

## Studies on Metal–Protoporphyrin–Iron(III) Complexes

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*Metal–protoporphyrin–iron(III) complexes are presented for the metal ions Al(III), Ba(III), Cd(II), Ca(II), Cr(III), Co(II), Fe(III), Mn(II), Ni(II), Pb(II), Ag(I), and Zn(II). These are distinct new species by comparison with similar Cu(II)–protoporphyrin–iron(III) complexes. Infrared spectroscopy provides evidence for the metal binding to protoporphyrin propionate groups. Mössbauer data and magnetic susceptibility data are reported for the complexes. There is evidence for interactions between the two metal nuclei.*

### Introduction

In 1923 Hamsik [1] reported the preparation of potassium, barium, calcium, silver, and lead salts of protoporphyrin IX–iron(III) but the stoichiometries were not fully established. We have reported studies on copper protoporphyrin–iron(III) complexes [2], one of which is a possible model for cytochrome *c* oxidase.

Such complexes formed between iron and the porphyrin group, or its derivatives, and with other macrocyclic ligands are useful models for haem proteins. Collman and co-workers [3–6] have synthesized a series of picket fence iron porphyrin complexes as models for the oxygen transport proteins.

The copper protoporphyrin–iron(III) [2] complex, though not an accurate mimic of the physical properties of cytochrome *c* oxidase, has some appeal-

ing features. Primarily no specialised architectural molecule has had to be prepared for the Cu–Fe complex formation. The studies we reported were in general agreement with other reports on model compounds [7–9] for cytochrome *c* oxidase, but all their models contained ligands that have not been found in naturally occurring systems.

The complexes we reported have the advantages that they are relatively easily prepared and in so far as the binding site for metal ions involves the haem carboxylate groups and may actually occur naturally as these groups are present in haem proteins.

We have extended our work on protoporphyrin–iron(III) complexes and report here studies on complexes containing the metals: aluminium, barium, cadmium, calcium, chromium, cobalt, iron, manganese, nickel, lead, silver and zinc.

### Experimental

Metal–protoporphyrin–iron(III) complexes were prepared which had stoichiometric ratios of 1:1 and 2:1 M:Fe. Haematin solutions were prepared from haematin (200 mg) (from Bovine blood, Sigma) which was first dissolved in NaOH (5 cm<sup>3</sup>, 1 mol dm<sup>-3</sup>). Complexes were prepared by similar methods to those reported previously [2]. The pH was adjusted by addition of dilute nitric acid until precipitation occurred (pH for each complex is given in Table I). The precipitates were filtered off and washed several times with water. The yields were about 90%. The compounds were used without further purification since it proved impossible to dissolve them to any extent without decomposition occurring.

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TABLE I. Elemental Analysis (%).<sup>a</sup>

	C	H	N	Fe	M	Precipitation pH
Co <sub>2</sub> haematin-H <sub>2</sub> O	50.3	4.4	6.6	7.1	12.9	6.4
C <sub>34</sub> H <sub>37</sub> Co <sub>2</sub> FeN <sub>4</sub> O <sub>9</sub>	(49.85)	(4.52)	(6.84)	(6.82)	(14.39)	
Co haematin-H <sub>2</sub> O	57.9	4.8	7.8	8.1	4.7 <sup>b</sup>	6.0
C <sub>34</sub> H <sub>35</sub> CoFeN <sub>4</sub> O <sub>7</sub>	(56.32)	(4.83)	(7.73)	(7.73)	(8.13)	
Zn <sub>2</sub> haematin-H <sub>2</sub> O	46.2	4.1	6.70	6.7	16.2	7.2
C <sub>34</sub> H <sub>37</sub> Zn <sub>2</sub> FeN <sub>4</sub> O <sub>9</sub>	(49.85)	(4.52)	(6.84)	(6.82)	(15.96)	
Zn haematin-H <sub>2</sub> O	52.7	4.4	7.5	6.7	6.9	6.9
C <sub>34</sub> H <sub>35</sub> ZnFeN <sub>4</sub> O <sub>7</sub>	(55.74)	(4.78)	(7.65)	(7.62)	(8.92)	
Ni <sub>2</sub> haematin-H <sub>2</sub> O	50.6	4.5	6.8	6.9	13.8	7.0
C <sub>34</sub> H <sub>37</sub> Ni <sub>2</sub> FeN <sub>4</sub> O <sub>9</sub>	(49.88)	(4.52)	(6.84)	(6.82)	(14.34)	
Ni haematin-H <sub>2</sub> O	56.3	5.0	7.9	7.1	6.7	6.3
C <sub>34</sub> H <sub>35</sub> NiFeN <sub>4</sub> O <sub>7</sub>	(56.25)	(5.10)	(7.72)	(7.70)	(8.09)	
Cd <sub>2</sub> haematinH <sub>2</sub> O	45.5	4.1	6.4	6.0	23.9	9.8
C <sub>34</sub> H <sub>37</sub> Cd <sub>2</sub> FeN <sub>4</sub> O <sub>9</sub>	(44.1)	(4.0)	(6.05)	(6.03)	(24.28)	
Cd haematinH <sub>2</sub> O	55.0	4.4	7.2	7.2	12.6	8.8
C <sub>34</sub> H <sub>35</sub> CdFeN <sub>4</sub> O <sub>7</sub>	(52.38)	(4.49)	(7.19)	(7.16)	(14.42)	
Fe(III)-haematinH <sub>2</sub> O	56.5	5.1	7.8	15.5		5.9
C <sub>34</sub> H <sub>35</sub> Fe <sub>2</sub> Al <sub>2</sub> N <sub>4</sub> O <sub>7</sub>	(56.06)	(4.87)	(7.78)	(15.54)		
Al <sub>2</sub> haematin-H <sub>2</sub> O	54.1	4.8	6.1	7.5	8.5	7.3
C <sub>34</sub> H <sub>37</sub> FeN <sub>4</sub> O <sub>9</sub>	(54.0)	(4.9)	(7.41)	(7.41)	(7.14)	
Ag <sub>2</sub> haematin-H <sub>2</sub> O	46.5	3.9	6.4	6.1		6.1
C <sub>34</sub> H <sub>37</sub> FeAg <sub>2</sub> N <sub>4</sub> O <sub>9</sub>	(44.48)	(4.03)	(6.10)	(6.10)		

<sup>a</sup>The calculated values (given in parentheses) for the 1:1 complexes are based on structure A with the addition of one water molecule. The calculated values for the 2:1 complexes are based on a structure containing one haematin moiety (minus two H<sup>+</sup> ions), two M<sup>2+</sup> ions, two OH<sup>-</sup> ions, and an additional water molecule. <sup>b</sup>This material appears always to be low on cobalt.

Analyses were carried out by the Micro Analytical Laboratory, Department of Chemistry, University of Manchester, and are given in Table I.

The number of water molecules is uncertain on the basis of these data, but one molecule has been included for calculating as this gives the best overall fit to the data in each case.

Complexes reported previously by Hamsik [1] were not analysed here. Cr and Mn complexes were also prepared but these have not been fully characterised though their i.r. and Mössbauer data are presented.

Infra-red spectra were recorded from mulls using a Perkin-Elmer 257 spectrometer. Electronic reflectance spectra of powders were recorded on a Unicam SP 700 spectrophotometer using alumina as reference. Magnetic susceptibilities of powders were measured by the Gouy method at room temperature. Tubes were packed several times to check for orientation errors.

The Mössbauer spectra of the powders were recorded on a conventional constant acceleration

spectrometer previously described [10–11]. The source was  $\approx 11$  mCi\* of <sup>57</sup>Co in rhodium (obtained from the Radiochemical Centre, Amersham) at 20 °C.

## Results and Discussion

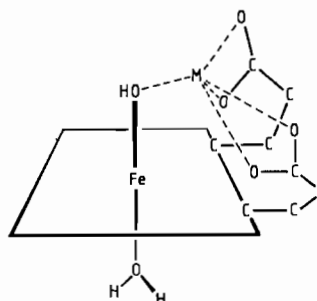
The analytical results for the metal–protoporphyrin–iron(III) complexes are presented in Table I. The analytical results though not marvellous nevertheless establish the stoichiometries of the complexes. We have previously shown for the copper protoporphyrin–iron(III) complexes that these materials are not coprecipitates of haematin and copper(II) hydroxide, and do not think it necessary to repeat the arguments here. Haematin usually precipitates at pH 4.6, the complexes in Table I all precipitate at the pHs' given in the Table. Most of these complexes precipitate at different pHs' than the corresponding metal hydroxides.

TABLE II. Infra-red Absorption Peaks in the Region 1800–1400  $\text{cm}^{-1}$ .<sup>a</sup>

Haematin	PP 1X	Na <sub>2</sub> -PP 1X	Metal-haematin complexes
1720 s	1700 s(a)		1720
1660 sh			1660 sh
1630 m			
1445 m	1435 sh	1550 vs(b)	1560 vs(b)
1410 sh	1415 sh	1435 sh	1445 sh
		1405 s(c)	1410 s(c)

<sup>a</sup>All spectra were obtained from mulls. PP1X = protoporphyrin IX. Strong peaks are assigned as: (a) C=O stretching of protonated carboxylic groups; (b), (c) asymmetric and symmetric C–O stretching vibrations of deprotonated carboxylic groups.

Infra-red spectra of the complexes in the region 1400–1800  $\text{cm}^{-1}$  are all similar and are given in Table II, and are similar to those for the copper complexes. Changes in the carbonyl stretching frequencies compared to haematin are observed. Such changes rule out the presence of unreacted haematin and are consistent with structures like A where the haem propionic carboxylate groups are bound to the metal.



Schematic representation of 1:1 metal protoporphyrin iron(III) complex showing the position of the metal atom above the haem plane and coordinated to the two propionic carboxylate groups and to an hydroxyl group which acts as a bridge to the Fe atom. A scale model of this structure indicates that when the metal is Cu the Cu–O bond length is about 1.97 Å. When the metal is Al(III), Cr(III) or Fe(III), there will be an additional OH<sup>−</sup> in the structure otherwise an additional H<sub>2</sub>O will be present.

Similar changes of the carbonyl stretching frequencies are found for disodium protoporphyrinate, compared to protoporphyrin-free acid (Table II).

The Mössbauer data for haematin,  $\mu$ -oxo-dimeric haematin and the metal haematin complexes are given in Table III, and the spectrum of the cadmium proto-

TABLE III. <sup>57</sup>Fe Mössbauer Parameters for the Haem Complexes.

	T K	$\delta/\text{mm s}^{-1}$	$\Delta/\text{mm s}^{-1}$	$\Gamma/\text{mm s}^{-1}$	
Haematin	298	0.20(2)	0.78(2)	0.24(4)	0.51(1)
	80	0.384(13)	0.8813	0.26(1)	0.31(2)
Haematin dimer	298	0.32(1)	0.58(2)	0.16(1)	0.16(1)
	80	0.40(1)	0.57(1)	0.17(1)	0.17(1)
Nickel protoporphyrin–iron(III) (1:1)	298	0.29(2)	0.67(2)	0.21(0)	0.23(1)
	80	0.40(1)	0.70(1)	0.19(1)	0.19(1)
Nickel protoporphyrin–iron(III) (2:1)	298	0.32(1)	0.70(1)	0.21(1)	0.23(2)
	80	0.40(1)	0.68(1)	0.18(0)	0.18(1)
Zinc protoporphyrin–iron(III) (1:1)	298	0.29(2)	0.68(2)	0.21(1)	0.20(1)
	298	0.40(1)	0.67(1)	0.19(0)	0.19(1)
Zinc protoporphyrin–iron(III) (2:1)	298	0.30(1)	0.64(1)	0.19(1)	0.18(1)
	80	0.40(1)	0.65(1)	0.18(1)	0.17(1)
Cadmium protoporphyrin–iron(III) (1:1)	298	0.30(2)	0.63(2)	0.18(1)	0.19(1)
	80	0.40(1)	0.64(1)	0.19(1)	0.18(1)
Cadmium protoporphyrin–iron(III) (2:1)	298	0.30(3)	0.62(1)	0.17(0)	0.18(0)
	80	0.39(2)	0.64(1)	0.19(0)	0.19(0)
Calcium protoporphyrin–iron(III) (1:1)	298	0.23(3)	0.69(3)	0.24(2)	0.35(4)
	80	0.39(2)	0.71(2)	0.24(1)	0.24(2)
Lead protoporphyrin–iron(III) (1:1)	298	0.26(3)	0.58(3)	0.16(2)	0.19(3)
	80	0.39(2)	0.64(2)	0.20(1)	0.18(1)
Lead protoporphyrin–iron(III) (2:1)	298	0.27(4)	0.55(4)	0.23(3)	0.27(3)
	80	0.40(2)	0.59(2)	0.20(1)	0.20(2)

(continued overleaf)

TABLE III. (continued)

	T K	$\delta/\text{mm s}^{-1}$	$\Delta/\text{mm s}^{-1}$	$\Gamma/\text{mm s}^{-1}$	
Aluminium protoporphyrin-iron(III) (1:1)	80	0.39(3)	0.65(2)	0.24(2)	0.22(3)
Aluminium protoporphyrin-iron(III) (2:1)	80	0.44(5)	0.62(5)	0.20(2)	0.19(2)
Chromium protoporphyrin-iron(III) (1:1)	80	0.38(3)	0.66(5)	0.21(2)	0.22(3)
Chromium protoporphyrin-iron(III) (2:1)	80	0.39(3)	0.67(4)	0.21(1)	0.20(1)
Cobalt protoporphyrin-iron(III) (1:1)	298	0.30(2)	0.63(2)	0.22(2)	0.22(2)
Cobalt protoporphyrin-iron(III) (1:1)	80	0.38(3)	0.70(3)	0.25(2)	0.24(3)
Cobalt protoporphyrin-iron(III) (2:1)	80	0.39(4)	0.67(3)	0.19(1)	0.23(2)
Manganese protoporphyrin-iron(III) (1:1)	80	0.40(2)	0.72(2)	0.32(1)	0.32(2)
Silver protoporphyrin-iron(III) (2:1)	80	0.38(2)	0.70(3)	0.25(1)	0.28(3)
Barium protoporphyrin-iron(III) (1:1)	80	0.44(3)	0.69(3)	0.31(2)	0.35(3)
Iron(III) protoporphyrin-iron(III) (1:1)	298	0.36(1)	0.69(1)	0.19(1)	0.18(1)
		0.34(1)	1.19(1)		0.14(1)
	80	0.46(1)	0.64(1)	0.20(1)	0.19(1)
		0.46(1)	1.07(1)		0.21(1)

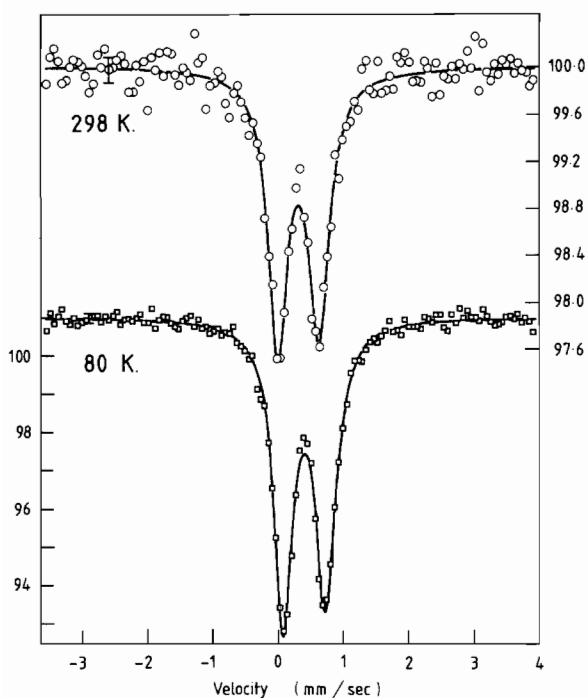


Fig. 1. Mössbauer spectrum of 1:1 cadmium-protoporphyrin-iron(III) complex at 80 K ( $\square$ ) and 298 K ( $\triangle$ ). At 80 K  $\delta = 0.40(1)$ ,  $\Delta = 0.65(1)$ ,  $\Gamma = 0.19(1)$   $\text{mm s}^{-1}$ . At 298 K  $\delta = 0.30(1)$ ,  $\Delta = 0.64(1)$ ,  $\Gamma = 0.19(1)$   $\text{mm s}^{-1}$ .

protoporphyrin-iron(III) complex is also shown in Fig. 1. The data for the metal protoporphyrin-iron(III) complexes are in keeping with the iron being high-spin iron(III) but with some indications of unusual spin coupling. The quadrupole splittings for the 1:1 complexes are  $0.64\text{--}0.72$   $\text{mm s}^{-1}$  at 80 K and for the 2:1 complexes  $0.62\text{--}0.71$   $\text{mm s}^{-1}$  at 80 K. The Mössbauer data are in keeping with those found for the copper protoporphyrin-iron(III) complexes. Though there is some asymmetry in many of the spectra there is no variation between 80 K and room temperature unlike other synthetic and biological ferrohaems [12–18]. The asymmetric spectra observed for haematin are explained in the same way as for haemin by Blume [19] in terms of a temperature dependent spin-spin relaxation process. The  $\mu$ -oxo-dimeric haematin (which gives symmetric Mössbauer spectra) contains two high-spin ( $S = 5/2$ ) iron(III) ions antiferromagnetically coupled through the oxygen bridge. As no electron spin fluctuations are possible under these conditions the Mössbauer spectrum is a symmetric doublet.

Though many of the complexes reported here have paramagnetic metals present as well as iron(III) not all (Ag, Ca, Ba, Pb, Zn and Cd do not) have unpaired electrons. Those that do may also be antiferromagnetically coupled and the extent to which the coupling takes place will cause spin-spin relaxation to be manifest in the Mössbauer spectra, *i.e.* the

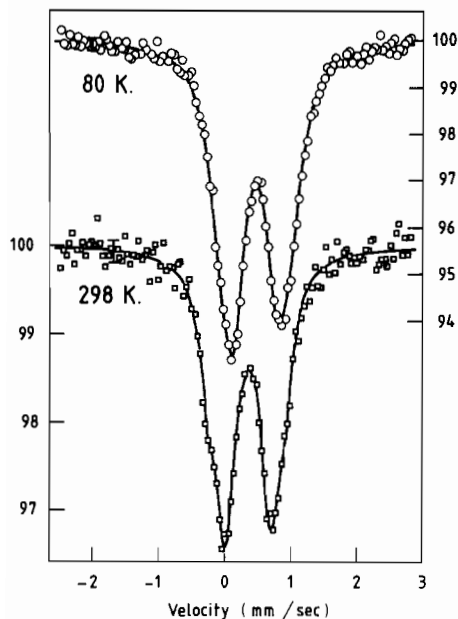


Fig. 2. Mössbauer spectrum of 1:1 iron(III)-protoporphyrin-iron(III) complex at 80 ( $\Delta$ ) and 298 K ( $\square$ ). At 80 K  $\delta = 0.45(1)$ ,  $\Delta = 1.07(1)$ ;  $\delta = 0.46(1)$ ,  $\Delta = 0.64(1)$ . At 298 K  $\delta = 0.34(1)$ ,  $\Delta = 1.19(1)$ ;  $\delta = 0.36(2)$ ,  $\Delta = 0.69(1)$ .

spectra become symmetric. However, as such a coupling could not involve all the electrons, some fluctuations of the electron spins may still be possible and cause the residual asymmetry observed in the spectra. Why the non-paramagnetic metals produce more symmetric Mössbauer spectra in Fig. 1 is not obvious but may be due to some sort of interaction with the protoporphyrin  $\pi$  clouds.

The quadrupole splittings found for the metal protoporphyrin-iron(III) complexes are larger than those found for  $\mu$ -oxo-dimeric haematin but lower than those of haematin. The isomer shifts are all similar. We have previously suggested for the copper complexes that the Cu atoms may be antiferromagnetically coupled to the haematin ion atoms via oxygen ligands, and obviously this would be possible for the paramagnetic metals presented here. These arguments would also be consistent with structure A.

The Mössbauer data for iron(III) protoporphyrin-iron(III) complex shows the presence of two iron(III) electronic environments, one of which is similar to the other metal protoporphyrin-iron(III) complexes reported here and is due to the protoporphyrin-iron(III) site, and the other with the larger quadrupole splitting (Fig. 2). This second site with a quadrupole splitting of  $1.07 \text{ mm s}^{-1}$  and isomer shift of  $0.46 \text{ mm s}^{-1}$  shows that the iron(III) is in a high spin state. Such an electronic environment would be expected for the M site in a structure such as A.

TABLE IV. The Magnetic Moments of Metal-Protoporphyrin Iron(III) Complexes.

Compound	$\mu_{\text{eff}}$ at R.T. (BM)
Haematin dimer	2.13
Nickel protoporphyrin iron(III) (1:1)	4.92
Zinc protoporphyrin iron(III) (1:1)	3.98
Cadmium protoporphyrin iron(III) (1:1)	3.92
Cadmium protoporphyrin iron(III) (2:1)	3.49
Calcium protoporphyrin iron(III) (1:1)	4.59
Silver protoporphyrin iron(III) (2:1)	4.35

Reflectance spectra for the cobalt and cadmium protoporphyrin-iron(III) complexes show the same features as those reported previously [2] for the copper protoporphyrin-iron(III) complexes. The band at  $11000 \text{ cm}^{-1}$  ( $900 \text{ nm}$ ) in haematin is much attenuated in these complexes. A much sharper band appears in the spectrum of haemin at  $11000 \text{ cm}^{-1}$ . The fact that this band is so attenuated in these metal-protoporphyrin-iron(III) complexes supports structure A where the OH ligand is bonding to both Fe(III) and other metal and as such would be expected to alter the electronic spectrum.

Room-temperature magnetic susceptibility data (Table IV) show curious values for the complexes. Though the 1:1 Cd:Fe complexes appeared to obey the Curie law over the temperature range 80–300 K, simple spin only treatments are entirely inadequate to interpret these results, especially when it is realised that the presence of metals that do not contain unpaired electrons seems to affect the  $\mu_{\text{eff}}$  values.

The results might suggest the presence of iron of spin lower than  $5/2$  but this is not compatible with the e.s.r. spectrum for copper-protoporphyrin-iron(III) reported previously, which was typical of high spin iron(III), or with the Mössbauer data which show only high-spin iron(III).

## Conclusions

All the studies reported here show that the complexes presented here are discrete compounds. The i.r. data provide evidence for the presence of  $\text{COO}^-$  groups. We postulate structure A for the 1:1 metal-protoporphyrin-iron(III) complexes and using molecular models, we have previously shown that such a structure could be formed incorporating Cu–O bond lengths (*ca.*  $1.97 \text{ \AA}$ ) and without strain.

It is worth noting that as all these complexes form so readily, the affinity of the carboxylate groups for metals must be high. Studies are currently in progress to examine the chelation properties of these carboxylate groups and the possible implications for biological systems.

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