Notes

Electronic Spectral Study of the Aqua ⇌ Hydroxo Equilibrium of Model Iron(III) Haems encapsulated in Aqueous Detergent Micelles

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Electronic spectral studies have been made on the aqua ⇌ hydroxo equilibrium in model six-co-ordinated iron(III) haems, [Fe(P)(py)L] (L = H_2O or OH; py = pyridine; P = meso-, proto-, or deutero-porphyrinate), encapsulated in different aqueous detergent micelles. The pK_a of the equilibrium is significantly affected by the hydrophobic interaction of the micellar cavity, and ranges from 7.1 to 10.3 depending on the porphyrin substitution and the micelle. The pK_a varies with the nature of porphyrin as meso ≥ deutero > proto, and with the micelles as sodium dodecyl sulphate > Triton X-100 > hexadecyltrimethylammonium bromide. The significance of these studies to similar acid ⇌ base equilibria in haemoproteins is discussed.

The sixth co-ordination position of iron(III) protoporphyrin (3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionic acid) (haem) in metmyoglobin and methaemoglobin is occupied by a water molecule or a hydroxide ion depending on pH of the solution.[1,2] In aqueous solution both metmyoglobin and haemoglobin exhibit an equilibrium between the acidic (aqua) and alkaline (hydroxo) forms over a range of pH 3—7. The pK_a of this equilibrium has been found to be sensitive to the origin of the protein and nature of the substituents on the haem, and it ranges from 7.4 to 10.9.[2-7] The hydrophobic environment of the haem cavity, hydrogen bonding, and the nature of substituents on the haem play important roles in the acid ⇌ base transition in haemoproteins.[8,9]

It has been suggested that micellar systems simulate the electrostatic and hydrophobic interactions of the haem cavity.[10] Several studies[8,11-15] have shown that haem is monodispersed when encapsulated in an aqueous detergent micelle. Thus, haem encapsulated in an aqueous detergent micelle would be inside a large macromolecular cavity whose interaction is primarily hydrophobic, which is likely to modify its reactivity.

These considerations led us to study by electronic spectroscopy the aqua ⇌ hydroxo equilibrium in six-co-ordinated aqua(pyridine)iron(III) porphyrin complexes encapsulated in different aqueous micelles. The Fe^{III} in these complexes is axially co-ordinated[16,17] to a water and a pyridine molecule, showing a similar structure to that of the haem in metmyoglobin. Bartocci *et al.*[18] have determined the pK_a of the aqua ⇌ hydroxo equilibrium in these complexes in aqueous pyridine solution as 10.5. Our present investigation was to examine the effect on this equilibrium of the size, nature, and hydrophobicity of the micelles as well as of substitutions on the porphyrin ring. We report here the results of our studies on aqua(pyridine)iron(III) complexes (Figure 1) derived from protoporphyrin, deuteroporphyrin, (3,7,12,17-tetramethylporphyrin-2,18-dipropionic acid), and mesoporphyrin (7,12-diethyl-3,8,13,17-tetramethyl-2,18-dipropionic acid) and their esters, encapsulated in aqueous anionic sodium dodecyl sulphate, cationic hexadecyltrimethylammonium bromide [$NMe_3(C_{16}H_{33})Br$], and neutral Triton X-100 micelles.

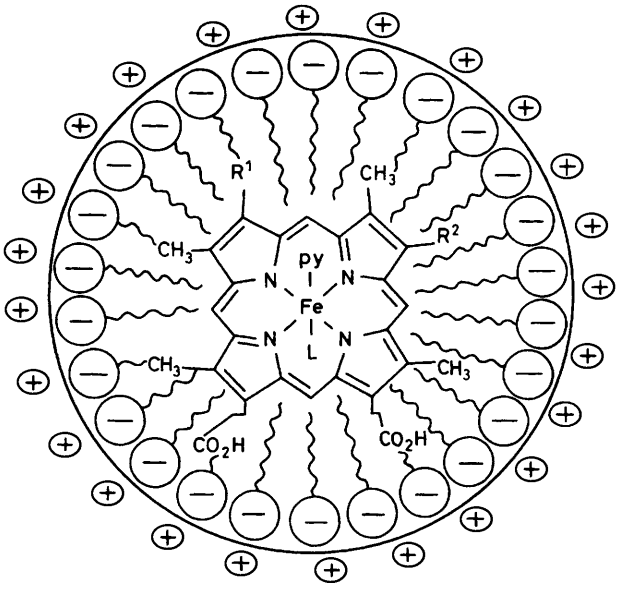

Figure 1. Model structure of haemin in pyridine—water inside an aqueous detergent micelle. L = H_2O for aqua- and L = OH for hydroxo-complex; R^1 = R^2 = CH=CH_2 for protoporphyrin IX, H for deuteroporphyrin IX, and Et for mesoporphyrin IX complexes

Experimental

Chloroiron(III) protoporphyrin IX (haemin chloride), [Fe(L^1)Cl], deuteroporphyrin IX dimethyl ester (H_2L^2), and mesoporphyrin IX dimethyl ester (H_2L^3) were obtained from Sigma Chemicals. Chloroiron(III) deuteroporphyrin IX dimethyl ester [Fe(L^2)Cl], and chloroiron(III) mesoporphyrin IX dimethyl ester, [Fe(L^3)Cl], were prepared from the ligand by known procedures.[19,20] Esterification of [Fe(L^1)Cl] was done by the methanol–H_2SO_4 method,[19,20] giving [Fe(L^4)Cl]. The complexes [Fe(L^2)Cl] and [Fe(L^3)Cl] were de-esterified by KOH in tetrahydrofuran (thf)[19,20] to give [Fe(L^5)Cl] and [Fe(L^6)Cl] respectively. The purity of the ligands and the compounds were checked by u.v.–visible, n.m.r. spectroscopy, and chromatographic methods.

Hexadecyltrimethylammonium bromide was purchased from E. Merck Co., sodium dodecyl sulphate and Triton X-100 from Sigma Chemicals and their purities were checked by n.m.r. spectroscopy. Iron(III) porphyrins in aqueous micellar solutions were prepared following the method of Simplicio and co-

Table. pK_a values for the aqua $\rightleftharpoons$ hydroxo equilibrium in [Fe(P)(py)L] (L = H_2O or OH) in different micelles and in the absence of micelles

System	pK_a (±0.1) Sodium dodecyl sulphate	Triton X-100	$NMe_3(C_{16}H_{33})Br$	Absence of micelle	Ref.
[Fe(L^1)(py)L]	9.8	9.4	7.7	10.5[a]	*b*
[Fe(L^4)(py)L]	9.2	8.4	7.1		*b*
[Fe(L^5)(py)L]	10.2	10.0	8.6	10.7	*b*
[Fe(L^2)(py)L]	9.7	9.0	8.2		*b*
[Fe(L^6)(py)L]	10.3	10.1	8.8	10.5	*b*
[Fe(L^3)(py)L]	9.8	9.1	8.2		*b*
Fe^{3+} Mb (aplysia)				7.6	*c*
Fe^{3+} Mb (sperm whale)				9.0	*c*
Horse-radish peroxidase				10.9	*c*

[a] Ref. 18. [b] Present work. [c] Ref. 2, p. 47.

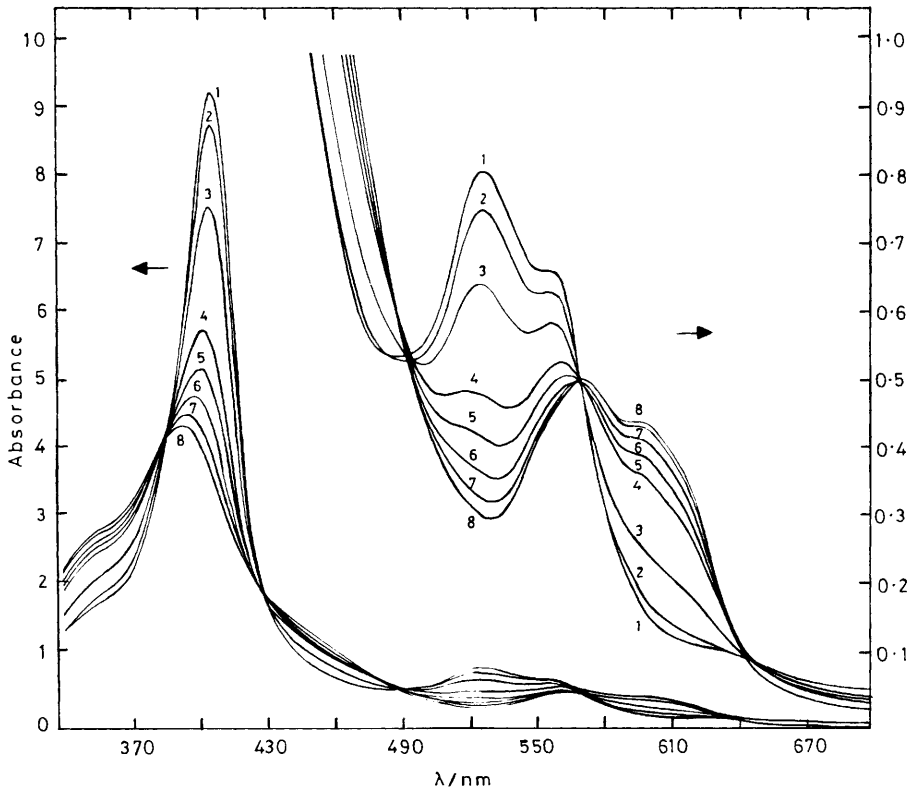

Figure 2. Spectral variations with pH for 6.5 × 10^{-5} mol dm^{-3} [Fe(L^1)(py)L] (L = H_2O or OH) in 18% (v/v) pyridine–water solutions encapsulated in a 5% $NMe_3(C_{16}H_{33})Br$ micelle at 26 °C: pH 5.17(1), 6.82(2), 7.33(3), 7.41(4), 7.79(5), 8.34(6), 8.86(7), and 9.94(8)

workers.[13–15] The resulting samples obeyed Beer's law over a concentration range of 2 × 10^{-3}—10^{-8} mol dm^{-3}, indicating the presence of monomeric haem inside the micelles.

The pH of the solutions was adjusted with dilute HNO_3 or dilute NaOH. The solutions were allowed to equilibrate at 45 °C in the dark before experiments. Optical spectra were recorded at *ca.* 25 °C on a Cary 17D u.v.–visible spectrophotometer and the pH was measured with a digital pH meter to within ±0.05 units.

Results and Discussion

Figure 2 shows a typical pH-dependent profile of the absorption spectrum of the aqua(pyridine)iron(III) protoporphyrin complex in $NMe_3(C_{16}H_{33})Br$. The spectra of the other porphyrin complexes are almost identical. At low pH absorption bands appear around 527 and 556 nm indicating the presence of the aqua form. At higher pH the spectra show bands at around 560 and 600 nm indicating the presence of the hydroxo form. Three isosbestic points occur at 495, 568, and 645 nm in the spectral range (450—700 nm), which suggest the presence of only two components, the aqua and the hydroxo forms in equilibrium (1) (P = porphyrinate, py = pyridine).

$$[Fe(P)(py)(H_2O)]^+\cdot micelle \rightleftharpoons [Fe(P)(py)(OH)]\cdot micelle + H^+ \quad (1)$$

The values of the pK_a obtained by fitting the pH titration curves are listed in the Table, which also includes for comparison the pK_a values of this equilibrium for those complexes in aqueous pyridine solutions in the absence of the micelles.

The changes in pK_a between the aqueous pyridine and

micellar solutions are not associated with any possible dimer–monomer equilibrium of the haem complexes. A study of the dimer–monomer equilibrium, following the procedure adopted by Simplicio,[13] showed that the aqua(pyridine) complexes exist as monomers even in the absence of micelles in the range pH 6.5—12.5. This is supported further by the observation that the spectra of the complexes in aqueous pyridine solutions are virtually unchanged[18] on addition of alcohols, known for their deaggregating behaviour.[21] The changes in pK_a are also not due to the influence of cations [*e.g.* $NMe_3(C_{16}H_{33})^+$], anions (*e.g.* SO_4^{2-} in sodium dodecyl sulphate), or the OH^- group (*e.g.* in Triton X-100), since the measurements on the protoporphyrin complex in 0.1 and 1.0 mol dm^{-3} Na_2SO_4 or NMe_4Br solutions give pK_a values in the range 10.3—10.5, indicating that cationic or anionic groups alone cannot significantly change the pK_a values.

The changes in pK_a must therefore arise from hydrophobic interactions inside the micelle. The hydrophobic environment of the micellar cavity evidently affects the aqua $\rightleftharpoons$ hydroxo equilibrium. In general, the pK_a in the micelle is lower than that in its absence. The effect of the hydrophobic interaction of the micellar cavity is further evident in the trend of variation of the pK_a with micelle. For all the complexes, the pK_a increases in the order anionic sodium dodecyl sulphate > neutral Triton X-100 > cationic $NMe_3(C_{16}H_{33})Br$, consistent with expectation based on consideration of the electrostatic charges in the Stern layer. The anionic micelle, in comparison with the neutral and cationic, stabilizes positive charge on the cationic aqua(pyridine)iron(III) complexes, and therefore higher pK_a values result.

The difference in pK_a values in $NMe_3(C_{16}H_{33})Br$ and in sodium dodecyl sulphate is quite large and significant. This is true for both the acid and ester forms of the complexes. The increase in the micellar size in $NMe_3(C_{16}H_{33})Br$ relative to sodium dodecyl sulphate is related to this difference. There is also a significant difference in the pK_a between the acids and esters. It is interesting that this difference is largest in neutral Triton X-100. A further trend in the variation of pK_a is observed with substitution at the R^1 and R^2 positions of the porphyrin. The trend is ethyl $\geqslant$ proton > vinyl, which is similar to that observed by McGrath and LaMar[7] in reconstituted myoglobin. It should be noted that this is qualitatively the opposite of the trend in electron-withdrawing power of the substituents.

It is clear from the Table that the range of pK_a found in this work for the acid $\rightleftharpoons$ base equilibrium of aqua(pyridine)-iron(III) porphyrins encapsulated in various micelles encompasses the values reported for various ferrichemoproteins. The Table also reveals that the sensitivity of the pK_a to the origin of the haemoprotein may be simulated by changing the nature of the hydrophobic interactions in the micelles and the substitution on the porphyrin ring. The nature of the hydrophobic interactions, electrostatic interactions from counter anions, as well as the surface charges of the surrounding macromolecule may be changed in a systematic, and perhaps continuous, manner in order to simulate the subtle differences in the structure and reactivity of haemoproteins derived from different sources.

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