obey Beer's law. This indicates the absence of any concentration-dependent self-association processes[24] in micelle-encapsulated heme peptides (see later). The pH of the solution was adjusted by a dilute NaOH or HNO_3 solution and measured by a digital pH meter with an accuracy of ±0.01 unit. The micellar solutions of heme peptides at adjusted pH were allowed to equilibrate for about 1 h at 50 °C before measurements were taken. The cyano complexes of the heme peptides were prepared by adding a concentrated micellar solution of NaCN to the heme peptide in micelles at pH 9.6 and again equilibrating at 40–50 °C. The ferrous heme peptide was prepared by adding a small amount (~50 μL) of a saturated solution of $Na_2S_2O_4$ to an alkaline (pH ~9.8) solution of the peptide in aqueous micelles.

NMR experiments were carried out on a Bruker 500-MHz FT NMR instrument. Peptide solutions 0.1–1 mM in SDS micelles in D_2O were used for the NMR studies. The pHs of the NMR samples were measured by using a microelectrode attached to the pH meter, and no solvent corrections were made to the pH values. For detecting the NMR signals from the heme peptide protons in micelles, it was necessary to simultaneously saturate all the proton signals of detergent and water by using a multiple-irradiation program.[20] Generally, 1000–5000 transients were acquired to give a good signal-to-noise ratio. The temperature of the NMR samples was varied and maintained (within ±0.5 °C) with an automated variable-temperature accessory. A positive sign is used for downfield shifts.

3. Results and Discussion

(i) Effect of Micelles on Aggregation of Heme Peptides. UV–visible spectra of the heme undecapeptide in the presence and absence of micellar solution are shown in Figure 2. The sharp increase in the absorbance in the Soret region of the heme peptide upon incorporation into the micelles indicates the monomeric

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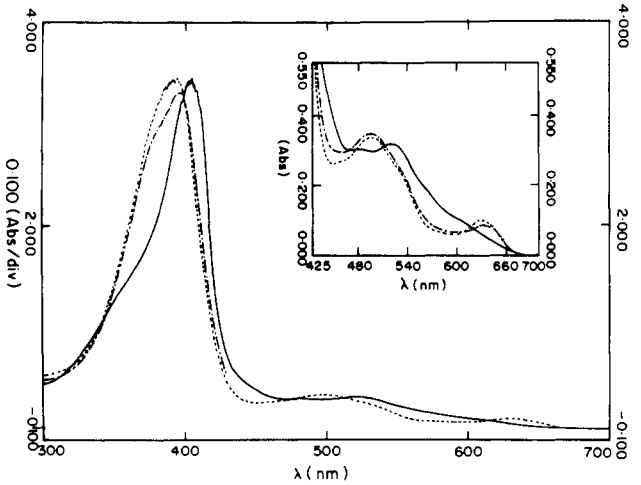

Figure 3. UV–visible spectra of 1.5×10^{-6} M heme undecapeptide in 5% aqueous SDS micellar solution at (i) pH 4.6 (-·-), (ii) pH 6.5 (---), and (iii) pH 9.8 (—).

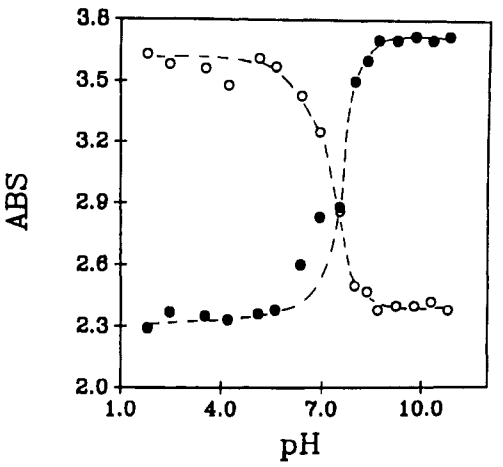

Figure 4. pH titration of the heme undecapeptide (ferric MP11) in 5% aqueous SDS micellar solution, showing the absorbance vs pH profile at 1.5×10^{-5} M peptide concentration: (a) 395 nm (○); (b) 408 nm (●).

nature of the heme peptide in micelles.[17,20] The histidine residue of both the heme peptides and the free NH_2 groups of the N-terminal valine and the lysine residues in MP11 (see Figure 1) are potent axial ligands on the heme iron. Previous studies[1] have proposed the existence of three proton-transfer equilibria in the ferric MP11 complex, which are assigned to (i) binding of histidine to the heme iron by replacing axial water from the diaqua complex (pK_a 3.4), (ii) replacement of the second water molecule by the α-NH_2 of valine (pK_a 5.8), and (iii) binding of the ϵ-amino group of lysine by replacement of the valine residue (pK_a 7.6). The aggregates of ferric MP11 in aqueous solution at ambient pH are formed by binding of the N-terminal valine or the ϵ-NH_2 group of the lysine residue of one peptide moiety to the iron of the other heme peptide.[9,15] One axial position on the iron is however always occupied above pH ~3 by the histidine residue in both the monomeric and the aggregated species. Figure 2 also shows a marked difference in the spectral features of the heme peptide in micellar solution compared to that in aqueous solution. The visible regions of the spectra (see inset in Figure 2) show a shift in the position of the band at 523 nm and an enhancement in intensity of the band at 630 nm upon incorporation of MP11 inside the micelles. This suggests that the nature of the coordination environments of the iron in ferric MP11 in the presence and absence of micelles are different. The nature of the UV–vis spectra of MP11 incorporated inside aqueous micelles at ambient pH is similar to that of metmyoglobin,[25] suggesting possible similarity in their coordination geometries, with the fifth and sixth axial sites being occupied by the histidine and water, respectively.

(ii) pH Dependence of Heme Peptides in Micelles. We have studied by UV–vis spectroscopy the proton-transfer equilibrium of ferric MP11 in aqueous SDS micellar solution over a pH range of 1.8–10.3 (Figure 3) and observed a pK_a at 7.2 (Figure 4), which corresponds to the aqua ⇌ hydroxo equilibrium in the ferric complex. Figure 5 shows the pH variation of NMR spectra of this peptide complex inside the SDS micelles. At very low pH (ca. 1.8), the paramagnetically shifted downfield regions of the spectra consist of several peaks in the range 55–80 ppm over a broad envelope (Figure 5a). As the pH is increased above ~2, a second set of resonances is observed to appear in this region (Figure 5b). On further increase in pH, the first set of resonances slowly decreases in intensity and finally disappears (pH 5–6), while the second set of resonances becomes well-defined in the 55–80 ppm range (Figure 5c). Increase in pH above ~7 causes rapid decrease in the intensity of these downfield signals, which finally disappear (at pH ≥10), and a new set of resonances in the 10–30 ppm range appears. These pH-dependent NMR spectra of the heme peptides were assigned by comparing the earlier assignments

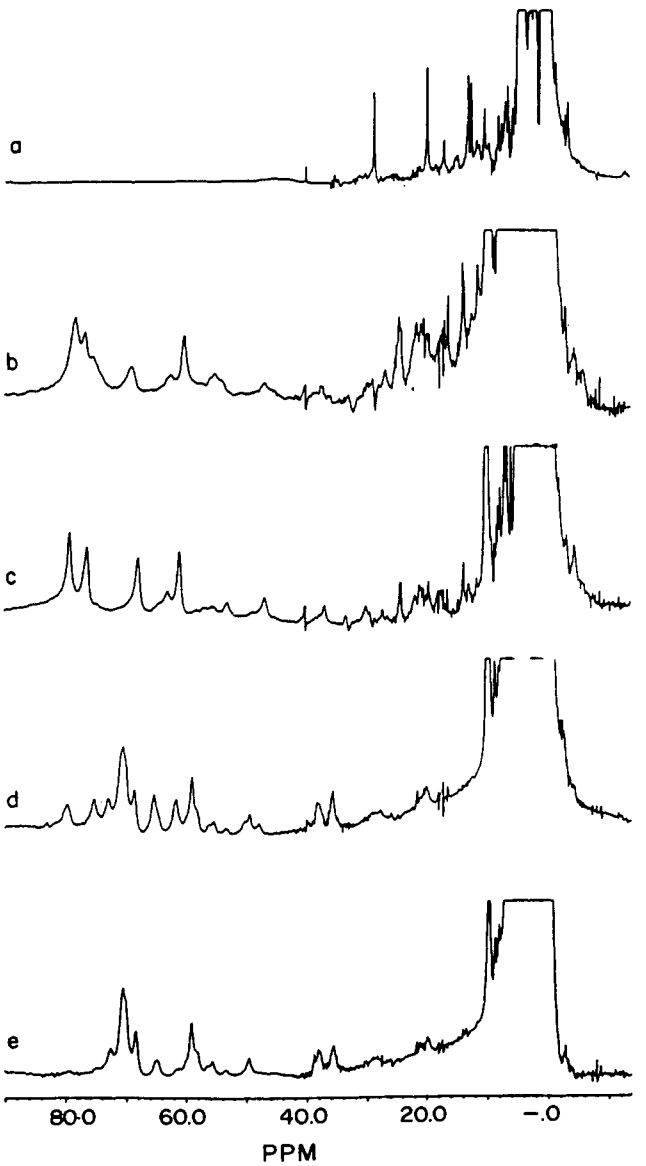

Figure 5. ^1H NMR spectra of the heme undecapeptide (ferric MP11) at pH (a) 9.8, (b) 6.9, (c) 5.6, (d) 2.4, and (e) 1.8.

of the NMR spectra of a heme protein and model ferric hemes[3,4] in aqueous solutions.

The set of downfield resonances in Figure 5 at the very low pH (<2) has close similarity to that of a high-spin diaqua hemin

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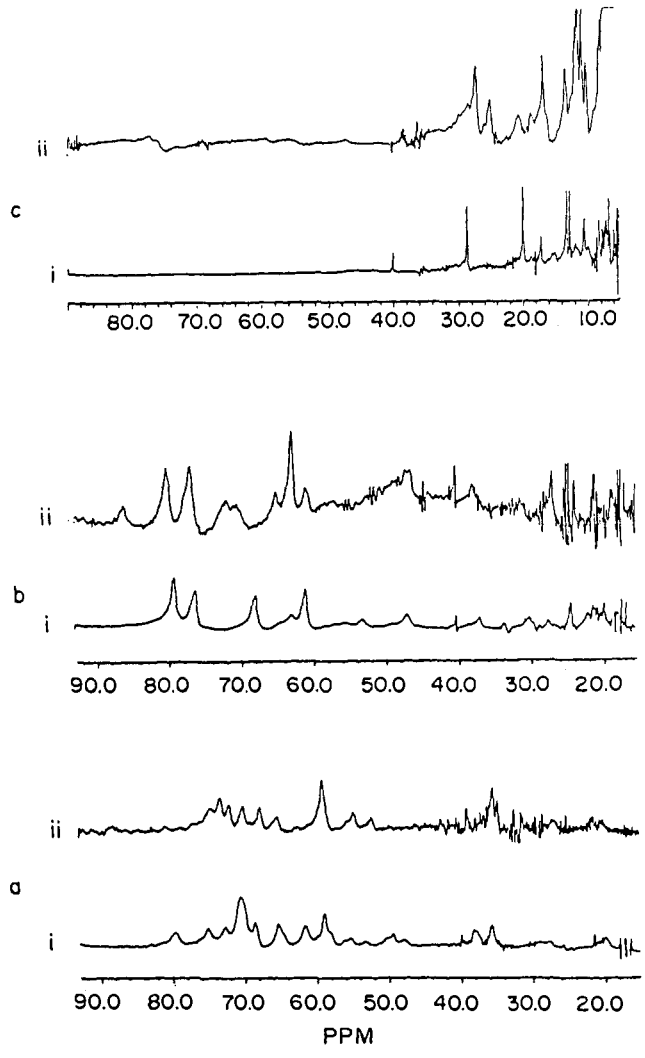

Figure 6. NMR spectra of (i) the heme undecapeptide (ferric MP11) and (ii) the heme octapeptide (ferric MP8) at pH (a) 2.4, (b) 5.6, and (c) 9.8.

complex in aqueous micelles, since diaqua hemin peptide complexes are likely to exist at such pH.[20] The other, set of downfield resonances appearing at a somewhat higher pH (pH 5–6) has similarity in pattern to the heme methyl signals in aqua metmyoglobins.[26] Between pH 2 and pH 5, these two species coexist in solution at equilibrium. The sharp signals at pH ~10 in the 10–30 ppm range (see Figure 5e) are characteristic of the heme methyl protons of low-spin ferric heme complexes[3] and may correspond to the hydroxo-ligated monohistidine adduct of MP11. In the intermediate pH range (7–10), an equilibrium between aqua ferric MP11 and hydroxo ferric MP11 exists in the micellar solution. The assignment of the paramagnetically shifted heme methyl signals and the axial ligation of MP11 was further confirmed by comparing its spectra with those of MP8 in aqueous micellar solutions (Figure 6). The close similarity in the spectra of MP8 to those of MP11 at all pHs suggests that both of the two peptides exist as high-spin diaqua species at very low pH and at pH above ~2 the histidine residue of the peptide chain is bound to iron in both cases. Moreover, since MP8 is known to exist as the monohydroxo species at pH ≥12,[6] a similar monohydroxo complex is expected to be present in the case of MP11 in aqueous micellar solutions at higher pH (pH ≥10). This is in conformity with our results of the UV–visible spectroscopic study.

The temperature dependence of the paramagnetic shift (IPS) of the heme methyl signals of ferric MP11 complexes at pH 5.6, 6.9, and 9.8 has been studied over the range 295–350 K. Figure

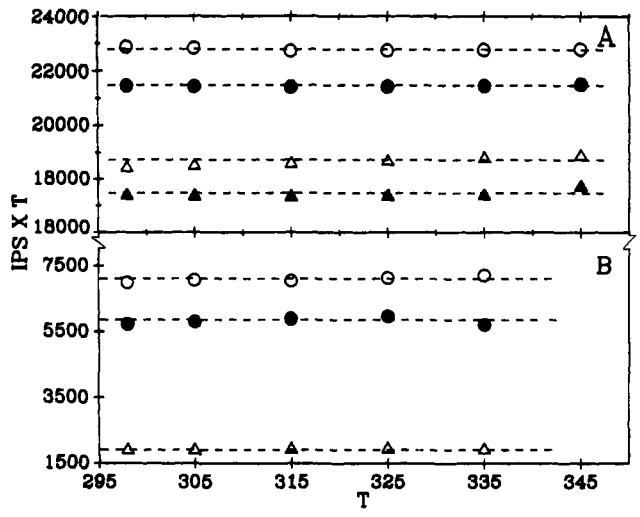

Figure 7. Temperature variation of the paramagnetic shift (the IPS × T vs T variations) of the ring methyl proton resonances of (A) the high-spin monohistidine monoaqua ferric MP11 complex in aqueous SDS micelles at pH 5.6 and (B) the low-spin ferric MP11 complex in micelles at pH 9.8.

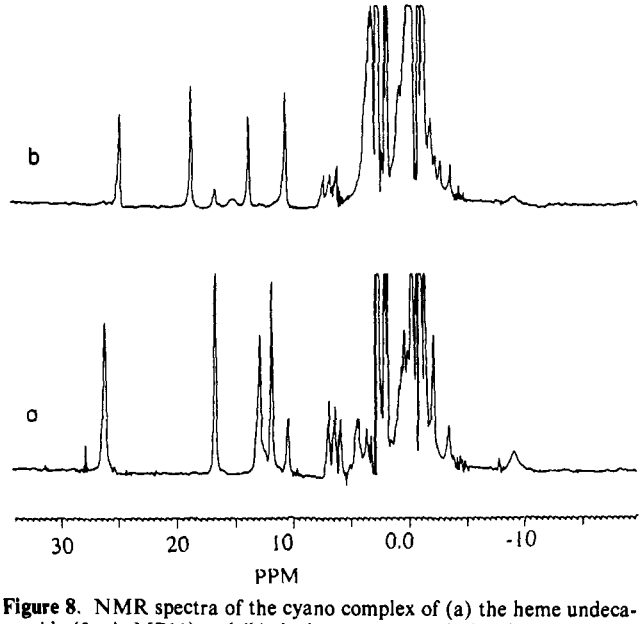

Figure 8. NMR spectra of the cyano complex of (a) the heme undecapeptide (ferric MP11) and (b) the heme octapeptide (ferric MP8) in 5% SDS aqueous micellar solution at room temperature.

7A shows that the paramagnetic shifts of heme protons in ferric MP11 at pH 5.6 (at which the monohistidine monoaqua complex is the major species in the micellar solution) closely obey the Curie law of temperature dependence, indicating that the contribution of the dipolar term to the paramagnetic shift (IPS) is negligibly small and the paramagnetic shift in the experimental temperature range is predominantly contact in origin.[3,27,28] The room-temperature magnetic moments (5.3 ± 0.2 and 5.6 ± 0.2 μ_B at pH 1.8 and 5.6, respectively) are consistent with its expected high-spin formulation. Since MP11 inside micelles is predominantly monomeric, the magnetic moment values for the complexes at pH 1.8 and 5.6 suggest that both the diaqua and the monohistidine monoaqua ferric MP11 complexes are high-spin ($S = {}^5/_2$) species. At pH ≥10, the complex in micelles has a magnetic moment of ~2.1 μ_B at room temperature, which is consistent with an assignment of a low-spin ground electronic state ($S = {}^1/_2$). The temperature dependence of heme methyl resonances at pH ≃10 also obeys the Curie law (Figure 7B).

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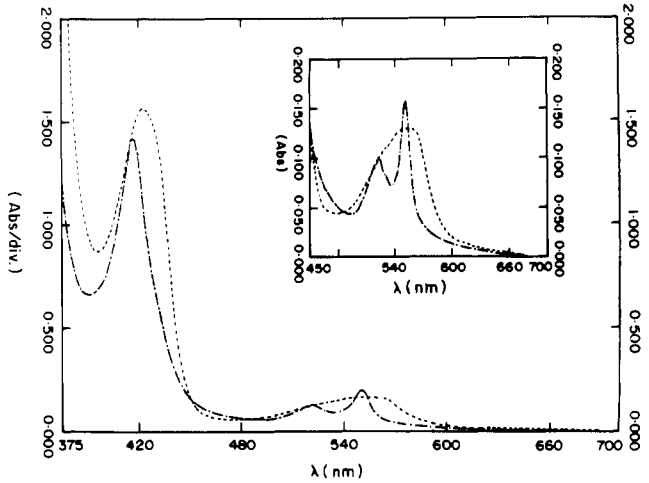

Figure 9. UV–visible spectra of the ferrous heme undecapeptide (ferrous MP11) in (a) aqueous solution in the absence of micelles (–·–) and (b) 5% aqueous SDS micellar solution (---).

(iii) Low-Spin Cyanide Complexes of Heme Peptides in Micelles. The NMR spectra of the cyano complexes of heme peptides in aqueous SDS micellar solution are shown in Figure 8. The assignments of the heme methyl protons for the cyano complexes are similar to those in the absence of micelles.[7] The paramagnetic shifts of the cyano heme peptide complexes lie in the region similar to those of other low-spin ferric porphyrin complexes.[3] Figure 8 shows that the nature of the NMR spectra of the cyano ferric MP11 complex is very similar to that of MP8, indicating similarity in the electronic structures of the two cyano complexes. Moreover, the heme methyl signals in low-spin cyano complexes of the heme peptides are found to be quite sharp, and like those of other low-spin hemin complexes, the line widths of these signals are also almost independent of the micellar environment.[21] The paramagnetic shifts of the heme methyl protons obey the Curie law in the temperature range of our study, which indicates that the second-order Zeeman contribution to the paramagnetic shift may be much smaller in cyano heme peptides compared to the cyano complexes of other ferric hemes.[3,12]

(iv) Ferrous Heme Peptides inside Micelles. Ferrous complexes of the heme peptide were obtained by dithionite reduction of the monohistidine monohydroxo complex (at pH ~9.8). The estimation of Fe(II) by the pyridine hemochrome method[3] showed that the reduction was ≥95% complete. The ferrous heme peptide obtained in this way was found to be stable for 2–3 days in the dark and in the absence of oxygen. Figure 9 shows the UV–visible spectra of the ferrous MP11 complex in aqueous and in SDS micellar solutions. The absorption spectrum of the ferrous MP11 complex in the absence of micelles (Figure 9a) is characteristic of six-coordinate ferrous hemes (hemochromes) with λ_{max} at 555, 524, and 415 nm.[29] On the other hand, the optical spectrum of the ferrous complex incorporated in aqueous micelles (Figure 9b) is quite different from the hemochrome spectra and is identical with that of the five-coordinate ferrous deoxy myoglobin complex with λ_{max} at ~560 and ~430 nm.[29] Ferrous MP11 in aqueous micellar solution may thus have a five-coordinate ferrous heme with the axial coordination site probably occupied by the histidine molecule, similar to the case of deoxy hemoglobins and deoxy myoglobins.[25,29] Treatment of the ferrous peptide complex in micellar solution with $K_3[Fe(CN)_6]$ slowly regenerates the ferric heme peptide with spectra similar to that in Figure 3 (ferric heme peptide inside micelles, almost 100% regeneration). Moreover, since ferrous MP11 is prepared by dithionite reduction of the ferric complex at pH ~9.8, the original ferric peptide species present at this pH must be the monohistidine monohydroxo complex in micellar solution. In the absence of micelles, the axial coordination of the ferric heme consists of two amino ligands (histidine and either the valine or the lysine residue); hence, on reduction, it gives the six-coordinate low-spin hemochrome species in simple aqueous solution.

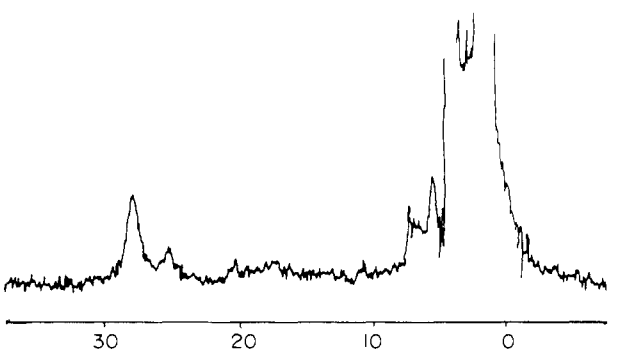

Figure 10. NMR spectrum of ferrous MP11 in SDS at 303 K.

The room-temperature NMR spectrum of the ferrous complex of MP11 in an aqueous SDS solution is shown in Figure 10. The paramagnetically shifted heme proton resonances in the ferrous peptide complex are broad and appear in the range 20–30 ppm. Further, the spectrum of the ferrous MP11 complex closely resembles that reported for five-coordinate ferrous hemes in aqueous CTAB solution,[29] suggesting a possible similarity in their coordination geometries. The heme proton resonances of ferrous MP11 are however more downfield shifted than those of the ferrous protoheme complex[29] but are closer to those in deoxymyoglobins[30,29] with respect to line widths and shifts. The room-temperature solution magnetic moment of ferrous MP11 was measured to be 4.5 ± 0.2 μ_B, which confirms its high-spin ($S = 2$) character. By analogy to the similar ferrous complexes of five-coordinate natural hemes, the paramagnetic shift in these complexes is also expected to be predominantly contact in origin with a σ-spin delocalization through the antibonding $d_{x^2-y^2}$ metal orbital.[29] The similarity in the line widths and spectral range of the heme protons in these ferrous complexes to the ferrous heme proteins suggests that, because of the larger size of the heme peptide, the mobility of the molecule becomes more restricted inside the micelles compared to the case of the protoporphyrin complex in micellar solutions, where the spectrum is better resolved.[29]

(v) Line Widths and Spread of the Heme Methyl Signals inside Micelles. We note from Figures 5 and 6 that in the high-spin ferric heme peptide complexes the line widths of the heme methyl protons are significantly large in the micellar solutions (~600 Hz) compared to those reported in aqueous solutions[3,20] (~200 Hz). In the low-spin ferric heme peptides, the change in line widths of heme methyl protons in micelles is not as dramatic as in the high-spin complexes (see Figure 8). The heme proton signals in ferrous complexes of heme peptides are quite broad and could not be well resolved. These changes in line widths of the ring methyl protons of different heme complexes in aqueous micellar solutions have general similarity to those of heme proteins.[30,31]

The broadening of the heme proton resonances is mainly due to increase in the total correlation time (τ_c) of the heme complex inside micelles. The increase in line width, $\Delta\sigma_{obs}$, of a paramagnetic complex inside micelles compared to that in a simple aqueous or nonaqueous solution is given as

$$\Delta\sigma_{obs} = [\sigma]_M - [\sigma]_F \simeq S(S+1)K_1(f([\tau_c]_M) - f([\tau_c]_F)) \quad (1)$$

where σ's are the line widths with the subscripts M and F referring to the micellar solution and simple aqueous or nonaqueous solution, respectively. K_1 is constant for a given proton in the porphyrin molecule and is given by the Solomon–Bolembergen equation.[29] $f(\tau_c)_M$ and $f(\tau_c)_F$ are the functions of correlation time, τ_c, where subscripts M and F correspond to the micellar solution and simple aqueous or nonaqueous solution, respectively. The increase in

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paramagnetic line width of the NMR signal of a given proton directly depends on the electron spin (S) of the paramagnetic atom. Apart from this, in the case of high-spin iron(III) as well as high-spin iron(II) hemes, the Curie spin effect on the line width also becomes quite significant at high magnetic field (e.g. 11.7 T, corresponding to 500 MHz in our case),[31] where the line width increases with the magnetic field and τ_c. The observed total line width thus increases as the total correlation time τ_c of the proton increases. The total correlation time is given by the electron spin relaxation time (τ_s) and the rotational correlation time (τ_r) as $1/\tau_c = 1/\tau_s + 1/\tau_r$. In the case of high-spin Fe(III) complexes ($S = {}^5/_2$), the electron spin correlation time τ_s is $\sim 10^{-10}$ to $\sim 10^{-11}$ s,[3,20] and that for low-spin Fe(III) complexes ($S = {}^1/_2$) is $\sim 10^{-12}$ s.[21] The typical value of τ_c for paramagnetic iron(II) porphyrins[3] is $\sim 10^{-13}$ s. The rotational correlation time (τ_r) of the heme complexes in simple solution is ca. 10^{-11} s,[3] while τ_r for the micelle protons is ca. 10^{-9} to 10^{-10} s[29] (which can be approximated as that for heme protons inside the micellar cavity). This increase in τ_r in micelles increases the $[\tau_c]_M$, and thus the line widths of the heme protons in micelles are expected to be larger than those of the protons in simple nonaqueous solutions. Since the τ_s values for high-spin Fe(III) complexes are comparable to the τ_r values, so the $[\tau_c]_M$ values for those systems are increased more significantly by the changes in the value of τ_r, leading to a large increase in the line width inside micelles.[31] However, τ_s for the low-spin Fe(III) species is much lower than τ_r in micelles; thus, changes in τ_r due to increase in viscosity inside the micellar cavity cause only a small increase in the total correlation time (τ_c) for these complexes, and since the total electron spin in low-spin iron is small ($S = {}^1/_2$), the increase in line widths of the heme proton signals in micellar solutions of low-spin heme complexes is much smaller compared to that in the case of high-spin Fe(III) porphyrin ($S = {}^5/_2$) complexes. Similarly, the increase in τ_r in micellar solutions only slightly affects the total correlation time, $[\tau_c]_M$, of ferrous hemes in micelles, but because of the large value of the unpaired electron spin ($S = 2$) and in the large magnetic field (11.7 T, large Curie spin contribution), the increase in the paramagnetic line widths of the heme protons in ferrous heme complexes is found to be quite significant. The value of electron spin relaxation time τ_s in heme proteins (and hence the NMR line width) varies considerably due to the variations in the anisotropic interactions of the heme moiety inside the protein cavity.[32] These interactions include H-bonding with the nearby protein residues, axial ligation with histidine and/or water, and possible formation of a salt bridge involving the propionic acid groups. Thus, the NMR line widths in heme proteins may increase also due to the increase in τ_s inside the heme cavity. However, this effect is expected to be small in the present system of micellar solutions, where increase in τ_r is more significant. The presence of a large peptide side chain is probably responsible for the larger rotational correlation time compared to the case of the protoheme complexes.[29] Thus, the heme methyl signals in the heme peptide complexes are relatively broader compared to those in the corresponding protoheme species. The ferrous complexes of heme peptides show an extremely broad spectrum mainly because of similar reasons.

The spread of the heme methyl signals has been a subject of extensive study in ferric as well as ferrous hemes and heme proteins.[4,29] It has been proposed[4,29] that the spread of the heme methyl proton signals increases as the symmetry of the porphyrin complex decreases. The spread of the heme ring methyl resonances in the high-spin monohistidine monoaqua ferric heme peptide inside aqueous micelles is larger ($\sim$19 ppm) compared to that for the analogous [Fe(PP)(DMSO)$_2$] in DMSO solution (8.8 ppm).[3] Moreover, the spread of the heme methyl proton signals in [Fe(PPIX)(H$_2$O)$_2$]$^+$ is also smaller (11 ppm) than that of the heme peptide complex. In the case of aqua metmyoglobin, the heme methyl spread is $\sim$40 ppm,[4] which is much larger even compared to that of monohistidine monoaqua ferric heme peptides in micelles. The spread of the heme methyl signals in cyano metmyoglobin[4] and ferricytochrome c cyanide[5] is comparable to that for cyano heme peptides in aqueous micellar solutions ($\sim$14.3 ppm; see Figure 8). The peptide side chain in heme peptide complexes may cause a significant increase in the in-plane asymmetry of these complexes compared to similar complexes of protoheme, which suggests the possible role of heme plane asymmetry in increasing the spread of the methyl signals.

4. Conclusions

The NMR and optical spectral studies on heme peptide complexes in aqueous SDS micelles suggested that the hydrophobic parts of the heme peptide complexes preferentially enter the micellar cavity as monomeric species. The pH dependence of NMR and UV–visible spectra gave a pK_a of 7.2, corresponding to the aqua–hydroxo transition in the ferric heme undecapeptide. At very low pH, the sole species was found to be the high-spin diaqua ferric heme peptide, and as the pH was increased to $\sim$5.6 the high-spin monohistidine monoaquo complex was stabilized. At extremely high pH ($\sim$9.8), the sole species was the low-spin hydroxo histidine complex. The pH dependence of the heme peptide complex in the range $\sim$6–10 was very similar to that in metheme proteins, where the aqua $\rightleftharpoons$ hydroxo transition is associated with a high-spin $\rightleftharpoons$ low-spin transition. The low-spin cyano complex of the heme undecapeptide in aqueous SDS solution was found to be predominantly low-spin in nature, and the effect of the structural asymmetry introduced by the peptide side chain in the heme undecapeptide and heme octapeptide complexes was manifested in the spread and nature of the spectra of the heme methyl protons. The ferrous complexes of heme peptides have been stabilized and characterized in aqueous SDS micellar solution. These ferrous heme peptides are five-coordinated high-spin species with the histidine residue of the peptide chain axially coordinated. The line widths of the heme methyl resonances were found to be similar to those of proteins. These studies indicate that heme peptides in aqueous micellar solution present good models for the heme cavity in both ferric and ferrous heme proteins.

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