Low-spin Iron(III) Porphyrins encapsulated in Aqueous Detergent Micelles: Proton- and Nitrogen-15 Nuclear Magnetic Resonance Studies

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Variable temperature ^1H and ^{15}N n.m.r. studies have been made on low-spin iron(III) protoporphyrin IX (3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionic acid) complexes, $[FeL(CN)_2]^-$ and [FeL(py)(CN)], encapsulated in aqueous detergent micelles. The haem is shown to be monodispersed in the micellar solution, which ensures the paramagnetic shift is free from the effect of aggregation. The effects of the hydrophobic interactions of the micellar cavity on the linewidth, and the magnitude and spread of the chemical shift, are discussed and compared with those in absence of micelles and in haemoproteins. The temperature dependence of the paramagnetic shift of the haem methyl proton shows deviation from Curie's law, which is discussed in terms of the electronic structure of haem. The effect of the size and hydrophobicity of the micelles is further shown in the ^{15}N n.m.r. signal of the bound CN^-, where the downfield shift follows the trend: absence of detergent < sodium dodecyl sulphate < Triton X-100 < hexadecyltrimethylammonium bromide.

Low-spin iron(III) complexes of synthetic and natural porphyrins have extensively been studied as models for the haem prosthetic group in low-spin haemoproteins.[1–6] In particular the paramagnetic isotropic proton n.m.r. shift (i.p.s.) of the haem has been recognised as a sensitive probe for study of the electronic structure of iron in such low-spin complexes in solution.[6–10] Previous proton n.m.r. studies on low-spin dicyano and pyridinecyano complexes of iron(III) porphyrins have however shown that the haems have a strong tendency to aggregate in solution,[11,12] which complicates the n.m.r. results owing to intermolecular interactions. Such aggregation is encountered both in organic and aqueous solutions.

One of the methods to de-aggregate the haems in solution is to incorporate them in a micellar cavity.[13–18] Previous spectroscopic studies have shown that low-spin dicyanide and pyridinecyanide complexes of iron(III) protoporphyrin IX (3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionic acid), [FeL(Cl)], can easily be solubilised in aqueous detergent micelles, and that they exist in monomeric form in the micelles.[13–15] Our own recent visible spectroscopic[16] and n.m.r.[17] studies show that the haems, in general, are monodispersed and stable when incorporated in aqueous detergent micelles such as sds (sodium dodecyl sulphate), ctab (hexadecyltrimethylammonium bromide), and TX-100 (Triton X-100). The proton n.m.r. spectra of high-spin iron(III) haems such as the diaqua and aquahydroxo species in aqueous sds micelles show that the haem methyl isotropic shift and its linewidth are independent of concentration in the range 0.1—1.5 mmol dm^{-3},[17] which indicates absence of aggregation of haems inside the micelles. Similar results have been observed for different iron(III) haems in aqueous ctab micellar solutions with haem concentration in the range 1—2 mmol dm^{-3}.[18] Minch and LaMar[15b] had earlier carried out a proton n.m.r. study on haemin dicyanide in aqueous ctab solution as a function of ctab concentration. They observed the formation of an adduct of haemin dicyanide with ctab at low detergent concentrations. However, their highest detergent concentration ($\leqslant$ 0.066 mol dm^{-3}) was about seven times the haemin concentration. Since the aggregation number of ctab is $\approx$61, the effective micellar concentration was less than that of the haemin complex, leading to a possibility of the presence of some haemin complex outside the micelle.

The present paper reports the temperature-dependent ^1H and ^{15}N n.m.r. spectra of two low-spin iron(III) protoporphyrin IX (L) complexes, namely $[FeL(CN)_2]^-$ and [FeL(CN)(py)] (py = pyridine), encapsulated in aqueous detergent micelles of sds, ctab, and TX-100. The effect of micellar interactions on the monomeric haem complexes has been investigated and compared with those with the metmyoglobin haem pocket. The concentration of detergent:haemin concentration ratio was kept above 140:1 to ensure that all the haemin cyanide is encapsulated inside the micelles (see Experimental section).

Experimental

The complex [FeL(Cl)] was obtained from Sigma Chemicals. The detergents ctab, sds, and TX-100 were purchased from E. Merck and Sigma, and their purity checked by analytical methods. The iron(III) porphyrins were incorporated in the micelles using published procedures.[13–17] Micellar solutions were prepared by warming at 50 °C a 5% solution of the detergents containing NMe_4Br (0.1 mol dm^{-3}) in 18% pyridine–water (for studies on the pyridinecyanide complexes), or in an aqueous solution at pH $\approx$9.6 (for studies on the dicyanide complexes). For n.m.r. experiments deuteriated pyridine and D_2O were used. Solid samples of [FeL(Cl)] were dissolved in this micellar solution, and the mixture allowed to equilibrate at 40—50 °C. The dicyanide complex was formed by adding a concentrated micellar solution of KCN to the porphyrin complex (also in micellar solution) and again equilibrating at 40 °C. The haemin pyridinecyanide complex was prepared by adding KCN solution to the 18% pyridine–water micellar solution of haemin chloride at a haemin:KCN molar ratio of 1:1.1.[19] The haemin dicyanide complexes in different types of micellar solutions were identified from their known visible spectra.[13–15] The Soret band maxima for haemin dicyanide (in absence of detergent, sds, TX-100, and ctab respectively) are at 418, 422, 429, and 431 nm, and those for the haemin pyridinecyanide are at 417, 418, 420, and 421 nm. Labelled $KC^{15}N$ was used for ^{15}N n.m.r. studies. For proton n.m.r. studies an external $SiMe_4$ standard was used, and an external $^{15}NO_3^-$ was used as reference for ^{15}N n.m.r. spectra.[20]

N.m.r. studies were carried out over a haemin concentration

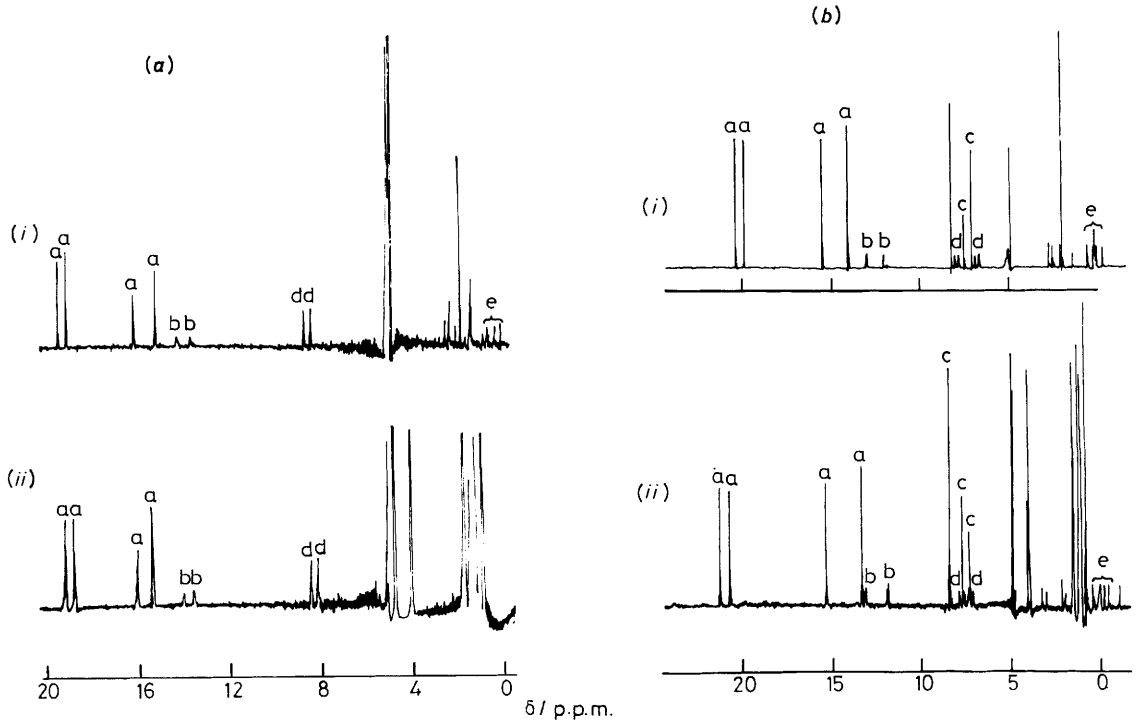

Figure 1. Proton n.m.r. spectra of (*a*) $[FeL(CN)_2]^-$ (0.1 mmol dm^{-3}) in (*i*) aqueous alkali (pH 9.6) and (*ii*) aqueous sds micellar solution, (*b*) [FeL(py)(CN)] (0.1 mmol dm^{-3}) in (*i*) 18% pyridine–water solution and (*ii*) sds micellar solution in 18% pyridine–water. Peak assignments (protons): a, haem methyl; b, vinyl CH; c, pyridine; d, propionic acid β-CH_2 and e, meso

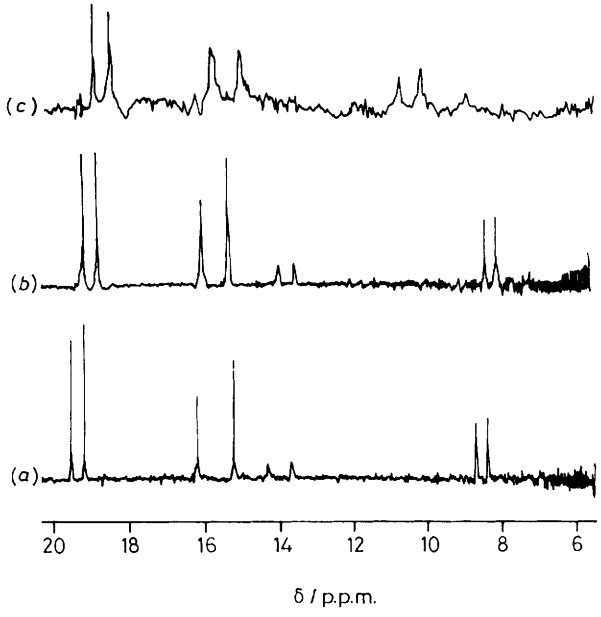

Figure 2. Proton n.m.r. spectra of $[FeL(CN)_2]^-$ (0.1 mmol dm^{-3}) in (*a*) aqueous alkali (pH 9.6), (*b*) aqueous 5% sds micellar solution, and (*c*) aqueous 5% ctab micellar solution

range of 0.01—2.0 mol dm^{-3}. The ratio of the concentration of detergent to that of haemin was 140:1 for 1.2 mol dm^{-3} haemin complex in 5% sds solution. The pH was determined with an accuracy of ±0.01 units with no solvent correction applied. The n.m.r. experiments were carried out on a Bruker 500 MHz instrument. To detect n.m.r. signals from the haem methyl protons it was necessary simultaneously to saturate all the proton signals of the micelle and water using a multiple irradiation programme. Generally, 1 000—5 000 transients were acquired to obtain a good signal-to-noise ratio. The temperature of the n.m.r. samples was varied and maintained (within ±0.5 °C) with an automated variable-temperature accessory.

Results and Discussion

The ^1H n.m.r. spectra of dicyanide and pyridinecyanide complexes in aqueous sds micelles at room temperature are shown in Figure 1. Included in the Figure are spectra of these complexes in the absence of micelles in aqueous alkaline solution (pH 9.6) for haemin dicyanide and in 18% pyridine–water for haemin pyridinecyanide. The spectra were recorded in the micelles over a haemin concentration range of 0.01—2.0 mmol dm^{-3} to check the effect of any possible aggregation. No dependence of the shift or linewidth of the haem protons on the concentration was observed. This lends further support to the monomeric nature of the haem complex inside the micelle. The assignment of haem methyl protons in these compounds is well documented in the literature.[21] Figure 2 compares the methyl region of the ^1H n.m.r. spectra of the dicyanide complex in sds and ctab and in the absence of micelles. A distinct upfield shift in the haem methyl proton resonances is observed upon incorporation of the haem inside the micelle. Further, the upfield shift increases from sds to ctab. The upfield bias in the isotropic shifts of haem protons may arise because of the change in hydrogen bonding ability of the 'solvent' inside the micellar cavity compared to that of water. The effect of solvent on the isotropic shift of haemin dicyanide had been demonstrated earlier,[22,23] which showed that with decreasing solvent hydrogen bonding ability in going from D_2O to dimethyl sulphoxide (dmso) the haem methyl signal moves upfield from δ −19.5 to −15.0. Since ctab is more hydrophobic[15] than sds, the change in shift in the case of micellar solutions seems to follow the increasing hydrophobicity of the micellar cavity.

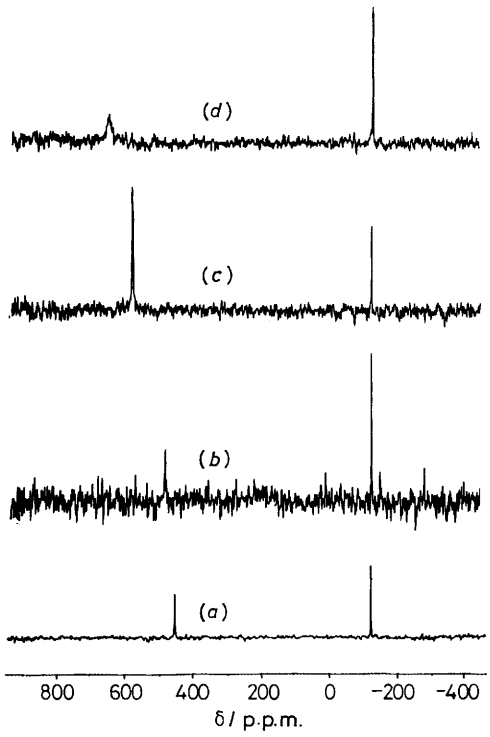

Figure 3. Nitrogen-15 n.m.r. spectra of $[FeL(C^{15}N)_2]^-$ (1.2 mmol dm^{-3}) in aqueous alkali (pH 9.6) (*a*), sds (*b*), TX-100 (*c*), and ctab micellar solution (*d*). The signal at δ −118 p.p.m. refers to free $C^{15}N$

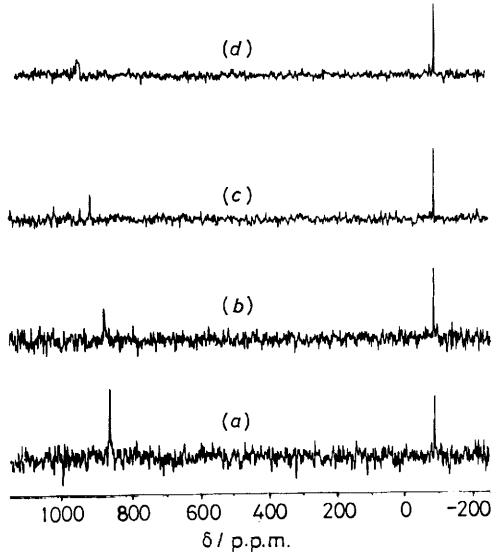

Figure 4. Nitrogen-15 n.m.r. spectra of $[FeL(py)(C^{15}N)]$ (1.2 mmol dm^{-3}) in 18% pyridine–water (*a*), sds (*b*), TX-100 (*c*), and ctab micellar solution in 18% pyridine–water (*d*). The signal at δ −118 p.p.m. refers to free $C^{15}N$

We note from Figure 2 that for the low-spin iron(III) porphyrins the linewidths of the haem methyl protons change significantly, though not dramatically, in the micellar solutions compared to those in simple aqueous solutions. For high-spin iron(III) porphyrins the increase in linewidths of haem proton resonances upon incorporation into micelles was however very prominent.[17] A possible origin of this behaviour may lie in the difference in the total correlation time τ_c. In the case of the high spin iron(III) complexes the electron spin correlation time τ_s is $\approx 10^{-10}$—10^{-11} s[24,25] while that for low-spin iron(III) complexes is $\approx 10^{-12}$ s.[26] The typical value for the rotational correlation time τ_r for these complexes is $\approx 10^{-9}$—10^{-10} s.[26,27] The total correlation time τ_c is given by equation (1). Since τ_s values for

$$\frac{1}{\tau_c} = \frac{1}{\tau_s} + \frac{1}{\tau_r} \quad (1)$$

high-spin iron(III) complexes are comparable to τ_r values so the resulting τ_c values for those systems are affected significantly by changes in the value of τ_r. However, since τ_s for low-spin iron(III) species is much lower than τ_r, changes in τ_r due to an increase in viscosity inside the micellar cavity would have only a small effect on the total correlation time for the low-spin iron(III) complex. Thus, as the rotational correlation time for the porphyrin complex decreases in micellar solution compared to that in simple solvents, linewidths of the haem proton signals only increase slightly in the case of the low-spin iron(III) complex whereas for the high-spin iron(III) porphyrin complexes a sizeable line broadening effect upon incorporation into micelles is observed. The linewidths of the haem methyl signals of low-spin cyanide complexes of iron(III) haemoproteins, *e.g.*, in metmyoglobin cyanide[5,23] likewise show little difference as compared with the model haemin cyanide complexes in simple solutions.

The spread of the haem methyl signals for metmyoglobin cyanide[23] is much larger compared to that for haemin cyanide in aqueous micellar solutions, as has also been found with haemin cyanide complexes in simple solutions.[10,23] This suggests that in the case of proteins the in-plane asymmetry is much more marked than that in simple aqueous[10b] or in aqueous micellar solutions. The haem methyl signals for haemin pyridinecyanide appear further downfield than those for haemin dicyanide. The increased asymmetry in haemin pyridinecyanide, owing to different axial ligands, is known to cause this downfield shift as observed in other hetero axial ligand low-spin haemins.[28,29] The asymmetry in haemin pyridinecyanide in aqueous micellar solution is likely to be more than that in simple aqueous solutions because of the presence of a bulky axial ligand (pyridine) inside the micellar cavity. Thus the spread of the haem methyl signals for haemin pyridinecyanide in aqueous micelles is greater than that in simple pyridine–water solutions leading to a downfield shift of the two downfield-shifted methyl signals and a small upfield shift of the other two methyl peaks [Figure 1(*b*)].

To probe further into the effect of the micelles on the paramagnetic shifts of haem, we investigated the ^{15}N n.m.r. spectra of labelled cyanide in $[FeL(C^{15}N)_2]^-$ and $[FeL(py)(C^{15}N)]$ in a number of detergents as well as in absence of detergent. Figures 3 and 4 summarise the results and display a pronounced systematic downfield ^{15}N shift of the bound cyanide signals on going from a solution without micelles through to sds to TX-100 to ctab micellar solutions. The $C^{15}N$ signal is known to be extremely sensitive to the solvent polarity.[20] It has been shown that the $C^{15}N$ shift of the bound cyanide in haemin dicyanide increases from 448 p.p.m. in D_2O to 732 p.p.m. in dmso.[30] A similar trend is also observed in the case of haemin pyridinecyanide complexes. The hydrophobicity of the micellar cavity increases with the size of the micelles,[27] and it is known that ctab forms a more hydrophobic micellar cavity than does TX-100 which in turn forms a more hydrophobic micellar cavity than does sds.[15] Thus the observed trend in large downfield shift of the bound $C^{15}N$ signal for haemin dicyanide as well as for haemin pyridinecyanide in micellar solutions is consistent with the increasing hydrophobic nature of the micelles. The signal for bound $C^{15}N$ in metmyoglobin cyanide appears in the range 931—945 p.p.m. downfield over the range pH 5.7—11.7.[31,32] That for haemin

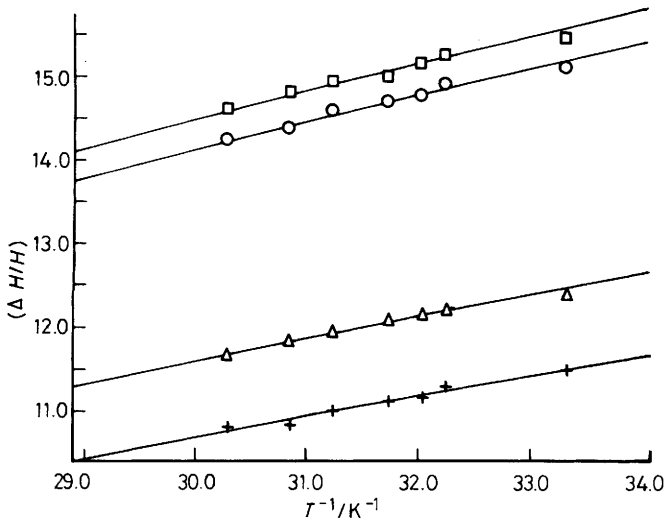

Figure 5. Temperature variations of the isotropic shift (i.p.s.) of the ring methyl proton resonances of $[FeL(CN)_2]^-$ in 5% aqueous sds solution

pyridinecyanide in ctab appears at 945 p.p.m. The large variation in the ^{15}N isotropic shift with micellar size suggests that the spin density at the axial cyanide ligand, directly bonded to the iron atom, is more sensitive to the micellar interaction. On the other hand, the porphyrin methyl and other protons do not show a similar marked effect upon encapsulation by micelles (*cf.* Figure 2).

The temperature dependence of the ring methyl resonances of these monomeric haem complexes in the hydrophobic micellar cavity of sds and ctab has been studied between 296 and 330 K, the temperature range being restricted due to the micellar solvent. A typical set of results for the dicyanide complex in sds is shown in Figure 5. A plot of $(\Delta H/H)T$ *vs.* T shows that the isotropic shift deviates from Curie behaviour in this temperature range. Such deviation has previously been seen for these complexes in organic and simple aqueous solvents as well,[6,26] but the origin of the deviation was not decided with certainty because of the possible effect of aggregation. Since these low-spin haems in the de-aggregated micellar solution also show similar deviation, the origin is most likely to be electronic in nature.

The isotropic shift consists of dipolar and contact terms,[33] which for the low-spin Fe^{3+} ion in axial symmetry are given by equations (2) and (3) where the symbols have their usual

$$[\Delta H/H]_C = \frac{Ag\beta S(S+1)}{3\gamma_N hkT} \quad (2)$$

$$(\Delta H/H)_D = \frac{1}{2}(K_{\parallel} - K_{\perp})\left(\frac{3\cos^2\theta - 1}{r^3}\right) \quad (3)$$

meaning.[33] In axial symmetry the ground state of the low-spin iron(III) haem is ^2E with $^2B_2$ (and $^4A_2$) as a close-lying excited state.[34] Equations (2) and (3) show that, while the contact term [equation (2)] obeys the Curie law, the temperature dependence of the dipolar term [equation (3)] is dependent on the temperature dependence of the magnetic anisotropy $(K_{\parallel} - K_{\perp})$. Horrocks and Greenberg[35,36] have calculated the magnetic anisotropy for low-spin iron(III) haems including the s.o.z. (second-order Zeeman effect) contribution (arising from the mixing between the ^2E and ^2B states) and have noted that the s.o.z. contribution leads to a non-Curie behaviour for the $(K_{\parallel} - K_{\perp})$. The isotropic shift would then deviate from a $1/T$ dependence as is indicated in Figure 5. We have also done a similar calculation of the isotropic shift using a crystal-field model with axial symmetry including the s.o.z., and find that the data of Figure 5 fit well if the excited state $^2B_2$ lies at ≈ 500 cm^{-1} above the ground ^2E state. A unique and reliable fit is however not possible here in view of the limited temperature range of the data. LaMar and Walker[8,25] have also made similar suggestions from the n.m.r. study of haemin dicyanide in aqueous solutions. Spin–orbit interactions of the $^4A_2$ excited state with the ground state could also lead to a departure from Curie behaviour, but the effect of such mixing would be small unless $^4A_2$ lies very close to the ^2E state.

Conclusions

The n.m.r. of low-spin iron(III) porphyrin dicyanide and pyridinecyanide in aqueous detergent micellar solutions show that the nature of the spectra of the haems in micelles remains very similar to those in the absence of micelles, *i.e.*, in simple aqueous or non-aqueous solutions. However, the haem methyl signals of haemin dicyanide show a small upfield shift in micellar solution compared to those in aqueous solutions. This small upfield shift has been explained in terms of the hydrophobicity of the micellar cavity. An increase in rotational correlation time τ_r owing to an increase in viscosity inside the micellar cavity does not affect the total correlation time τ_c of low-spin haemins to any significant extent, because τ_c for these complexes is dominated by the electron-spin correlation time τ_s. Hence the haem methyl signals in micellar solution do not show any marked increase in linewidths compared to those in aqueous solutions.

The ^{15}N n.m.r. spectra of the dicyanide as well as the pyridinecyanide complexes of haemin show that the $C^{15}N$ ligand is more sensitive to the nature of the solvent than are the ring methyl protons. Increase in hydrophobicity around the haemin cyanide causes an increasingly downfield shift in the ^{15}N signal of the bound $C^{15}N$. The difference in hydrophobicity of different micellar solutions is manifested by the increased downfield shift of the bound $C^{15}N$ signal of haemin cyanides in the order: absence of detergent < sds < TX-100 < ctab. Parametric ligand-field calculations of the proton n.m.r. shifts of haemin dicyanide in sds micelles support mixing of the $^2B_2$ excited state into the ^2E ground state of the compound giving rise to a substantial s.o.z. contribution.

The spread of haem methyl signals of haemin cyanide complexes in aqueous detergent micelles is similar to that in simple aqueous solutions, while in the case of metmyoglobin cyanide the spread is much larger due to the in-plane asymmetry in the protein.[37] However the electronic structure of haem both in the low-spin haemoprotein as well as in haemin cyanide in aqueous detergent micelles is characterised by mixing between low-lying Kramers doublets through s.o.z. interactions.

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