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Mössbauer Studies on Protoporphyrin IX Iron(III) Solutions

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The Mössbauer spectra of frozen aqueous solutions of protoporphyrin IX iron(III) over the pH range 6.7 to 14.4 and of solids precipitated from protoporphyrin IX iron(III) solutions at pH 5.5 and 4.4 are reported. These spectra, supported by electronic spectra, show that protoporphyrin IX iron(III) exists principally in two forms, monomeric and μ-oxo-oligomeric, depending on the pH of the solution. At pH > 8 only the μ-oxo-oligomer exists but at pH < 8 there is a mixture of the two forms. The existence of μ-oxo-oligomer persists even in protoporphyrin IX iron(III) solids precipitated at pH 5.5 and 4.4. A new method of preparation of μ-oxo-oligomer is reported. The i.r. spectrum supported by a chemical analysis shows that this compound is a Na salt.

Introduction

As part of a continuing program to understand the chemistry of the protoporphyrin–iron(III) moiety we are interested in the solution chemistry of this metalloporphyrin [1, 2]. There have been extensive solution [3–26] studies on aggregation of this porphyrin in the literature, though there is much confusion and contradiction. As shown below a great many discrepancies remain, and the reason for this work is to help to clear some of these.

The Fe(III) μ-oxo-oligomer [26] (often described as μ-oxo-dimeric haematin) is the aggregate that has been the recipient of much recent attention. It forms from just about any Fe(III) porphyrin in the presence of base. Its existence in solution was postulated by Shack and Clark [27] in 1947.

Cohen [24] reported dimeric Fe(III) porphyrin hydroxides of Fe(III) tetraphenylporphyrin and Fe(III) deuteroporphyrin dimethyl ester. His studies included Mössbauer spectroscopy, infra-red, molecular weight determinations and magnetic susceptibility at room temperature. Fleischer and Srivastava [28] have presented infra-red evidence and X-ray evidence for the structure. The structure of the μ-oxo-oligomer of Fe(III) tetraphenylporphyrin was determined by Hoffman *et al.* [29]. It was found that the interaction between the phenyl rings prevented the close approach to the Van der Waals distance of separation (3.4 Å), which was determined for the μ-oxo oligomer of Mn(III) phthalocyanine [30]. For the μ-oxo oligomer of Fe(III) tetraphenylporphyrin the paramagnetic Fe(III) nuclei are antiferromagnetically coupled via the nearly linear oxo linkage [31–34]. The Fe–O–Fe angle is 174.5° and despite the strong antiferromagnetic coupling between the iron atoms, the iron is displaced 0.50 Å from the mean plane of the porphyrin nitrogens towards the axial oxo ligand.

Magnetic work by Blauer and Ehrenberg [3] gave very high magnetic moments when the formation of μ-oxo oligomers might be expected. Rawlinson and Scutt [4] were able to achieve some lowering of the magnetic moment of Fe(III) protoporphyrin presumably through antiferromagnetic coupling, but only at very high concentrations of base.

Walter [6] found Fe(III) deuteroporphyrin dimethyl ester disulphonate dimers below pH 13, and he formulated the species as containing two water molecules as additional ligands on a μ-oxo dimer. Above pH 13, he formulates a new species in which hydroxide ions are formed from the water molecules. White [35] suggests that knowing the structures of high spin Fe(III) complexes [7] of porphyrins, it may be that below pH 13 no ligands are held by the high-spin oligomer, while above pH 13, two added hydroxides give rise to a low-spin oligomer.

Berdnaski and Jordan [9, 10] found Fe(III) protoporphyrin dimeric in the entire pH range 7–13 but found that the dimer existing above 12.5 is smaller than that at lower pH. Such additions of hydroxide ions to the outside coordination sites of the oligomer should induce a low-spin iron, which in turn must make each iron atom come into the plane of the porphyrins.

We report here the results of a Mössbauer investigation of ^{57}Fe enriched protoporphyrin-iron(III) in frozen solution of the pH range 6.0 to 13.1.

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Experimental

Preparation of ^{57}Iron–Protoporphyrin IX

This compound was prepared according to the method of Caughey [36]. A refluxing solution of Na_2PPIX in glacial acetic acid in N_2 atm was treated with ferrous acetate solution (prepared by dissolving ^{57}Fe in glacial acetic acid) and the mixture refluxed until the reaction was complete. The hot solution was then diluted with hot saturated NaCl aqueous solution, and then with cold distilled H_2O. The precipitate was collected and washed several times with dilute acetic acid and H_2O, and dried. The ^{57}Fe was supplied by A.E.R.E. stable isotope division.

Preparation of ^{57}Iron–PP1X Solution

5 mg of enriched ^{57}Fe–PP1X was dissolved in 0.5 ml 1 N NaOH solution and then diluted to 5 ml with distilled water. The solution was centrifuged to get rid of the insoluble particles. The pH of the solution was adjusted as required by adding NaOH pellet for $pH > 12$ or by adding 1 N HCl for $pH < 12$. These solutions were frozen in perspex cells for the Mössbauer experiments.

Preparation of the Sodium Salt of the μ-Oxo-oligomer of Protoporphyrin IX Iron(III)

To 200 mg of haematin in a 500 ml beaker, 5 ml of 1 N HNO_3 was added, followed by 200 ml of acetone. The solution was then filtered from the undissolved particles. To this solution, 0.3 N NaOH solution was added drop by drop until a green precipitate appeared. The amount of 0.3 N NaOH solution used was 20 ml. The precipitate was collected and washed several times with a solvent made up from 95% acetone + 5% H_2O, and then dried in an oven for several hours at 110 °C. *Anal.* Calcd. for $C_{68}H_{60}Fe_2N_8O_9Na_4$: C, 61.03; H, 4.49; Fe, 8.35; N, 8.38; Na, 6.88; Found: C, 61.4; H, 4.7; Fe, 8.3; N, 8.35; Na, 7.1.

Instrumentation

Electronic spectra were recorded on a Beckman DU 7 spectrophotometer in 1 cm^2 cells, using solutions of haematin concentrations 50.5 μ*M*. Infra-red spectra were recorded from nujol mulls using a Perkin-Elmer 257 spectrometer.

The Mössbauer spectra were recorded on an instrument previously described [37]. The source was ^{57}Co (10 mCi) in rhodium (Radiochemical Centre, Amersham) at 20 °C. The spectrometer was operated in a saw tooth mode and the spectra computer fitted. The spectrometer was calibrated with a 25 μ*M* thick natural iron reference absorber. All isomer shifts are referred to this as zero shift.

Results and Discussion

The Mössbauer data obtained in this work are tabulated in Table I.

TABLE I. Mössbauer Parameters of Solids Isolated from Frozen Solutions of Protoporphyrin IX Iron(III) Solution.

pH	δ/mm s^{-1}	Δ/mm s^{-1}	Γ/mm^{-1}		% Abs. Area	
4.4†	0.41(1)	0.58(1)	0.15(1)		20(3)	
solid	0.42(2)	1.06(2)	0.36(2)	0.46(5)	45(3)	35(4)
5.5†	0.41(1)	0.58(1)	0.15(1)		30(2)	
solid	0.41(3)	1.01(7)	0.37(2)	0.71(9)	36(2)	34(5)
6.0†	0.41(1)	0.58(1)	0.15(1)		7(2)	
frozen solution	0.41(1)	1.03(7)	0.45(2)	0.61(6)	53(2)	41(4)
6.7†	0.41(1)	0.58(1)	0.15(1)		32(7)	
frozen solution	0.40(5)	1.00(8)	0.26(1)	0.27(1)	43(5)	25(6)
8.0	0.39(1)	0.60(1)	0.23(1)		100(4)	
frozen solution						
9.4	0.39(1)	0.60(1)	0.23(1)		100(4)	
frozen solution						
12.0	0.41(1)	0.58(1)	0.18(1)		100(5)	
frozen solution						
13.0	0.40(1)	0.58(1)	0.18(1)		100(3)	
frozen solution						
14.4*	0.41(1)	0.58(1)	0.15(1)		100(5)	
frozen solution						

All spectra run at 80 K. †These spectra fitted by holding δ, Δ and Γ for the μ-oxo-oligomer constant and only varying the intensity of this site. *Calculated pH, see footnote in text.

In frozen solution at pH 6.0 and 6.7 the spectra obtained can be computer fitted to a combination of protoporphyrin iron(III) and to μ-oxo oligomer of protoporphyrin iron(III) [1, 2]. These results are significant as they show the presence of the μ-oxo oligomer in solutions that were purposely aged at these pH values for two days and centrifuged before their frozen solution Mössbauer spectra were obtained. Solids obtained from similar solutions at lower pH values could be fitted similarly (Table I), and this indicates the extreme stability of the μ-oxo oligomer even at low pH.

At higher pH values the Mössbauer spectrum of frozen solutions is that of Fe(III) μ-oxo oligomer, and this spectrum persists at pH's up to and including 13.25.* Even at pH 13.25 there was no Mössbauer evidence for any other species present in contrast to the work of Walters [6] on Fe(III) deuteroporphyrin dimethyl ester disulphonate dimers.

The asymetric spectra observed for protoporphyrin IX iron(III) are explained in the same way as for haemin by Blume [38] in terms of a temperature-dependent spin–spin relaxation process. The μ-oxo oligomer (which gives symmetric Mössbauer spectra) contains two high-spin (S = 5/2) iron(III) ions antiferromagnetically coupled through the oxygen bridge. Since no fluctuations of the electron spins are possible under these conditions the Mössbauer spectrum is a symmetric doublet. We note that the Mössbauer parameters we report for μ-oxo oligomer are similar to other μ-oxo species reported previously [1, 2, 24, 39]. It must be noted that though all solutions contain no precipitate before freezing, on the event of freezing (even rapid freezing), equilibria may shift during cooling [40]. If this happens, then Mössbauer parameters for the frozen solution reflect the structure not of the initial room temperature solution, but of the solution at the solidification temperature.

In connection with the mechanism of rapid freezing it must be appreciated that some segregation of the Mössbauer nucleus may form one amorphous phase species and aggregations in the glasslike phase may be produced. This process can in theory be followed by means of paramagnetic spin relaxation in solutions that contain iron(III), as an increasingly closer approach of the iron(III) atoms increase the spin–spin interactions and the frequency of spin relaxation. This results in the disappearance of the magnetic hyperfine structure. Our data shows no such hyperfine structure. However, we note that such structure was only found for haemin solutions at very low temperatures (4.2 K) with very good counting statistics. Even at 4.2 K Lang *et al.* [41] found a major component of their spectrum was similar to that of crystalline haemin. This they attribute to Fe sites on which the electron spins are relaxing rapidly. Our low pH spectra, where monomeric protoporphyrin IX iron(III) is present, could be similarly explained.

In our low pH spectra, aggregation of the monomers via vinyl group interactions for example, could account (by the close mutual approach of the protoporphyrin IX units this would necessitate) for the facile rapid relaxation of electron spins. This is the only evidence we have (and this tentative) for greater aggregates than that of the μ-oxo oligomer.

Previous work from this laboratory and other workers have shown the affinity of the protoporphyrin carboxylate groups for metal ions [1, 2, 42]. If our Mössbauer data is that of a crystallized out solid then it is likely to be a Na^+ salt of the Fe(III) μ-oxo oligomer. The recorded spectra are similar to those we have previously reported for the solid dimer precipitated at very high pH. If species such as those proposed as possibilities by Brown *et al.* [12] *viz.*:

H_2O–Fe(por)–O–Fe(por)–OH

and

HO–Fe(por)–O–Fe(por)–OH

had been present then it is likely that such species would only be present at pH 13 and be the most likely to be isolated from such pH values. However, it is unlikely that such species would crystallize at pH values around 6. It is therefore more likely, especially when account is taken of the low protoporphyrin concentrations used here, that the frozen solutions have not deposited solid μ-oxo oligomer but are still homogeneous. Yet the Mössbauer parameters for the frozen solution spectra show the same parameters as solid μ-oxo oligomer [1, 2]. This would suggest that in solution the structure of the μ-oxo oligomer contains no additional ligands. Caughey *et al.* [43] reported that they have been unable to detect ligand binding irons to the Fe–O–Fe linkage. Indeed they do not expect such ligand binding. They argue that with the iron displaced towards the oxygen by 0.5 Å it would be difficult for any additional ligands to be attached to the iron. No interaction between the Fe(III) protoporphyrin dimethyl ester μ-oxo oligomer and imidazole in chloroform solution was found [44].

However, Gallagher and Elliot [45] report the formation of an Fe(II) protoporphyrin dimer in NaOH/pyridine solution. Their proposed structure is $[Fe(III)P)py)OH]_2$ which is indistinguishable by methods they used from a μ-oxo oligomer with pyridine as additional ligands on the outside. Strong and Hartzell [8] report a bis-imidazole hydroxo-bridged species with Fe(III) TCPP.

White [35] finds the arguments of Caughey persuasive with regard to any high-spin iron μ-oxo oligomers. He states "the only reasonable manner in which additional ligands would be added *trans* to the

*This is a pH meter reading. The calculated pH from the amount of NaOH used to prepare this solution was 14.397.

Fe–O–Fe linkage is through formation of a low spin oligomer". We agree with this view and indeed no evidence for low-spin iron(III) is found in the Mössbauer spectra reported in this work.

Electronic Spectra

Previous reports [12, 19, 25, 46–48] of electronic spectra of protoporphyrin IX iron(III) have concentrated on aggregation of porphyrins around pH 7. Fig. 1 shows spectra of the Soret region of protoporphyrin IX iron(III) in the pH range 5.88 to 13.04.

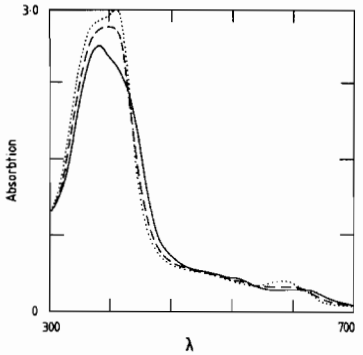


Fig. 1. Absorption spectra of protoporphyrin IX iron(III) solutions of 50 μM at pH s —— 5.88, – – – 8.2, ····· 13.04.

According to previous work [46–48] aggregated states are characterised by broad spectral bands of relatively low intensity whereas the monomeric species give rise to sharp intense Soret bands with maxima at rather longer wavelengths. All the spectra we report are of solutions containing 50.5 μm of protoporphyrin IX iron(III), and all are of freshly prepared solutions recorded within 10 minutes of preparation (Table II).

The spectra at high pH are similar to those reported by Maehly and Åkeson [19], whereas those recorded at pH's below 7 show a maximum at lower wavelength. The total bandwidth in the Soret region does not change much over the entire pH range studied. We interpret the spectra showing a Soret maximum at 365 nm (lower pH) to contain predominantly monomer (as confirmed in the Mössbauer data) and those that contain a Soret maximum at 387 nm to contain predominantly μ-oxo oligomer (indeed, only μ-oxo oligomer is found in the Mössbauer data). Figure 2 shows the presence of an

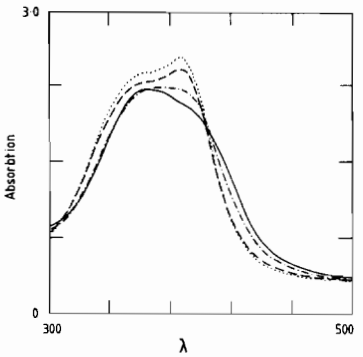


Fig. 2. Absorption spectra of protoporphyrin IX iron(III) solutions of 50 μM at pH s ····· 12.9, – – – – 10.4, – · – · – · 7.5, —— 5.54.

isosbestic point (401 nm) indicating two major components. We have little evidence for greater aggregation than that of the μ-oxo oligomer in our studies though we did not do any time-dependence studies. We also note that our Mössbauer data was collected on frozen solutions that were 30 times more concentrated than those used for the electronic spectra.

The ability of metal cations to bind the propionic acid groups [1, 2, 42] has been overlooked by most workers in the field. Such binding between the acid groups of neighbouring molecules via cations could be a major initiator of aggregation. Brown *et al.* [46]

TABLE II. Electronic Spectra.

pH	λ_1	ϵ mM	λ_2	ϵ mM	λ_3	ϵ mM	λ_4	ϵ mM
5.9	365	52.04					634	4.36
6.9	366	52.04					631	4.71
7.6	369	53.30					633	4.51
8.2	373	55.60	384	55.60	608	5.09	632	4.71
9.4	373	*	386	57.40	608	5.74		
10.4	374	*	387	58.16	608	5.96		
11.1	374	*	385	58.46	608	5.98		
11.9	374	*	387	57.92	608	5.86		
12.6	374	*	387	58.89	608	5.96		
13.0	374	*	386	59.23	609	5.84		

[a] λ_1 and λ_2 are Soret bands. λ_1 and λ_4 are due to the monomer of protoporphyrin IX iron(III). λ_2 and λ_3 are due to the μ-oxo oligomer of protoporphyrin IX iron(III). *There is a component due to λ_1 whose intensity diminishes as the pH increases.

commenting on results of Abraham *et al.* [49] suggest that in aqueous solution aggregation may involve the charged carboxyl groups in porphyrin side chains. They further suggest that cations may hold two porphyrin molecules together, but neither link these suggestions nor speculate into the nature of possible cations.

From Table II is it noticeable that both protoporphyrin IX–iron(III) and the μ-oxo oligomer show a different distinctive band between the 600–650 nm region at 632 and 608 nm respectively. Again isosbestic points are seen associated with these bands (Fig. 3).

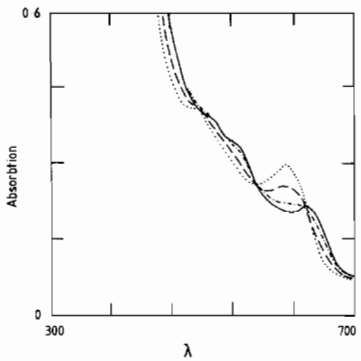


Fig. 3. Absorption spectra of protoporphyrin IX iron(III) solutions of 50 μM at pHs —— 5.88, – · – · – 7.57, – – – – 8.2, ····· 13.04.

Although these two bands are not very intense they are obviously useful in characterising the components of protoporphyrin IX iron(III) solutions.

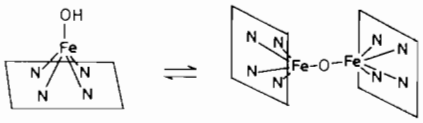
We have previously reported the reflectance electronic spectra for both protoporphyrin IX iron(III) and its μ-oxo oligomer [1]. We reported the presence of a band at 900 nm in protoporphyrin IX iron(III) which is absent in its μ-oxo oligomer. So in all there are three major bands in the electronic spectrum of protoporphyrin IX iron(III) and only two major bands in that of its μ-oxo oligomer.

Infra-red Spectra

Infra-red spectra for μ-oxo oligomer have been reported by Brown *et al.* [50]. We have prepared the μ-oxo oligomer by their aqueous method as well as our own. Our work suggests that both methods result in the formation of sodium salts, since the band at 1720 cm^{-1} (in haematin) [1] which is due to the C=O stretching of the propionate groups has disappeared and two new bands at 1560 and 1410 cm^{-1} due to asymetric and symmetric C–O stretching frequencies of deprotonated carboxylic groups are present. Unfortunately, Brown *et al.* [50] do not provide chemical analysis for their materials.

Conclusion

From the Mössbauer data and electronic spectra it would appear that the aqueous chemistry of protoporphyrin IX iron(III) solutions can be explained as that of a binary system where the concentration of any intermediate is very small indeed. The system is pH and time dependent. The μ-oxo oligomer has been shown to be very stable and is dominant at pHs above 7.0 and can exist for long periods of time below pH 7. The system is best understood in the following scheme



No stability at pHs above 7

Stable at pH 7 or above, also stable below pH 7 for long periods

In this scheme we represent the monomeric protoporphyrin IX iron(III) as a five coordinate high spin iron(III) species in solution. Though previously many workers have considered it to be a six-coordinated species, Torrens *et al.* [39] rightly state "Monomeric 6-coordinated iron(III) hydroxo complexes are unknown except perhaps for protein derivatives such as methemoglobin and metmyoglobin, where the large protein moiety sterically prevents dimerisation."

Support for the protoporphyrin IX iron(III) being five coordinate in solution comes from known crystal structures of high spin Fe(III) complexes [29, 51–55].

We note that Brown *et al.* [12, 46] found for a solution of concentration 100 μM only 1% of the protoporphyrin IX iron(III) was present as monomer at pH 7. Our fresh solutions of half this concentration showed greater amounts in their electronic spectra. Mössbauer data for solutions of concentration about 1500 μM show the presence of monomeric protoporphyrin IX iron(III) in large amounts at pH 6.7.

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