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A Spectrophotometric Investigation on Iron(III)-protoporphyrin-IX in Water/Alcohol/Pyridine Solvent Systems

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In view of their outstanding biological role, Iron porphyrin complexes have been the subject of continuous interest for several decades [1–3]. Extensive investigations have been performed on the axial coordination chemistry [4] as well as on the redox properties [5] of several Iron(III) porphyrin systems. Most of the studies have been complicated by the puzzling aqueous chemistry of these systems, particularly of the iron(III) ones. In these systems, the complex axial coordination chemistry is often further complicated by the occurrence of polymerization [6]. Both phenomena are sharply dependent on the type of porphyrin (*i.e.*, on the nature of the substituents on the porphyrin ring) and on several experimental variables. As a consequence, the identification of the species being investigated may often be a non-trivial problem, as shown by the abundance of different formulations of the same experimental system by different authors.

We would like to report here on the definition of the predominant species present in aqueous solutions of iron(III)protoporphyrin-IX chloride (chlorohemin) containing various concentrations of alcohols, pyridine, and hydroxide ions.

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Experimental

Chlorohemin was obtained from Fluka AG and was used without further purification. The other chemicals used were commercial products of reagent grade. The solvents used were commercial products of spectrograde quality.

Spectrophotometric measurements were performed with Perkin-Elmer mod. 323 (double beam) and Shimadzu QV 50 (single beam) spectrophotometers. pH measurements were performed with a Radiometer digital mod. PHM 52 pH-meter.

Procedures

Freshly prepared solutions, made by dissolving solid chlorohemin in the appropriate solvent system, were always used. The use of aged solutions gives rise to erratic and poorly reproducible results.

Results and Discussion

Chlorohemin (Fe(III)PPCl) was dissolved in solvent systems containing various concentrations of water, pyridine, alcohols, and NaOH. Four standard solvent systems used were (a) pure pyridine, (b) 98% v/v pyridine/water, (c) 18% v/v (2.3 *M*) pyridine/water and (d) 18% v/v pyridine/40% v/v alcohol/42% v/v water. The spectra obtained by dissolving Fe(III)PPCl in the above solvents are shown in Fig. 1. In all of the above solvents the absorption spectrum was observed to be strongly dependent on pH. The

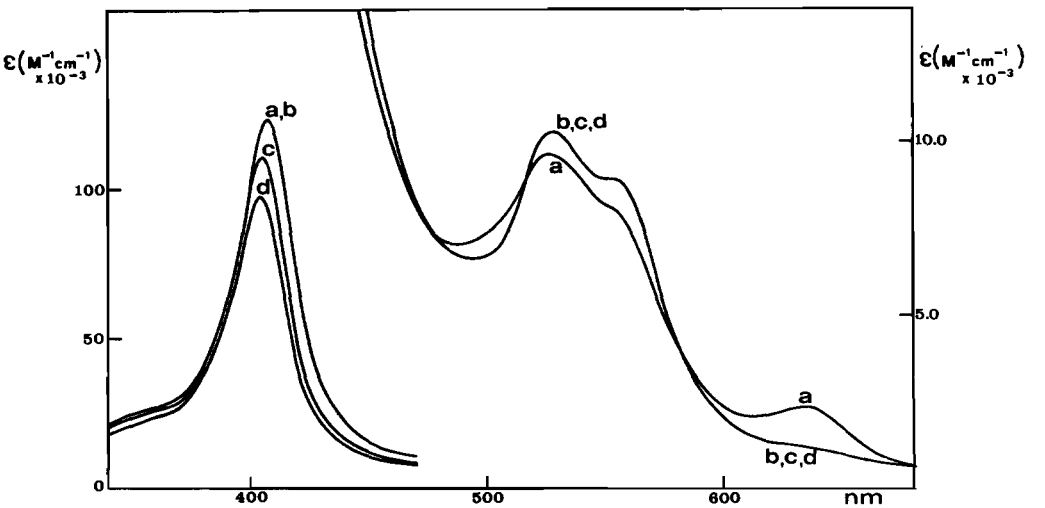


Fig. 1. Absorption spectra of Fe(III)PPCl in a) pure pyridine; b) 98% v/v pyridine/water; c) 18% v/v pyridine/water; d) 18% v/v pyridine/42% v/v water/40% v/v ethanol.

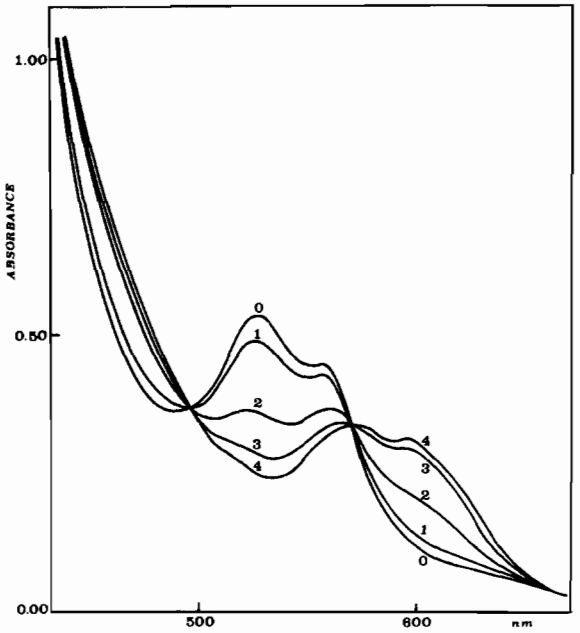


Fig. 2. Spectral variations of 6.2×10^{-5} *M* Fe(III)PPCl in 18% v/v pyridine/water as a function of measured pH; 0) pH = 8.50, 1) pH = 9.04, 2) pH = 10.44, 3) pH = 11.42, 4) pH = 11.70.

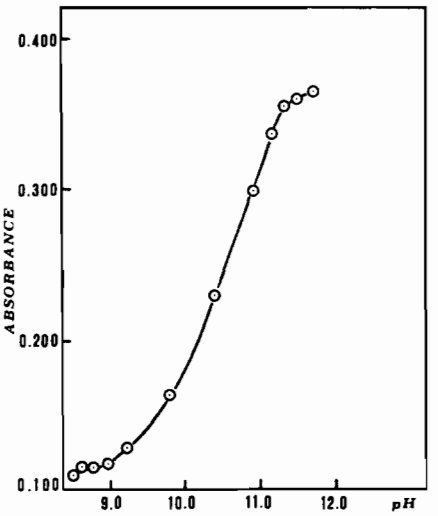
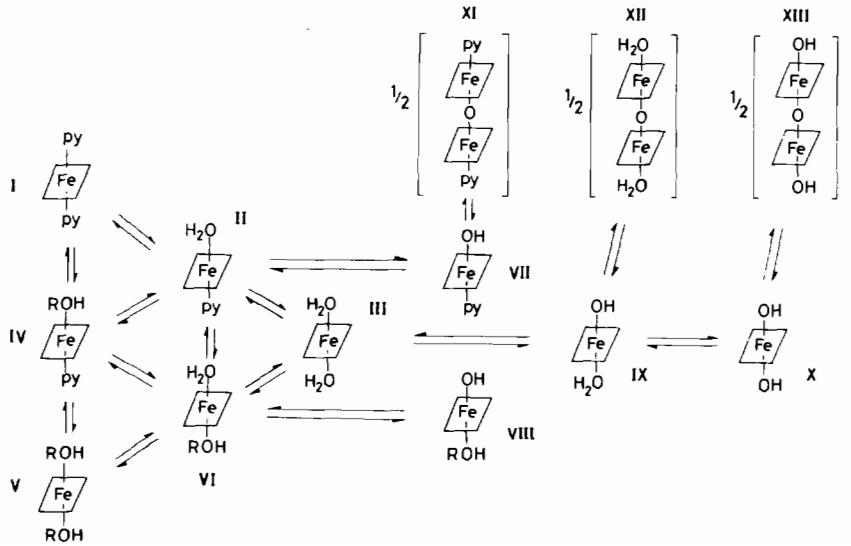


Fig. 3. Spectrophotometric titration curve of 6×10^{-5} *M* Fe(III)PPCl in 18% v/v pyridine/water, at 600 nm.

spectral changes of Fe(III)PPCl in *c* solvent as a function of measured pH are reported in Fig. 2. The spectral titration curve, taken at 600 nm in solvent *c* is reported in Fig. 3. Spectral titrations carried out in solvent *d* gave results identical to those of Figs. 2 and 3, except for slightly different end spectra obtained when the alcohol is ethanol, methanol and 2-propanol.

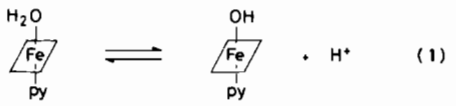
A number of plausible equilibria in the experimental conditions used in this work are shown in Scheme I, in which the porphyrin ring is represented as a square in the equatorial plane of the tetragonal structure. The dimers are depicted as μ-oxo bridged complexes [7]; although both carboxylate bridging through propionyl ring side chains [8] and hydrophobic interactions [9, 10] have been proposed as alternative paths for dimer formation, the μ-oxo structure (which has been crystallographically established for related tetraphenylporphyrine complexes [11]) is the most likely and accepted one [12]. As far as the axial coordination equilibria are concerned, besides the obvious ones involving water and pyridine, also the possibility of alcohols acting as axial ligands has been considered. This coordination has been often postulated [8, 13] in order to account for the well-known monomer stabilizing effect of alcohols [8], and NMR line broadening techniques have been used to measure exchange rates of coordinated ethanol in one case [14]. As far as the acid–base



equilibria are concerned, those involving the ring propionic groups have been omitted for clarity since these groups are no doubt completely deprotonated in the whole pH range investigated.

As a starting point for discussion, we make the obvious assumption that in neat pyridine the complex is in the bispyridine form (I), with a typical spectrum shown in Fig. 1a (bands at 527, (554) and 636 nm). The effect of adding water to the pyridine solution is as follows: the spectrum changes sharply (Fig. 1b, bands at 528 and 556 nm) upon addition of the first few percents of water but then remains practically constant on further addition of water up to the solubility limit of the complex (Figs. 1b, 1c). This clearly indicates that only one main species is present in 98–18% pyridine/water by volume and that this species must be formed from the bis-pyridine complex (I) by substitution of at least one pyridine ligand. The spectrum of this species is different from that of bis aquo (III) complex which can be measured by dissolving Fe(III)PPCl in micellar aqueous solutions of detergents [1, 15]. It is also different from that of the monohydroxo complex (IX) obtainable in water/alcohol solutions at pH > 8 [8, 16] as well as from that of the dimer (XII) stable in alakaline aqueous solutions [1, 16]. Thus, the relevant species present in 98–18% v/v pyridine solvent must be a mixed-ligand complex containing one pyridine, *i.e.*, species II, VII, or XI. Since the spectrum of the water/pyridine solutions is virtually unchanged by substituting substantial amounts of water by alcohols (spectra *c* and *d* in Fig. 1), the presence of a dimer XI can be ruled out. Therefore, we conclude that in water/pyridine solvent (98–18% v/v pyridine), species II and/or VII are present. In water/pyridine/alcohol the same species seem to be present, although the possibility of species IV having a very similar spectrum to that of species II cannot be ruled out.

In this connection, the results obtained by varying pH in 18% v/v pyridine/water seem to be of particular interest. The spectral variations as a function of pH (Fig. 2) clearly indicate the presence of an acid–base equilibrium involving a species with $pK_a \sim 10.5$ (Fig. 3). We assign the observed acid–base equilibrium to that between the pyridine-aquo (II) and pyridine-hydroxo (VII) species (eq. 1). The fact that



(1)

the same pK_a is obtained in the presence and in the absence of alcohols seems to indicate that species II and VII predominate even in 18% v/v pyridine/40% v/v alcohol/42% v/v water without appreciable alcohol coordination occurring in this solvent. The effect of alcohols on the end spectra of the titrations are considered to be solvent effects on the spectrum of VII. As expected, this effect is lower for the more weakly solvating t-butanol with respect to the other alcohols.

It is to be noted that other acid–base equilibria have been reported for Fe(III)protoporphyrin-IX species in aqueous solvent systems. These equilibria, however, are definitely different from that studied here, since they refer to species III $\rightleftarrows$ IX ($pK_a = 6.1$ [15], 6.7 [17]) and IX $\rightleftarrows$ X ($pK_a \sim 13$ [16]). The presence of equilibrium 1 in the 9–10.5 pH range had been previously suggested by Davis and Martin [18], on the basis of a polarographic study carried out in a solvent system comparable to that of the present work. These authors, however, observed some complications (*i.e.*, the presence of more than one electroreducible pyridine-containing species), which are definitely not apparent in our spectrophotometric study. In a recent study [19] carried out in 10% DMF/90% water, the equilibrium constant for the pyridine complexation step III $\rightleftarrows$ II is reported to be 37.2, a value which is in agreement with the essentially complete formation of the mono-pyridine species in the range of pyridine concentrations (higher than 18% by volume) spanned in the present work. Also, Degani and Fiat [20] suggested, on the basis of NMR studies, the formation of aquo-pyridine complexes in the 5–20% pyridine concentration range.

In conclusion, our observations indicate that in aqueous solutions containing 18–98% pyridine by volume, two Iron(III)protoporphyrin-IX species are present, namely the aquo-pyridine (II) and the hydroxo-pyridine (VII) complex, depending on pH, with a pK_a for equilibrium 1 of 10.5. In such a pyridine concentration range, the presence of alcohols does not seem necessary to avoid the formation of dimers.

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