Notes

Proton Nuclear Magnetic Resonance and Optical Spectra of Six-co-ordinated High-spin (S = 2) Bis(tetrahydrofuran)(3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionato)iron(II) encapsulated in Aqueous Detergent Micelles

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Proton n.m.r. (500 MHz) and optical spectral studies have been made on the six-co-ordinated high-spin iron(II) haem [FeL(thf)$_2$] (L = 3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionate, thf = tetrahydrofuran) encapsulated in aqueous hexadecyltrimethylammonium bromide micelles. The results are compared with those of the five-co-ordinated high-spin iron(II) haem reported previously. The optical spectrum of the six-co-ordinated species show the Soret band at 415 nm and two other bands at 540 and 564 nm, while for the five-co-ordinated species the Soret band shifts to longer wavelength and a single broad band at 555 nm was obtained. The n.m.r. spectra show a larger downfield shift for the methyl- and *meso*-proton resonances and a smaller spread of the four methyl resonances for the six-co-ordinated complex as compared to those of five-co-ordinated high-spin haems.

In deoxyhaemoglobin and deoxymyoglobin the high-spin (S = 2) iron(II) ion is found in a five-co-ordinated square-pyramidal geometry above the plane of the porphyrin and displaced towards the fifth ligand.[1,2] On binding a sixth ligand the iron atom is pulled into the plane of the porphyrin, where it is found as a low-spin species in octahedral geometry.[1,2] The out-of-plane displacement of the iron atom in the deoxyproteins was believed to be due to the large size of high-spin (S = 2) iron(II) as compared to that of the N_4 hole in the porphyrin plane.[2] This led to the general belief that the high-spin state is always associated with the out-of-plane displacement and five-co-ordination of the iron atom in porphyrins.

However, if two equivalent weak-field axial ligands are bound, high-spin six-co-ordinated haems may be obtained where the iron atom may be found in the plane of the porphyrin.[3] There are several examples of high-spin six-co-ordinated iron(III) porphyrin complexes,[3,4–6] but the only well characterized analogous iron(II) complex is [Fe(tpp)(thf)$_2$] (tpp = 5,10,15,20-tetraphenylporphyrinate, thf = tetrahydrofuran) which was reported to be stable only in the crystalline state.[7,8] In these six-co-ordinated complexes the iron atom is rigorously centred in the porphyrin plane[3,8] and the large high-spin ions are accommodated in the haem plane by porphyrin ring-core expansion with significant non-bonded repulsion between the axial ligands and the pyrrole N atoms of the porphyrinato core.[3,8–10]

Recently we have shown that six-co-ordinated high-spin complexes are easily formed when weak axial ligands such as H_2O or thf are bound to iron porphyrins encapsulated in detergent micelles.[4–6,11] A Mössbauer study of [FeL$_2$(thf)$_2$] [L = 3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionate (dianion of protoporphyrin IX)] in frozen 5% aqueous solution showed isomer shift [0.91(2) mm s^{-1}] and quadrupole splitting [2.49(2) mm s^{-1}] values[11] similar to those of the high-spin six-co-ordinated tpp analogue.[7,8] A resonance-Raman study of haem in aqueous thf showed an expanded porphyrin core with the high-spin Fe^{II} centred in the haem plane.[12] However, in the absence of the detergent (*e.g.* in aqueous thf) about 50% of the molecules are found to be in the form of aggregated species.[11] The detergent micelles stabilize monomeric species in aqueous solution as found earlier.[4–6,13] In view of the recent interest in the six-co-ordinated high-spin haems as haemoprotein models,[3,7–12] we report here the ^{1}H n.m.r. and electronic spectra of the complex [FeL(thf)$_2$] in aqueous $NMe_3(C_{16}H_{33})Br$ solution. To our knowledge this is the first such study on a high-spin six-co-ordinated iron(II) haem in aqueous solution.

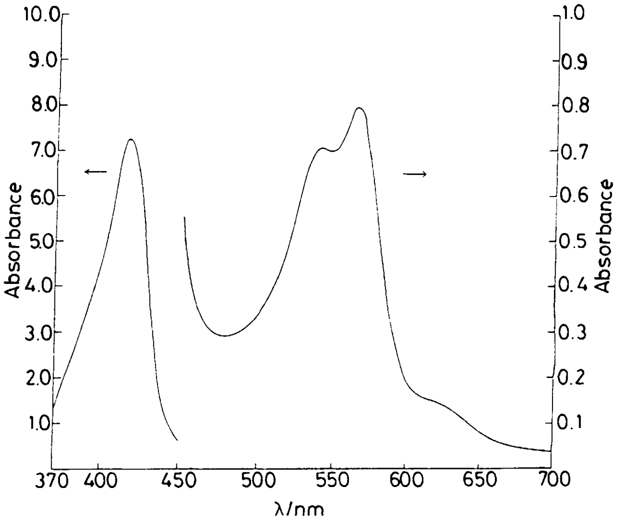

Figure 1. Electronic spectrum of 6.5 × 10^{-5} mol dm^{-3} [FeL(thf)$_2$] encapsulated in 5% aqueous $NMe_3(C_{16}H_{33})Br$ micelles at 298 K and pH 10.0

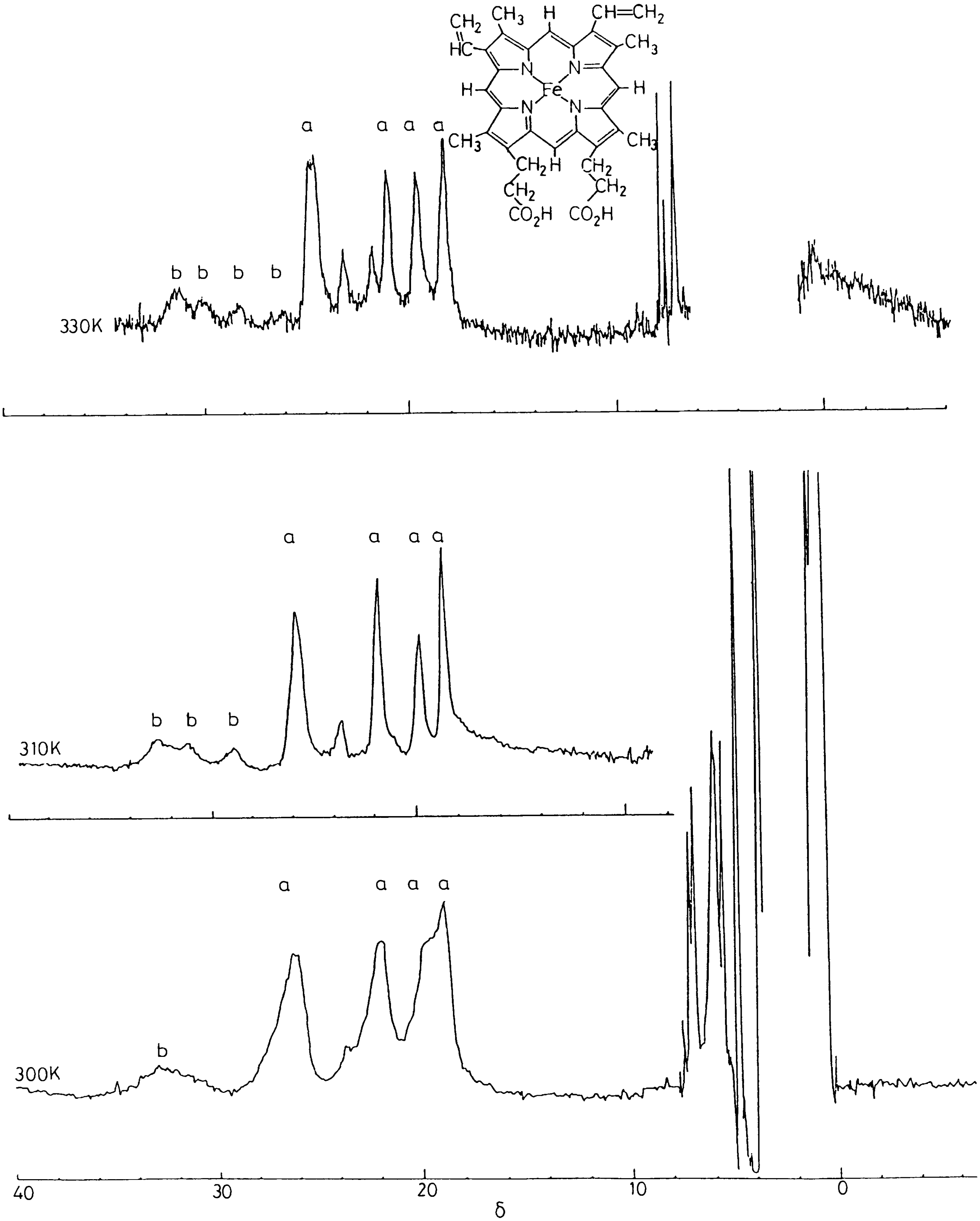

Figure 2. Proton n.m.r. spectra (500 MHz) of [FeL(thf)$_2$] at 330 (top) and 300 K (bottom) in 5% aqueous NMe$_3$(C$_{16}$H$_{33}$)Br solution. Assignments: a, haem CH$_3$; b, *meso*-H

Experimental

Iron(III) protoporphyrin IX chloride (haemin chloride) was purchased from Sigma Chemicals, $NMe_3(C_{16}H_{33})Br$ from E. Merck Co. A solution of the iron(III) complex (0.01—1.0 mmol dm^{-3}) in 5% aqueous $NMe_3(C_{16}H_{33})Br$ micelles[4—6] at pH 10.0 was prepared by dissolving it in an alkaline, thoroughly deoxygenated, $NMe_3(C_{16}H_{33})Br$ solution. The solution was allowed to stand for about 2 h at 40—50 °C and then to equilibrate with excess of tetrahydrofuran for another 3 h prior to reduction of the complex by sodium dithionite ($Na_2S_2O_4$).

All operations were carried out under a nitrogen atmosphere and all reagents (including water–D_2O) were deoxygenated. A minimum amount of a saturated aqueous solution of $Na_2S_2O_4$ was used to reduce the iron(III) complex. For the optical spectrum, a *ca.* 10^{-5} mol dm^{-3} solution of the iron(III) porphyrinate was taken in a quartz cuvette fitted with a rubber septum, and the reduction was carried out by injecting the dithionite solution, with gentle shaking, until a red solution was obtained. The sample for n.m.r. studies was prepared by reducing a *ca.* 1 mmol dm^{-3} solution of the iron(III) porphyrinate in an n.m.r. tube fitted with a rubber septum which was previously flushed with nitrogen gas.

Experimental details for the optical and n.m.r. spectral measurements are as reported previously.[13] To get a good signal-to-noise ratio in the n.m.r. spectra, about 4 000—5 000 transients were required over a spectral width of *ca.* 45 kHz with 8K data points. In order to observe the paramagnetically shifted porphyrin protons, the micelle- and the water-proton signals needed to be saturated. A microprogram for multi-frequency irradiation was used to irradiate all these proton signals.

Results and Discussion

The optical spectrum of $[FeL(thf)_2]$ in 5% $NMe_3(C_{16}H_{33})Br$ aqueous solution is shown in Figure 1. This spectrum is clearly different ($\lambda_{max.}$ at 415, 540, and 564 nm) from those of high-spin ($S = 2$) five-co-ordinated or intermediate-spin ($S = 1$) four-co-ordinated species in the same detergent solution.[13] Gradual addition of thf to the four-co-ordinated [FeL] in $NMe_3(C_{16}H_{33})Br$ solution, which shows a split Soret band,[13] led to a sharp band at 415 nm when excess of thf was added. The spectrum in Figure 1 is similar to that found for six-co-ordinated haems[14] indicating co-ordination of two molecules of thf to [FeL]. Optical spectral study at various concentrations showed no evidence for aggregation[15] of the haem in 5% aqueous $NMe_3(C_{16}H_{33})Br$. Thus the species in solution is a monomeric six-co-ordinated haem with thf as the axial ligands.

The ^1H n.m.r. (500 MHz) spectra of $[FeL(thf)_2]$ in 5% aqueous $NMe_3(C_{16}H_{33})Br$ at various temperatures are shown in Figure 2. Though the room-temperature (298 K) spectrum is broad, four sharp well resolved lines are observed at >300 K. These spectra agree quite well with those reported for deoxyhaemoglobin[16] and other high-spin ($S = 2$) haems.[13] However, the downfield shift of the proton resonances of the six-co-ordinated species $[FeL(thf)_2]$ is larger than that reported previously for other high-spin ($S = 2$) haems. Assignment of the various resonances is based on the relative intensity and multiplicity and by comparison with the assignments for high-spin iron(II) haems[17] and haemoglobin.[16,18]

The porphyrin proton resonances in the n.m.r. spectrum of $[FeL(thf)_2]$ in aqueous $NMe_3(C_{16}H_{33})Br$ are much broader than those observed for high-spin haems in organic solvents[17] and are similar to those observed for haemoglobin.[16—18] A possible cause of such line broadening for haems encapsulated in a hydrophobic cavity was discussed recently.[13] As in the case of high-spin five-co-ordinated species,[13] the isotropic shift of the methyl and *meso* protons shows a linear temperature dependence in the range 310—330 K indicating that only one spin state is populated in this range.[13]

There are three significant differences in the n.m.r. spectra of the five- and six-co-ordinated high-spin iron(II) haem complexes. First, the spread of the haem methyl resonances for the six-co-ordinated complex $[FeL(thf)_2]$ is much smaller (*ca.* 6.5 p.p.m.) than that found for the five-co-ordinated high-spin species (*ca.* 16 p.p.m.) studied under identical conditions.[13] The smaller spread of the haem methyl signals for the former may be due to a smaller haem in-plane asymmetry.[13,16—18] Secondly, the average shift of the four haem methyl resonances has a larger downfield bias for the six-co-ordinated species (*ca.* 21 p.p.m.) than that of the five-co-ordinated haem (*ca.* 17 p.p.m.).[13] A similar dependence of the methyl proton resonances on co-ordination number was found for the iron(III) porphyrinates,[19] where the larger downfield shifts for the six-co-ordinated complex were attributed to the added effect of π-spin transfer on a dominant σ-spin transfer mechanism.[19] Thirdly, the broad and weak resonances near 30 p.p.m. downfield, resolved as four broad and weak peaks at 320 K (Figure 2), are likely to be the *meso*-H signals.* For the five-co-ordinated high-spin haem the *meso*-H peaks appear at δ 0—6.[17] The large downfield bias of the *meso*-H resonances for the high-spin six-co-ordinated haems studied here agrees quite well with the similar observation for haemin complexes.[19]

It is interesting that for the haem complexes where the iron atom lies in the plane of the porphyrin (*viz.* four-co-ordinated intermediate-spin,[13,20] six-co-ordinated low-spin,[13] and six-co-ordinated high-spin) the downfield shift of the *meso* protons is larger than that of the methyl-proton resonances. However, for the high-spin five-co-ordinated haems where the iron atom is out of the porphyrin plane the downfield shift of the methyl-proton resonances is larger than that of the *meso*-proton resonances.[17]

Acknowledgements

The n.m.r. work reported was done at the 500-MHz FT-NMR National Facility. We thank the staff members for their help. We also thank Professor Samaresh Mitra of the Tata Institute of Fundamental Research, Bombay, for his help and encouragement.

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* Assigned by comparison with the proton n.m.r. spectra of various iron-(II) and -(III) haems reported previously.[13,17—20]

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Received 10*th November* 1989; *Paper* 9/04823E