Octaethylporphyrinate Haem Complexes encapsulated inside Aqueous Detergent Micelles: a Spectroscopic Study

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NMR and optical spectral studies have been made for monomeric aqua and hydroxo complexes of iron(III) 2,3,7,8,12,13,17,18-octaethylporphyrin in aqueous sodium dodecyl sulphate (sds) detergent micellar solutions. Both the aqua and hydroxo complexes are found to be high spin ($S = \frac{5}{2}$). The pK_a for the aqua $\rightleftharpoons$ hydroxo equilibrium was found to be 4.8. Iron(II) complexes of octaethylporphyrin in different spin states and co-ordination geometries have also been stabilized in aqueous micellar solution. The detailed structural disposition of the porphyrin complex and the conformation of the surfactant molecules inside the micellar cavity have also been investigated using ^{13}C NMR spectra of aqueous sds detergent micelles containing the complex. The average values of the distances of the different carbon centres in a surfactant molecule from the iron obtained from spin–lattice relaxation enhancement measurements were fitted to a spherical model of micellar structure to obtain the average configuration of the detergent molecules and the position of the complex inside the micelles.

Biomimetics of the protein pocket in haemoproteins are of considerable challenge and continued interest.[1–5] The electronic properties and biochemical functions of the haem prosthetic group in haemoproteins have been found to depend on the nature of the protein environment surrounding the haem group.[4–6] Previous studies [2–6] have suggested that the diversity in the physicochemical properties of the haem complexes in various proteins stems largely from the hydrophobic interactions and steric effects imparted on the haem moiety by the protein cavity.

Model studies on different metalloporphyrin complexes have proposed that the hydrophobicity of the protein pocket has a major influence on the properties of the haem prosthetic group.[4,7] Moreover, porphyrins and metalloporphyrin complexes show a strong tendency to aggregation in simple aqueous and non-aqueous solutions.[1,8,9] Recent studies in our laboratory [10–14] have shown that natural haem complexes can be stabilized in monomeric forms inside a hydrophobic micellar cavity with different spin and oxidation states of iron. The electronic structure and co-ordination geometry of the natural haem complexes encapsulated inside micelles have been studied using NMR [10–13] and other spectroscopic techniques.[14–17] These studies show that such complexes with different spin and oxidation states of iron and different axial ligands, encapsulated inside aqueous detergent micelles, provide very good models for the corresponding haemoproteins.

The effects of haem paramagnetism on the NMR spectra of micelles have been used to determine the structural disposition of the haem complex inside the micellar cavity.[18] Studies of the proton and ^{13}C NMR spin–lattice relaxation time enhancement showed that iron(III) protoporphyrin complexes encapsulated inside aqueous sodium dodecyl sulphate (sds) micellar solutions reside ≈ 6 Å inside the micellar Stern surface.[18] The structural disposition of these complexes suggested that the polar propionic acid side chains are directed towards the micellar Stern layer.[18] Because of the presence of these propionic acid groups in protoporphyrin complexes, the iron(III) haem moiety cannot go to the centre of the micellar core.

In order to get a deeper insight on micellar interactions with the iron porphyrin complexes one needs to study synthetic porphyrin complexes in micellar environments. Iron(III) octaethylporphyrin complexes (Fig. 1) are well known for their strong tendency to form aggregates in solutions.[1,8,9] Moreover, since these species are not soluble in water, studies have so far been limited mainly to non-aqueous solvents.[1] Thus, studies on these species in a non-aggregated state in aqueous micellar solution would aid our understanding of their chemistry in a protein-like environment. Furthermore, these synthetic complexes, unlike the protoporphyrin analogues, do not contain polar propionic acid groups, and hence it is expected that the position of the haem moiety inside the micellar cavity would be different in this case.

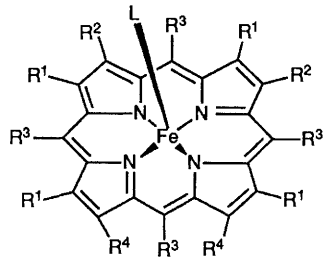

Fig. 1 Schematic structure of five-co-ordinated iron(III) porphyrin complexes: (*a*) octaethylporphyrin; $R^1 = R^2 = R^4 = Et$, $R^3 = H$; (*b*) protoporphyrin IX, $R^1 = Me$, $R^2 = CH{=}CH_2$, $R^4 = CH_2CH_2CO_2H$, $R^3 = H$; L = iron(III) axial ligand (Cl, H_2O, OH, *etc.*)

In this study the stabilization of various iron complexes of octaethylporphyrin (Fig. 1) in aqueous sds micellar solutions has been investigated. NMR and optical spectral methods coupled with solution magnetic moment measurements have been used to study the electronic properties of these synthetic haem complexes in micellar solution at various spin and oxidation states and in different co-ordination geometries of the metal ion and the results compared with those for the corresponding natural analogue. NMR spin–lattice relaxation rate enhancements of the surfactant atoms have been used to determine the structural disposition of the symmetrical iron octaethylporphyrin molecule and the average configuration of the detergent molecules inside the micellar cavity.

Experimental

2,3,7,8,12,13,17,18-Octaethylporphyrin (H_2oep) and sds (sod-

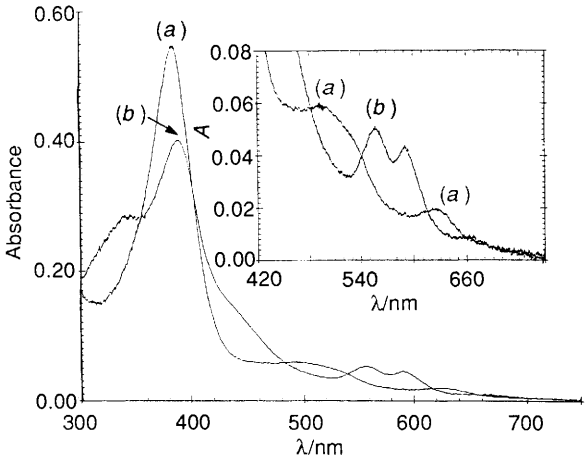

Fig. 2 Optical spectra of 7×10^{-6} mol dm^{-3} iron(III) octaethylporphyrin in 5% aqueous sds micelles: (*a*) at pH 2.1, the aqua complex, $[Fe(oep)(H_2O)]^+X^-$ ($X = \frac{1}{2}SO_4$), and (*b*) at pH 9.8, the hydroxo complex [Fe(oep)(OH)]

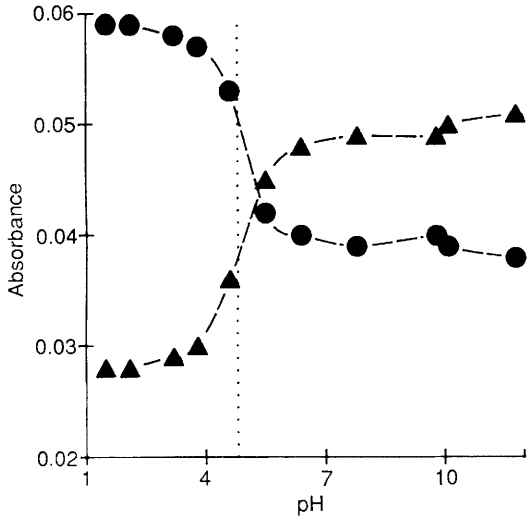

Fig. 3 Absorbance *vs.* pH curve for the aqua $\rightleftharpoons$ hydroxo equilibrium for 7×10^{-6} mol dm^{-3} iron(III) octaethylporphyrin in 5% aqueous sds micelles at $\lambda = 494$ (●) and 555.6 nm (▲); $pK_a = 4.8$

ium dodecyl sulphate) were obtained from Sigma Chemicals. The purity of these chemicals was checked by analytical methods.[19] The complex $[\{Fe(oep)\}_2O]$ was prepared from the ligand using the known procedure.[20] Micellar solutions were prepared[10–18] by warming at 50 °C a 5% solution of the detergent containing 0.1 mol dm^{-3} tetramethylammonium bromide in an aqueous solution at pH ≈ 9–10. The iron(III) porphyrin complex was incorporated inside the micelles using the following procedure. Solid samples of $[\{Fe(oep)\}_2O]$ were dissolved in the minimum amount of acetone, added to the aqueous micelles and finally acetone was evaporated by boiling the solution. The resulting micellar solution of iron(III) octaethylporphyrin was allowed to equilibrate at 40–50 °C for *ca.* 30 min and the completion of micelle encapsulation of the haem complex was checked by monitoring optical spectra (see later). The pH of the iron(III) porphyrin complex was adjusted by slow addition of small amounts of dilute H_2SO_4 to its micellar solution and again equilibrating at ≈40 °C for 30 min. Micellar solutions of octaethylporphyrin complexes prepared in this way follow Beer's law over a range of $\approx 5 \times 10^{-8}$ to $\approx 1 \times 10^{-3}$ mol dm^{-3}, indicating that the micelle-encapsulated haem is predominantly monomeric in nature. Micellar solutions of monomeric iron(III) octaethylporphyrin complexes were also prepared from [Fe(oep)Cl] using the above procedure. The two preparations, however, gave identical results.

Optical spectral studies were carried out on a Shimadzu UV-2100 ultraviolet–visible spectrophotometer at *ca.* 25 °C. The pH of the micellar solution was determined with an accuracy of ±0.001 unit using an Orion Research microprocessor-controlled digital pH meter. No solvent corrections were made to the pH. NMR studies were carried out using deuteriated solvents over a haem concentration range from 0.001 to 0.1 mmol dm^{-3}, on a Bruker 500 MHz FT-NMR instrument. In order to observe the paramagnetically shifted porphyrin protons, the signals of the micelle and water protons needed to be saturated. A microprogram for multi-frequency irradiation was used to irradiate all the micelle peaks and water signal. About 4000–5000 transients were acquired over a spectral width of ≈45 kHz with 8192 data points to get a good signal-to-noise ratio in the proton NMR spectra of the iron porphyrin complexes. The proton chemical shifts reported are with respect to $SiMe_4$ as an external standard and downfield shifts are taken as positive. The NMR spin–lattice relaxation times were determined by an inversion-recovery technique using a 180–τ–90 pulse sequence.[21] For inversion-recovery experiments a relaxation delay of ≈5 T_1 for complete relaxation followed by a variable delay τ (between the 180 and 90 pulses) was applied. The error in evaluation of T_{1obs} was estimated to be <5%. Proton NMR experiments were carried out at 500.137 MHz with 15.5 μs 90° pulse width, ^{13}C NMR at 125.77 MHz with 8.3 μs as the 90° pulse width. For ^{13}C T_1 experiments broad-band decoupling of ^1H was used. Solution magnetic moments in the micellar solutions were determined by the Evans method[22] adapted for proteins.

Results and Discussion

Aqua and Hydroxo iron(III) *Haem Complexes inside Micelles.*—Fig. 2 shows ultraviolet–visible spectra of iron(III) octaethylporphyrin at different pH. The pink complex obtained in the micelles at pH 2.1 [Fig. 2(*a*)] shows the following λ_{max} values (nm) and molar absorption coefficients ($\times 10^5$ dm^3 mol^{-1} cm^{-1}): 383.2 (0.8), 494.0 (0.08) and 622.4 (0.028). This complex was identified as the aqua complex $[Fe(oep)(H_2O)]^+$. The green hydroxo complex [Fe(oep)(OH)] [Fig. 2(*b*)] was obtained at pH ≥ 9.8, and by the following λ_{max} values (nm) and molar absorption coefficients ($\times 10^5$ dm^3 mol^{-1} cm^{-1}): 338.8 (0.4, sh), 392.0 (0.57), 555.6 (0.07) and 590.6 (0.06). Since octaethylporphyrin complexes are insoluble in water, both of these complexes cannot be prepared in the absence of micelles. Previous attempts to prepare the hydroxo complex of oep resulted in the formation of the μ-oxo dimer in non-aqueous solutions.[23] The [Fe(oep)(OH)] obtained in aqueous micellar solutions shows marked spectral differences from the μ-oxo dimer formed in the absence of micelles. This further supports the monomeric nature of the iron(III) oep complex inside the sds micelles. The general nature of the spectra of these aqua and hydroxo species resembles those of the corresponding natural iron(III) haem complexes[10] in micelles and the metmyoglobins.[6] The pK_a for the aqua $\rightleftharpoons$ hydroxo equilibrium was determined by ultraviolet–visible spectral study of the iron(III) octaethylporphyrin complex in sds solutions (Fig. 3). This equilibrium corresponds to a single proton transfer with a pK_a of 4.8 (Fig. 3). The pK_a for this equilibrium in sds micelles for the iron(III) complex of protoporphyrin IX (3,7,12,17,tetramethyl-8,13-divinylporphyrin-2,18-dipropanoic acid) is 5.6.[16] The aqua complex is cationic (or zwitterionic) while the hydroxo complex is neutral, and thus the stabilization of the hydroxo species occurs more in the hydrophobic micellar cavity compared to the aqua complex, and thereby the aqua $\rightleftharpoons$ hydroxo equilibrium favours the formation of the hydroxo complex in micellar solution at a given pH compared to that in the absence of micelles. However, in the case of the natural haem complexes, although this effect is the most prominent, the protonation or deprotonation of the propionic acid side chains which are directed towards the micellar surface may cause partial charge

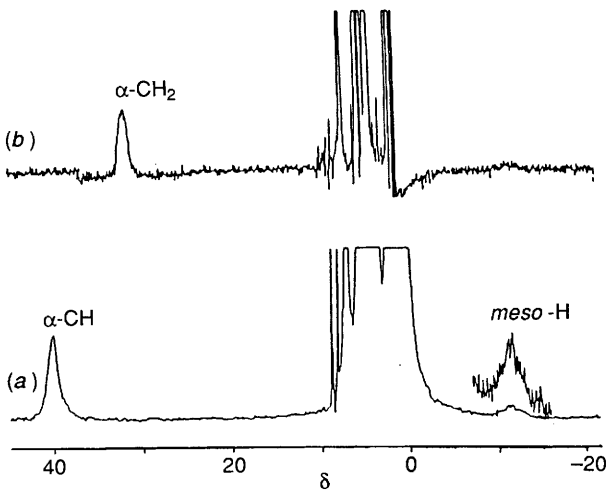

Fig. 4 NMR spectra of 0.1 mmol dm^{-3} iron(III) octaethylporphyrin complexes in 5% aqueous sds micelles: (*a*) $[Fe(oep)(H_2O)]^+X^-$ ($X = \frac{1}{2}SO_4$) at pH 2.1 and (*b*) [Fe(oep)(OH)] at pH 9.8

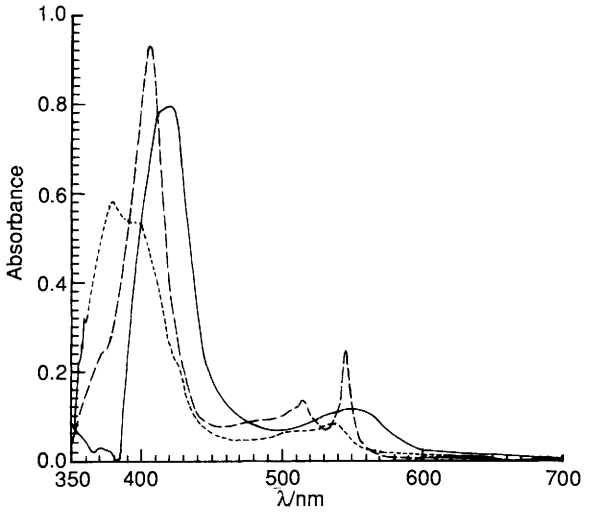

Fig. 5 Optical spectra of $\approx 7 \times 10^{-6}$ mol dm^{-3} iron(II) octaethylporphyrin complexes in 5% aqueous sds micelles (*a*) with no axial ligand (four-co-ordinate) (- - - -) (*b*) with axial pyridine (six-co-ordinate) (— — —) and (*c*) with axial 2-methylimidazole (five-co-ordinate (——)

neutrality in case of both the hydroxo and aqua complexes of natural haems. Thus the pK_a of natural haem complexes, although significantly decreased in micellar solutions[14] from that in the absence of micelles, is however, expected to be slightly larger than for these synthetic haems.

Fig. 4 shows the proton NMR spectra of the aqua and hydroxo complexes of iron(III) octaethylporphyrin in 5% aqueous sds solution. The close similarity in the nature of the NMR spectra of the aqua and hydroxo species with known high-spin iron(III) octaethylporphyrin complexes[1,2] suggests that these species also have high-spin ($S = \frac{5}{2}$) electronic ground states. Assignment of the resonances is based on reported spectra of similar high-spin iron(III) octaethylporphyrin complexes.[1,2,24,25] The downfield-shifted broad signal corresponds to the α-CH_2 of oep. The *meso*-H signal of the aqua complex appears at ≈ 10 ppm upfield [Fig. 4(*a*)], however in case of the hydroxo complex [Fig. 4(*b*)] the *meso*-H signal could not be resolved possibly due to line broadening. The α-CH_2 resonance of the aqua complex appears at more downfield than that of the hydroxo species. A similar change in the position of the haem proton resonances was previously observed for natural haem complexes.[10] Solution magnetic moment measurements gave $\mu_{obs} = 5.8 \pm 0.2$ β for the aqua and 5.7 ± 0.2 β for the hydroxo complex at room temperature. The paramagnetic shift for the high-spin iron(III) haem complex is given by equation (1) where

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

A is the hyperfine coupling constant, β is the Bohr magneton and *D* is the zero-field-splitting parameter. Other symbols have their usual meanings.[10] The first and second terms in equation (1) are the contact and dipolar contributions to the paramagnetic shift.[1,10] The observed paramagnetic shift for both complexes was found to obey the Curie law indicating a pure electronic ground state with a large contact contribution to the shift.[1] The observed magnetic moments and temperature dependence of the paramagnetic shift are consistent with both complexes having predominantly high-spin ($^6A_1$) ground states.[10]

The position of the *meso*-H NMR signal for iron(III) porphyrin complexes has previously been used to identify the co-ordination number around the iron centre in haem complexes.[2,25] It has been shown[25] that five-co-ordinated high-spin iron(III) haem complexes show a highly upfield (≈ 55 ppm) *meso*-H signal {*e.g.* [Fe(oep)Cl]}, while for the corresponding six-co-ordinated species this signal appears downfield {$[Fe(oep)(MeOH)_2]^+$}. Theoretical calculations[26] on high-spin iron(III) haems, however, showed that the hyperfine coupling constant for the *meso*-H decreases in magnitude upon increasing co-ordination number from five to six. Moreover, the dipolar interaction also was shown to decrease in magnitude upon increase in co-ordination number.[26] The change in the resonance position actually depends on the change in the electron-spin density at the *meso*-H site and on the position of iron with respect to the haem plane. In the present case, the appearance of *meso*-H signal in the upfield region (at -10 ppm) suggests that the iron atom in the aqua complex resides above the mean porphyrin plane and one water molecule is axially bound to the iron forming a five-co-ordinated aqua complex, $[Fe(oep)(H_2O)]^+$. Owing to the higher hydrophobicity of the oep ring, the octaethylporphyrin complex in aqueous detergent micelles is more stable inside the micellar cavity compared to the corresponding natural haems. This suggests that the micellar environment around the haem in aqueous sds solutions of octaethylporphyrin complexes is more hydrophobic than is the case with corresponding natural analogues. In case of the aqua complexes of natural iron(III) haems, two H_2O molecules have been proposed to be axially ligated to the six-co-ordinated iron.[10] The protohaem molecule resides close to the micellar Stern surface,[18] and hence a six-co-ordination completed by two axial water molecules may be possible. In case of the aqua complex of oep the iron atom binds to only one water molecule, indicating that the concentration of water around the haem is very small, *i.e.* the oep complex inside the micelles, unlike the natural haems, may be embedded deep inside the hydrophobic core. Since, the *meso*-H signal for the hydroxo complex is not identified in the present system, the co-ordination number of iron in the hydroxo species could not be assigned. However, since the aqua and hydroxo complexes are in fast proton-transfer equilibrium in the present case, I propose a five-co-ordination geometry for the [Fe(oep)(OH)] complex inside sds micelles. Thus, although six-co-ordination geometry is proposed for the aqua and hydroxo complexes of iron(III) protoporphyrin in sds micellar solution, the corresponding octaethylporphyrin complexes, owing to the high hydrophobic nature of the oep, moiety may exist as five-co-ordinated species inside the micellar cavity.

Reduction of the [Fe(oep)(OH)] complex in sds solution by sodium dithionite (50 μl saturated aqueous solution) gave the iron(II) complex of octaethylporphyrin inside micelles (Fig. 5). The estimation of the amount of Fe^{II} by the pyridine

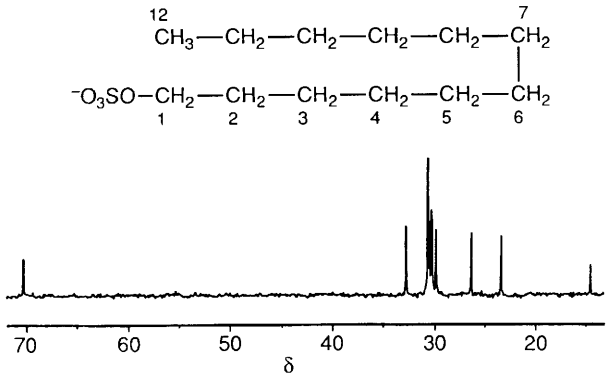

Fig. 6 Carbon-13 NMR spectra of 5% aqueous sds micelles containing 0.1 mmol dm^{-3} [Fe(oep)(OH)], see text for assignment [18]

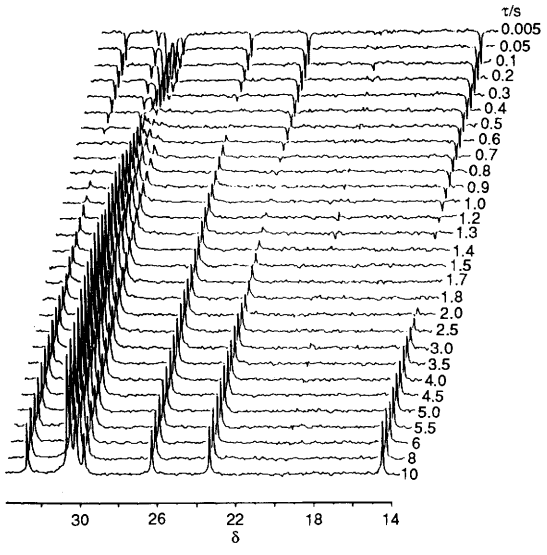

Fig. 7 Carbon-13 NMR inversion-recovery spectra of the surfactant molecules containing 0.1 mmol dm^{-3} [Fe(oep)(OH)] in 5% aqueous sds micelles

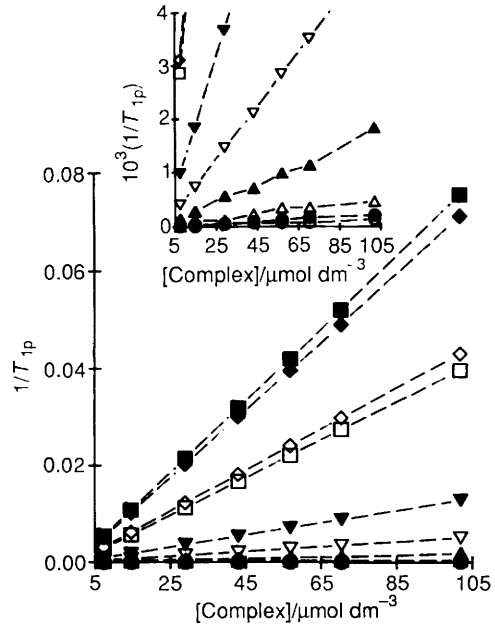

Fig. 8 Plots of the paramagnetic relaxation enhancement $1/T_{1p}$ *vs.* concentration of [Fe(oep)(OH)] for C^1 (○), C^2 (●), $C^{3,4}$ (△), $C^{5,6}$ (▲), C^7 (▽), C^8 (▼), C^9 (◇), C^{10} (◆), C^{11} (□) and C^{12} (■)

haemochrome method [19] showed that the reduction is more than 90% complete. The optical spectrum of this iron(II) complex [Fig. 5(*a*)] clearly resembles those of four-co-ordinated iron(II) haem complexes and is very similar to that observed for the iron(II) protohaem complex [12] in $NMe_3(C_{16}H_{33})Br$. The appearance of a split Soret band is typical of the four-co-ordinate iron(II) porphyrins.[12] The micelle-encapsulated bis-(pyridine)iron(II) octaethylporphyrin [Fig. 5(*b*)] was prepared by addition of pyridine to the four-co-ordinate iron(II) complex in aqueous sds solution. Similar addition of excess of 2-methylimidazole (2-mim) gave the mono ligated adduct, [Fe(oep)(2-mim)], in the micelle [Fig. 5(*c*)]. The similarity of its spectrum with that of the reported 2-mim adduct of iron(II) protohaem complex in aqueous $NMe_3(C_{16}H_{33})Br$ suggests five-co-ordination for this species. The solution magnetic moment of the four-co-ordinate iron(II) octaethylporphyrin was found to be 3.6 ± 0.2 β which suggests an intermediate spin ($S = 1$) ground electronic state for this species. The magnetic moment of the five-co-ordinate 2-mim adduct is 4.8 ± 0.2 β which is characteristic of high-spin ($S = 2$) iron(II) porphyrin complexes. The pyridine adduct was found to be diamagnetic with zero magnetic moment. Iron(II) complexes of natural porphyrins cannot be stabilized in aqueous sds micelles, and much larger aqueous $NMe_3(C_{16}H_{33})Br$ micelles [12,13] were needed to stabilize various iron(II) protohaems. This indicates that the environment around the octaethylporphyrin complex in aqueous sds is more hydrophobic than that of the analogous natural haem species, suggesting that the oep moiety might be embedded much deeper inside the micellar hydrophobic cavity than is the protoporphyrin molecule.

Structure of the Micellar Cavity.—The critical micellar concentration (c.m.c.) of the sds micelles containing the iron(III) octaethylporphyrin complex (5×10^{-5} mol dm^{-3}) was determined from the concentration dependence of the ^1H NMR shifts of the sds protons [27] to be 9.1 mmol dm^{-3}. The concentration dependence of the conductivity of the surfactant solution gave a similar c.m.c. value.[27] The c.m.c. was found to be independent of the iron(III) haem concentration (in the range ≈1 μmol dm^{-3} to 0.1 mmol dm^{-3}). Moreover, its value in the absence of the paramagnetic haem complex is similar to that in its presence.[18,27] This indicates that the presence of iron(III) haems does not have any significant effect on the thermodynamics of the formation of micelles.

Previous studies have demonstrated that paramagnetic enhancement in the ^{13}C NMR spin–lattice relaxation time can be very successfully used to deduce the position of the haem group inside the micellar cavity as well as the average conformation of the detergent molecules inside the micelles.[18] Fig. 6 shows the ^{13}C NMR spectra of sds in presence of 0.1 mmol dm^{-3} [Fe(oep)(OH)]. The resonances are quite well resolved and can easily be monitored to deduce the structural features of the micelles. Assignments of ^{13}C NMR spectra of aqueous sds micelles are documented in the literature.[18–29] The ^{13}C signal at δ ≈ 70 corresponds to the CH_2 group directly attached to the polar head (C^1) and that at δ 14.4 arises from the CH_3 tail group (C^{12}). The peaks in the δ 35–23 region correspond to the aliphatic chain CH_2 groups. Comparison of the ^{13}C NMR spectra of sds carbons in the presence of different concentrations of the iron(III) porphyrin complex showed that there is very small change in the chemical shifts of the micellar carbons in the presence of the paramagnetic complexes (0.1 mmol dm^{-3}). This apparent insensitivity [18] in the chemical shift of the surfactant carbons to the presence of paramagnetic complexes may be due to the large values of the chemical shifts in the ^{13}C NMR spectra of the micelle carbons (total range of spectra δ 0–70) compared to the paramagnetic dipolar shifts (≤1 ppm).

Fig. 7 shows a typical ^{13}C NMR inversion-recovery experiment for sds containing [Fe(oep)(OH)] at pH 9.8. Although the presence of paramagnetic iron(III) haem complexes was found to affect the ^{13}C chemical shifts of sds micelles to a very

small extent, the spin–lattice relaxation rates for sds carbons show a significant systematic variation with increasing concentration of the complex [Fe(oep)(OH)] (Fig. 8). The experimental relaxation rates of the carbon nuclei increase with the concentration of the complex, and the relaxation enhancement for the C^5–C^{12} carbons was found to be more than that for the other carbon centres in the sds molecule. A similar trend in relaxation enhancement with paramagnetic concentration was also found with the iron(III) complexes of protoporphyrin inside sds micellar solution.[18] The relaxation enhancements (T_{1p}) by paramagnetic ions are given[30] by equation (2) where T_{1obs} and

$$\frac{1}{T_{1p}} = \frac{1}{T_{1obs}} - \frac{1}{T_{1A}} = \frac{1}{T''_{1A}} + \frac{P_m}{T_{1M} + \tau_M} \quad (2)$$

T_{1A} are the spin–lattice relaxation times observed in the presence and absence of iron(III) octaethylporphyrin respectively, T''_{1A} is the outer-sphere contribution to the relaxation, T_{1M} is the paramagnetic relaxation time of the micellar carbon with iron(III) haem complex inside the micelles, τ_M is the rate of exchange of haem between the inside and outside of the micelles and P_m is the total mole fraction of micellar nuclei interacting with a paramagnetic centre. The value of P_m is given by the ratio of the total number of detergent molecules interacting with the iron(III) haem to the total number of detergent molecules present in the solution. The outer-sphere contribution to the relaxation rates ($1/T''_{1A}$) has been shown to be very small ($< 6\%$) under the present experimental condition. In the fast-exchange limit (*i.e.* $T_{1M} \gg \tau_M$), the paramagnetic relaxation enhancement[30] can be used to determine T_{1M} by neglecting τ_M in equation (2).

The overall shape of the micelles depends on the total detergent concentration and ionic strength of the solution.[27] At very high detergent concentrations and high ionic strengths micelles tend to become non-spherical in shape leading to drastic changes in the micellar phase properties.[31] Previous studies suggested that at sds concentrations less than 0.3 mol dm^{-3} the micelles are predominantly spherical.[28] Earlier studies[32] with sds micelles containing the dye Orange OT showed an aggregation number of 131; sds micelles containing the iron porphyrin molecule (which is approximately similar in size to Orange OT) have been suggested to have a similar aggregation number.[17] Moreover, in the present case, since the c.m.c is almost independent of the addition of paramagnetic iron(III) haem complexes, the overall shape of the sds micelles can be assumed to remain spherical[33,34] with an average aggregation number, $\bar{N}$, of 131 per micelle. Thus the iron(III) octaethylporphyrin complex encapsulated inside a detergent micelle can be considered to be interacting with $\bar{N}$ detergent molecules.

The equilibrium associated with the encapsulation of iron(III) haem inside micelles is $L_{\bar{N}} + M \rightleftharpoons ML_{\bar{N}}$ and considering the single-step model for micelle formation,[35] equation (2) can be written as (3) where $[M]_0$ is the total concentration of

$$\frac{1}{T_{1p}} = \frac{\bar{N}[L_{\bar{N}}][M]_0}{[L_{\bar{N}}](\bar{N})[L_{\bar{N}}] + \text{c.m.c.}} \cdot \frac{1}{T_{1M}} \quad (3)$$

paramagnetic heme and $[L_{\bar{N}}]$ is the concentration of micelles. Synthetic octaethylporphyrin complexes are insoluble in water thus its iron(III) complex in the present case remains encapsulated inside the micelles, indicating that the value of the dissociation constant, K_D, for the micelle–haem adduct is negligibly small (≈ 0). The value of T_{1M} was calculated using equation (3), where $\bar{N}$ was taken as 131 and c.m.c as 9.1 mmol dm^{-3}. The T_{1M} obtained in this way was used to evaluate the structural properties of the micelles encapsulating the porphyrin complex.

The spin–lattice relaxation time, T_{1M}, is related to the distance (R_{IS}) of the relaxing nucleus (carbon) from the paramagnetic ion by the Solomon–Bloembargen equation[30,36] which in the present case can be written as (4) where γ_I is the

$$\frac{1}{T_{1M}} = \frac{2}{5} \cdot \frac{\gamma_I^2 g^2 S(S+1)\beta^2}{R_{IS}^6} \cdot f(\tau_c) \quad (4)$$

nuclear gyromagnetic ratio and S the total electronic spin of the paramagnetic complex. The interaction between surfactant molecule and paramagnetic iron is predominantly dipolar in nature[18] hence the contact contribution to the relaxation has been neglected in equation (4). Moreover, the major contribution to the total correlation time τ_c arises from the electron-spin relaxation time, T_{1e} of the iron(III), thus $f(\tau_c)$ in the present case can be replaced by T_{1e}.[1] The value of T_{1e} used was 3.4×10^{-10} s, for high-spin [Fe(oep)(OH)].[37]

The average configuration of the detergent molecules and the relative position of the iron centre inside the micelles can now be deduced using the distance parameter R_{IS} obtained from equation (4). Neutron scattering studies[38] showed that the radius of the hydrocarbon core in the pure sds micelles is 17.40 Å with an aggregation number of 74. Incorporation of the large porphyrin moiety inside the micelles is expected to affect the size of the hydrophobic core. The increase in the aggregation number (131) of the sds micelles is suggestive of such a situation. A non-spherical (ellipsoidal) shape of the micelles may be possible for this type of system, however because of the rapid fluctuations and exchange processes involved in the micellar structure the statistically averaged shape of the micelles encapsulating the haem would remain almost spherical and the structural information derivable from the present study will correspond only to the static, time-averaged, spherical structure. Moreover, since the thermodynamics of micellization, *viz.* the c.m.c., does not show any significant change due to the presence of the haem complex in the present experimental range of concentrations, we can assume that the micellar phase does not change drastically in its shape. Since the volume of the micelles is proportional to the aggregation number, the radius of the micellar core containing the haem complexes would be 21 Å.[18] The aliphatic chain of the detergent molecules has conformational fluctuations inside the micellar cavity[39] and the relative position of the paramagnetic haem with respect to the surfactant nuclei inside the micellar cavity is also not constant for every surfactant unit. Owing to these conformational differences the absolute distances of a particular atom in a detergent molecule from the metal centre are different for different detergent units in the micellar cavity, although only a single peak corresponding to an atom in all amphiphile units in the micelles was seen owing to very fast dynamic fluctuations in the micellar cavity.

The distance (R_{IS}) determined by relaxation enhancement studies thus is a measure of a statistically averaged distance parameter. Assuming a smooth structure for the micellar surface[36] with a uniform density distribution, the average distances of the micellar carbon and the iron centre of the porphyrin complex were evaluated by assuming a spherical micellar model and using equation (5)[18,28] where R_a and R_M

$$\frac{1}{R_{IS}^6} = \frac{R_a^2 + R_M^2}{(R_a^2 - R_M^2)^4} \quad (5)$$

($R_a \neq R_M$) are the average distances of the micelle atom and the metal atom from the centre of the micelle (Fig. 9). The total radius of the hydrophobic core of the spherical micelles containing the haem complex was taken as 21 Å and the value of R_M was determined by putting $R_a = 21$ Å for the outermost carbon (C^1) atom. The distance of the iron(III) ion from the centre of the micelle was found to be ≤ 1 Å for [Fe(oep)(OH)]. This further confirms that the iron(III) haem resides inside the micellar cavity. The radius of the complex is ≈ 6 Å.[40] Thus, unlike natural protohaem complexes, the iron(III) octaethylporphyrin in micellar solution remains deeply embedded near the centre inside the hydrophobic region of the micelles. The results shown in Fig. 9 suggest a structural disposition of the detergent molecules and the haem complex inside the micelles

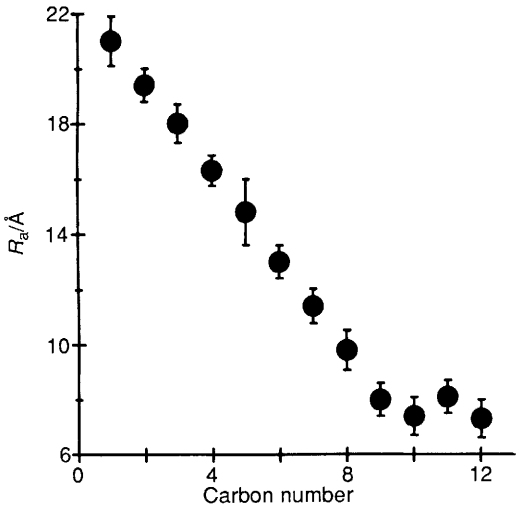

Fig. 9 Plot of the average distance of the micellar carbon (R_a) from the centre of the micelles *vs.* the atom number in the surfactant (numbering starts from the hydrophilic head group carbon)

whereby the aliphatic tail of the detergent molecule is randomly distributed inside the hydrophobic micellar core in such a way that the average distance of the non-polar terminal carbons (C^8–C^{12}) from the paramagnetic iron centre remains almost constant. This indicates a radial distribution of these terminal carbons near the core of the micelles. A similar structure was deduced for sds micelles encapsulating iron(III) protoporphyrin complexes.[18] The average conformation of the surfactant molecules in the sds micelles determined by Cabane[28] using an extra-micellar paramagnetic probe is also in accord with the present results. This further shows that the presence of haem moiety does not cause any significant change in the average structure of the micelles.

Conclusion

Aqua and hydroxo iron(III) complexes of octaethylporphyrin have been stabilized in aqueous detergent micelles as monomeric species. Octaethylporphyrin complexes are insoluble in water; thus solubilization in micellar solutions provides a very easy means to study these complexes in an aqueous protein-like environment. The electronic structure of these synthetic iron(III) haems showed that both the aqua and the hydroxo complexes have high-spin ($S = \frac{5}{2}$) ground states. NMR studies suggest that only one water molecule is axially bound to the iron centre in the aqua iron(III) complex of octaethylporphyrin in micelles. The stabilization of iron(III) complexes of octaethylporphyrin, such as four-co-ordinate intermediate-spin ($S = 1$), five-co-ordinate high-spin ($S = 2$) and six-co-ordinate diamagnetic species inside aqueous sds solution suggests that the porphyrin molecule is embedded deep inside the micellar core. The detailed structural disposition of the iron(III) octaethylporphyrin complex inside aqueous detergent micelles has been determined from the ^{13}C nuclear spin–lattice relaxation of the surfactant atoms. The results suggest that the porphyrin complexes are embedded near the centre inside the micellar Stern layer. The structural parameters were deduced from the average distances between the surfactant carbons and the haem iron centre, which gave only a statistically averaged picture of the micelles rather than the exact structure of the aggregate. The average conformation of the surfactant molecules does not show any significant variation due to the presence of the porphyrin complex inside the micelles.

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