On the Nature of Protoporphyrin(IX) Iron(III) in Aqueous Solution At and Below the pH of Precipitation

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Protoporphyrin(IX) iron(III), $[PPIXFe(III)]^+$, precipitates below pH 5.9 in aqueous solution [1–4]. The precise nature of the $[PPIXFe(III)]^+$ species around and at the precipitation pH has been the subject of debate and speculation [5]. Silver and Lukas [3] in studies on the PPIXFe(III) suggested that the species at low pH was a five-coordinate species in which the Fe(III) was bound to hydroxide, based on evidence from Mössbauer and electronic absorption spectroscopy. More recently, Miller *et al.* [6] reported studies on substituted tetraphenylporphyrinato iron(III) in aqueous solutions at various pH values. In these studies they noted that the visible spectra of substituted water soluble tetra(*p*-phenyl)-porphinato iron(III), $[TPPFe(III)]^+$, species at low pH are those of six-coordinate $[(Por)Fe(III)(H_2O)_2]^+$ (where Por = substituted Tpp^{2-}) species. These authors noted that it was unfortunate that the visible spectra of $[PPIXFe(III)]^+$ and other pyrrole substituted porphyrins were different to those of $[(Por)Fe(III)(H_2O)_2]^+$, as the coordination of the former species could not be verified from visible spectra [6].

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Many workers [7–12] have shown that $[PPIXFe(III)]^+$ in aqueous solution aggregates (even at low concentrations in aqueous solution) at all pHs.

Recently Mazumdar *et al.* [13] in a study aimed at using six-coordinate $[PPIXFe(III)L_2]^+$ species as models for ferric haemproteins, presented an electronic absorption spectrum of their heam species in aqueous detergent micelles at pH 2.6. The spectral details are presented in Table I along with those of some water soluble substituted TPPFe(III) species. The similarity of the electronic absorption spectra of these species to that of $[TPPFe(III)(H_2O)_2](ClO_4)$ in THF [17] allows the suggestion that all these methine substituted porphyrins have two water molecules bound to the iron at low pH [6]. These spectra are also similar to that of $[PPIXFe(III)(H_2O)_2]^+$ in the detergent micelles [13] consistent with a six-coordinate environment in keeping with the given formulation.

As the electronic absorption spectra of $[PPIXFe(III)]^+$ aqueous solutions around/below pH 5.9 are clearly different to that (in detergent at pH 2.6) reported by Mazumdar *et al.* [13], then these do not contain simple $[PPIXFe(III)(H_2O)_2]^+$ species. In studies on PPIXFe(II) solutions Silver *et al.* [18] have shown that the aggregation of the PPIXFe(II) units modifies their electronic structure using evidence obtained from Mössbauer spectroscopy. It appears that in $[PPIXFe(III)]^+$ aqueous solutions aggregation of the monomers does not favour six-coordinate diaquo species, but the modified electronic structure rather prefers five-coordinate high-spin PPIXFe(III)L ($L = OH^-$) complexes.

It is obvious that the aggregation of these species then leads easily onto the formation of μ-oxo bishaem species as the pH is raised.

The most likely routes to this are shown in equation forms as follows:

TABLE I. Electronic Absorption Spectra for some Iron(III) Porphyrin Aqueous Solutions

Compound	pH	Soret band γ (nm)	β band γ (nm)	Relative peak height	Reference
FePPIX[a]	5.9	365	634	52:4:–	3
FeTPPS	2.3	392	528(680)[b]	56:5:1	14, 15
FeTNPS	2.5	397	525	45:5:–	6
$Fe(SP)_2(SMeP)_2P$	2.8	392	525(647)[b]	46:4:1	6
Fe(TMpyP)	1.0	398	524(650)[b]	40:4:1	16
$FePPIX(H_2O)_2$[a,c]	2.6	392	500,532,630	30:3:2.7:1	13

[a]This porphyrin has peripheral substituent groups on the pyrrole rings and these might be expected to influence the electronic spectra. [b]Some porphyrins show a distinct third band in the spectra. Because of this relative peak heights are included and normalised so that where no third band is tabulated, its intensity, if present, is much less than 1 in relation to the relative intensity of the Soret band. [c]This sample was 5×10^{-5} M in a 5% aqueous sodium dodecyl sulphate micelle.

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$$[PPIXFe(III)(H_2O)]_n^+ \longrightarrow [(PPIXFe(III)_2O]_{n/2} + \tfrac{n}{2}H_2O + nH^+$$

or

$$[PPIXFe(III)(H_2O)]_n^+ \longrightarrow [PPIXFe(III)(OH)]_n^0 + nH^+$$

$$[PPIXFe(III)(OH)]_n^0 \longrightarrow [(PPIXFe(III)_2O]_{n/2} + \tfrac{n}{2}H_2O$$

Neither of these schemes utilises a six-coordinate species of the type $[PPIXFe(III)(H_2O)(OH)]$ such as the one observed at high pH by Mazumder *et al.* [13].

It is also possible from the facts collected in this letter to understand that it is the tendency of monomeric $[PPIXFe(III)]^+$ species to aggregate in aqueous solution that leads to its precipitaion below pH 5.9. In addition, insight into the role of the protein in haemproteins is possible. By forced separation of $[PPIXFe(III)]^+$ units into isolated pockets in the haemproteins the protein segregates the units and facilitates conditions favourable to six-coordination.

The hydrophobic interactions between the porphyrin and the amino acid side chains in the haemprotein pockets are stronger than the porphyrin–porphyrin interactions of a metalloprotein aggregate. Thus the haems in a protein are dispersed into monomers, which are further stabilised by formation of six-coordinated species. To a limited extent this property is simulated in the hydrophobic interiors of a micelle but not in ordinary aqueous solutions (particularly near the precipitation pH).

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