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Intermediate Spin Protoporphyrin(IX) Iron(III) Complexes

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Abstract

'Intermediate' spin iron(III) has been identified, using Mössbauer spectroscopy, in materials containing protoporphyrin(IX) iron(III). These materials were all precipitated from acid pH in the presence of a variety of ligands. The implications of the results are discussed both in comparison to other known 'intermediate' spin porphyrins and for their relevance to haem proteins.

Introduction

Over the last few years intermediate spin porphinato iron(III) complexes have been the centre of much speculation [1–8]. An understanding of their properties may be of use in understanding a number of biologically important haem containing proteins [9–11]. Complexes that fall into this group are not common and in fact nearly all the complexes so far studied have been found to be more accurately described as quantum-mechanical $S = 3/2$, $5/2$ spin-state admixtures [1–8, 12, 13] at room temperatures.

In fact to produce a pure $S = 3/2$ intermediate spin state as has been pointed out, should be straightforward [12]. The species that must display an $S = 3/2$ spin state would be an Fe(III) porphyrin complex that has no axial ligands. Its formulation would be $[\mathrm{Fe(Por)}]^+$ (Por = porphyrin). The resulting tetragonal ligand field would cause one d orbital (the $d_{x^2-y^2}$, the orbital directly facing the porphyrinato nitrogen atoms) to be raised in energy above the other four. This orbital would then be empty in a d^5 state and a pure $S = 3/2$ state would then exist [12].

Scheidt *et al.* have demonstrated that the synthesis of a bare cationic porphyrin complex of the type $[\mathrm{Fe(Por)}]^+$ is not easy [12]. They have shown that there is probably no such thing as a truly non-coordinating ligand [1, 12, 14], though in the complex $[\mathrm{Fe(TPP)(FSbF_5)}]$ the $\mathrm{SbF_6}$ is bonded through a Fe–F bond of 2.105(3) Å, the iron is only a small distance out of the mean porphyrin plane, a nearly pure $S = 3/2$ spin state is found [12].

Toney *et al.* [13] have characterized two perchlorate derivatives of (2,4,6-trimethoxyphenyl)porphinato iron(III) that, they claim are true intermediate spin complexes at room temperature [13]. These complexes, however, have been discussed by others [12] and shown to still have properties (magnetic moments in the solid state) that are consistent with some admixed $S = 3/2$, $5/2$ character at least in the solid state.

These complexes and most of the other well characterized $S = 3/2$, $5/2$ spin admixed species are non naturally occurring methine substituted porphyrins. The only pyrrole ring substituted iron(III) porphyrins showing such a spin state admixture is octaethylporphyrin iron(III) (OEPFe(III)) which is also a man-made porphyrin. The side groups on the pyrrole rings in (OEPFe(III)) are electron donating.

Much of the work of these laboratories has been aimed at the understanding of protoporphyrin(IX) iron (PPIXFe) chemistry, as PPIXFe is one of the most widespread haems found in nature [15–21]. The total number of substituents on the pyrrole rings of PPIX is eight. These are made up of 4 methyl groups (electron donating), one per pyrrole ring, 2 vinyl groups (electron withdrawing) and 2 propionic acid chains (electron withdrawing when protonated) again one per pyrrole ring. Therefore PPIX is much less electron-rich than OEP. It is therefore of great interest to ascertain if PPIXFe(III) is able to sustain an $S = 3/2$ spin state or even an $S = 3/2$, $5/2$ spin admixtures. We report here the results of Mössbauer spectroscopic studies on materials precipitated at low pH from PPIXFe(III) solutions.

Results

The Mössbauer data (Table I and Figs. 1–4) on the precipitated solids all contained evidence of at least two separate iron electronic environments. The first of each of these pairs of sites is typical of an $S = 5/2$ high spin iron(III) complex with an isomer shift in the range 0.34 to 0.48 mm s^{-1} and a quadrupole splitting in the range 1.00 to 1.38 mm s^{-1}. Quadrupole splittings of this size in $S = 5/2$

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TABLE I. ^{57}Mössbauer Spectra of Precipitates Formed from PPIXFe(III) Solutions Made up in Dilute NaOH as Set out Below

Precipitate Number	Starting PPIXFe(III) species	Solutions present	Salts present	Acid used to precipitate solid	δ (mm s^{-1})[a]	Δ (mm s^{-1})	Γ (mm s^{-1})[b]		A (%)			
1	$(PPIXFe)_2O$	$H_2O + NaOH$	Excess KI	Few drops conc. H_2SO_4	0.33(2)	2.98(4)		0.39(3)			31.4(2.9)	
					0.34(1)	1.03(1)	0.22(1)		0.26(1)	36.7(1.4)		32.0(15)
2	$(PPIXFe)_2O$	$H_2O + NaOH$	–	Few drops conc. CF_3COOH	0.39(3)	3.15(5)		0.24(5)			4.7(1.6)	
					0.43(1)	1.38(2)	0.28(1)		0.51(1)	49.8(0.7)		45.6(1.1)
3	$(PPIXFe)_2O$	$H_2O + NaOH$	Excess K_2S	Few drops conc. H_2SO_4	0.37(1)	2.91(2)		0.31(2)			46.6(3.6)	
					0.39(5)	1.10(10)	0.35(3)		0.54(6)	28.2(2.3)		25.8(3.2)
4	$(PPIXFe)_2O$	$H_2O + NaOH$	Excess $NaClO_4$	Few drops conc. H_2SO_4	0.37(1)	3.32(1)		0.21(1)			64.1(3.2)	
					0.48(6)	1.32(20)	0.43(6)		0.48(11)	21.3(3.3)		14.6(3.7)
5	PPIXFeCl	$H_2O + NaOH$	Slight excess $NaCl_4$	Few drops conc. H_2SO_4	0.37(2)	3.26(3)		0.28(2)			31.5(1.3)	
					0.40(1)	1.12(1)	0.29(1)		0.36(1)	39.0(1.0)		29.6(1.1)
6	$(PPIXFe)_2O$	$H_2O + NaOH$	–	Few drops conc. H_2SO_4	0.41(1)	3.17(2)		0.41(1)			22.3(1.9)	
					0.46(2)	1.21(2)	0.29(1)		0.38(2)	43.3(1.3)		34.5(1.5)
7	$(PPIXFe)_2O$	$H_2O:C_2H_5OH$ (1:1) + NaOH	–	Few drops conc. H_2SO_4	0.41(1)	3.20(4)		0.29(3)			18.8(2.4)	
					0.43(2)	1.16(2)	0.34(1)		0.46(3)	49.5(1.8)		31.7(2.3)
8	PPIXFeCl	$H_2O + NaOH$	–	Few drops conc. H_2SO_4	0.37(1)	3.22(2)		0.25(2)			25.1(2.1)	
					0.45(1)	1.24(3)	0.35(1)		0.51(3)	42.7(1.8)		32.2(2.4)

[a] Relative to iron foil. All spectra were collected at 78 K.

[b] Half width at half height (two line widths given when doublet fitted as two single lines).

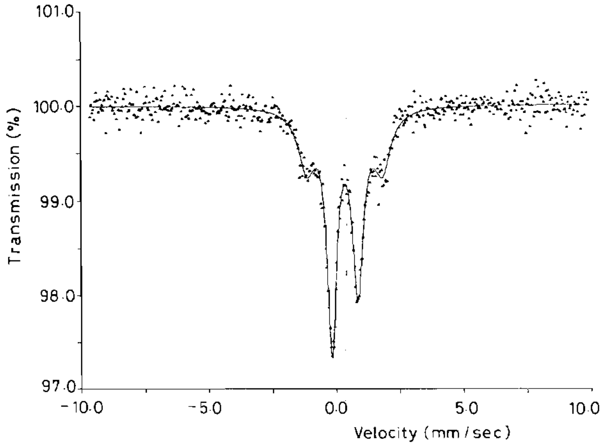

Fig. 1. ^{57}Fe Mössbauer spectrum of precipitate 1 from Table I.

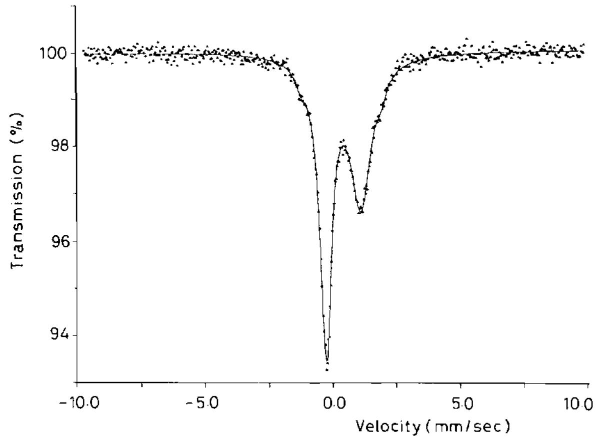

Fig. 2. ^{57}Fe Mössbauer spectrum of precipitate 2 from Table I.

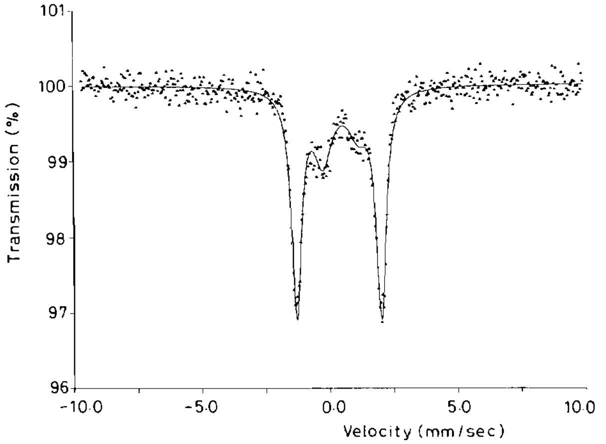

Fig. 3. ^{57}Fe Mössbauer spectrum of precipitate 4 from Table I.

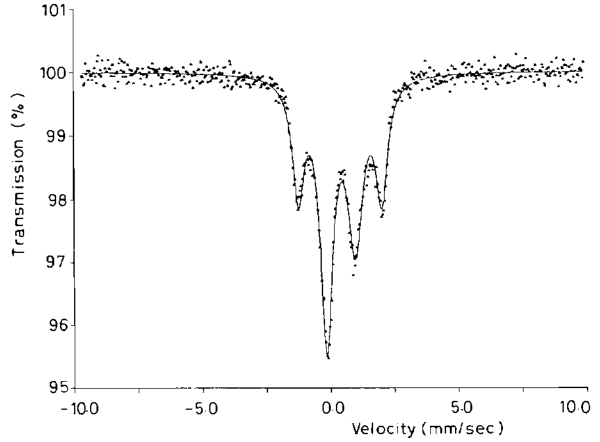

Fig. 4. ^{57}Fe Mössbauer spectrum of precipitate 5 from Table I.

complexes are common for weak axial ligands, stronger axial ligands generate smaller values 0.46 to 0.9 mm s^{-1}. However it is not possible to state directly whether the $S = 5/2$ porphyrinato iron(III) complex is five or six coordinate from the Mössbauer data alone. A number of $S = 5/2$ six-coordinate structures have been solved in which the porphyrin core has expanded to accommodate a high spin iron(III) ion in the plane [22]. Although there may be some evidence to indicate the high spin six-coordinate complexes have larger quadrupole splittings than five coordinate complexes, more data are needed to confirm this finding [14, 23].

PPIXFe(III) complexes (Table I) are pyrrole substituted porphyrins. Previous Mössbauer data on confirmed six-coordinate high spin ferric complexes are confined to methine substituted ferric porphyrins (Table II). There seem to be differences in the quadrupole splittings between methine [14, 24, 25] and pyrrole ring substituted complexes (see Tables I and II).

TABLE II. ^{57}Fe Mössbauer Parameters of some High Spin Ferric Compounds Relevant to This Work

Compound	T (K)	δ^a (mm s^{-1})	ΔE_q (mm s^{-1})	Reference
$[Fe(TPP)(H_2O)_2](ClO_4)$	78	0.33	1.69	24
$[Fe(TPP)(Me_2SO)_2](ClO_4)$	4.2	0.45	1.22	14
$[Fe(TPP)(EtOH)_2](BF_4)$	4.2	0.42	1.89	25

^{a}Relative to iron foil.

The second site seen in each of the Mössbauer spectra presented here (Table I) is characterized by an isomer shift in the range 0.33–0.41 mm s^{-1} and a quadrupole splitting in the range 2.9–3.3 mm s^{-1}. Such Mössbauer data have previously been found for complexes that were either 'pure' $S = 3/2$ iron(III) porphyrin species or spin admixed 3/2, 5/2 iron(III) porphyrin materials (Table III) [1, 2, 5, 12, 13, 26–28, 32–34].

TABLE III. ^{57}Fe Mössbauer Parameters of some 'Intermediate Spin' Ferric Compounds Relevant to This Work

Compound	T (K)	δ^a (mm s^{-1})	ΔE_q (mm s^{-1})	Reference
1. $\mathrm{Fe(TPP)(ClO_4)\cdot 0.5}$*m*-xylene	77	0.38	3.48	1, 26
2. $\mathrm{Fe(TPP)(C(CN)_3)}$	78	0.30	3.18	2
3. $\mathrm{Fe(TPP)(FSbF_5)\cdot C_6H_5F}$	77	0.39	4.29	12, 32
4. $\mathrm{Fe(TPP)(B_{11}CH_{12})\cdot C_7H_8}$	b	0.33	4.12	33
5. $\mathrm{Fe(OEP)(BF_4)}$	115	0.36	3.45	27
6. $\mathrm{Fe(OEP)(ClO_4)}$	115	0.37	3.52	3, 5
7. $\mathrm{[Fe(OEP)(EtOH)_2]ClO_4}$	115	0.36	3.32	5
8. $\mathrm{[Fe(OEP)(3\text{-}Clpy)_2]ClO_4}$	77	0.42	2.68	8
9. $\mathrm{[Fe(OEP)(3\text{-}Clpy)]ClO_4}$	77	0.36	3.23	34
10. $\mathrm{[Fe(TPP)(2,4,6\text{-}OCH_3))](ClO_4)\cdot CHCl_3}$	295	0.41	3.55	13
11. $\mathrm{[Fe(TPP(2,4,6\text{-}OCH_3))](ClO_4)\cdot 2THF\cdot CHCl_3}$	295	0.39	3.28	13
12. Chromatium cytochrome *c'*	42	0.29	2.91	28

^{a}Relative to iron foil. ^{b}Exact temperature not given but temperature range given in 4.2–128 K.

Discussion

It has been found that in the (porphyrinato)-iron(III) complexes previously studied if the starting material has a high-spin ferric structure, the $S = 3/2$, $5/2$ admixture results through weak coordination to perchlorate ions [13]. Porphyrin core contraction that accompanies an increased attraction between the iron atom and the pyrrole nitrogens increases the energy of the $d_{x^2-y^2}$ orbital making it less easily occupied by the fifth d electron, with the result that the electron preferentially occupies the d_{xy} orbital [1, 5, 22]. Other factors that will affect this are:

(1) Additional charge on the pyrrole nitrogen atoms. (Such an effect could be caused by an increase in electron density in the porphyrin 3e(π) molecular orbital which will repel the $d_{x^2-y^2}$ orbital [13]. This could be caused by electron donatory substituent groups strategically placed on the porphyrin periphery.)

(2) Ligands that are weak or sterically hindered (either by themselves or by the substituents on the porphyrin) should also favour the $S = 3/2$ component in the admixture [13].

All these factors *i.e.* weak axial ligands, hindered approach to the iron atom, and strongly donating peripheral substituents on the porphyrin were all features of the (perchlorato)(tetrakis(2,3,6-trimethoxyphenol)porphinato)iron(III) complexes found to be nearly true intermediate spin compounds [12, 13]. The four methyl groups on the PPIXFe(III) periphery are electron donating. Moreover, the vinyl and propionate groups of PPIX can also move out of the mean porphyrin plane possibly forming a form of picket fence [18, 20, 21], so causing steric hindrance to bulky axial ligands and concomitantly with the vinyl groups moving out of the porphyrin plane their electron withdrawing effect will diminish [23]. Thus large weak ligands might be expected to favour true intermediate spin states in PPIXFe(III) complexes. It is pointless to argue whether the large quadrupole splittings found in complexes reported here represent pure $S = 3/2$ or spin admixted $S = 3/2$, $5/2$ iron(III) electronic species. It is however, obvious from this work that a spin state being at least partially $S = 3/2$ is possible in the chemistry of PPIXFe(III).

The actual quadrupole splittings for the $S = 3/2$, $5/2$ site found for these complexes will be dependent on the nature of the fifth/sixth ligand. The species in precipitate 1 (Table I) is likely to have two very weak I^- ligands bonded in the fifth and sixth positions. It is worthy of note that the structure of $\mathrm{[Fe(TPP)(F)_2]^-}$ is known [29] this is a high spin Fe(III) complex. In this structure the F^- atoms are axial bonded to the Fe(III) centres and H-bonded to 2-methylimidazolium cations. The resulting Fe–F bond lengths of 1.966(2) Å are much larger than that of the five-coordinate high-spin [Fe(TPP)F] complex (1.79(3) Å) [30]. It is not unreasonable that a $\mathrm{[Fe(PPIX)I_2]^-}$ complex would be an intermediate spin complex. Precipitate 2 probably has a $\mathrm{CF_3COO^-}$ ion as its sole axial ligand. Precipitate 3 (Table I) is interesting, the likely sulphur ligand species present in these conditions would be $\mathrm{SH^-}$, but the work of English *et al.* [3] shows that a five-coordinate species containing this ligand would be a low spin iron(III) complex. It is likely, therefore, that a mixed hydrogen bonded ligand of the type $\mathrm{SH^-\cdots HSO_4^-}$ is the axial ligand present.

Precipitates 4 and 5 (Table I) have similar large quadrupole splittings and have in common $\mathrm{ClO_4^-}$ present as the main candidate for the axial ligand.

Precipitates 6–8 all have quadrupole splittings around 3.20(3) mm s^{-1} and have $\mathrm{SO_4^{2-}}$ or $\mathrm{HSO_4^-}$ as a possible common candidate for the fifth axial ligand.

These results suggest an ordering of quadrupole splitting depending on the nature of the axial ligand that increases from $SH^- \cdots (H)SO_4^- < 2I^- < CF_3CO_2^-$ or $HSO_4^- < ClO_4^-$, when the porphyrin is PPIX. Unfortunately such a range of ligands has not yet been studied for other porphyrins (*cf.* TPP and OEP, Table III).

An examination of the Mössbauer data in Table I and III is interesting. A surprising variation in the isomer shift can be seen. The structures of entrie 1–4, 6, 8 and 9 in Table III are known [1, 2, 8, 12, 33, 34] of these entries 1, 4 and 9 contain non planar porphyrins that could be described as saddle-shaped. This is most obvious in the case of entry 4 [33]. We note that Gupta *et al.* [33] do refer to the importance of saddle-shaped porphyrins. Saddle-shaped porphyrins have often been associated with π radical cations [35, 36] in ferric metalloporphyrins if the porphyrin is antiferromagnetic coupled to the metal [35]. If the coupling is ferromagnetic, then the porphyrin is planar [35]. Obviously in the case of the data in Table III, the oxidation state of the complexes does not allow full π radical cations to be considered. However it could well be that the ligand orbital donates charge to the metal. Such charge donation might well be expected to effect isomer shifts.

Intermolecular antiferromagnetic coupling of moderate magnitude has been recently shown to be the rule in five coordinate complexes when association into dimers occurs in the crystal lattice [33].

Finally it should be noted that the findings reported here for the PPIXFe(III) species have direct model potential for chromatium cytochrome c' [28]. Although cytochrome c' does not contain simple PPIXFe(III), it is closely related (it in fact contains a modified PPIX bound covalently to the polypeptide by thioether bonds between two cysteines and the vinyl side chains).

Conclusions

From this work it can be seen that PPIXFe(III) can form $S = 3/2$, $5/2$ spin admixed species with weak axial ligands, but whether such a spin mixture has a role in enzymes containing pure PPIXFe(III) remains to be established.

Experimental

The μ-oxo-bishaem of protoporphyrin IX iron(III) $(PPIXFe(III))_2O$ was prepared by first dissolving 200 mg at PPIXFeCl in dilute NaOH. The acetone was added till a precipitate formed and the solid $(PPIXFe)_2O$ was isolated by filtration. For the experiments discussed in this work 200 mg of either $(PPIXFe)_2O$ or PPIXFeCl were dissolved in aqueous alkali in the presence or absence of another salt or solution (see details in Table I), a few drops of concentrated H_2SO_4 were then added and precipitates immediately formed. The precipitates were washed with excess water (pH ~ 6.0). These precipitates were then placed in sample holders and transferred to the Mössbauer spectrometer. The precipitate that was analysed was No. 1 of Table I. Using the % areas obtained from the Mössbauer spectrum, the material was formulated as $0.66\ PIXFeI + 0.34\ HPPIXFeI_2 + 4H_2O$. *Anal.* Calc.: C, 47.52; H, 4.77; N, 6.52; Fe, 6.49; I, 19.78; O, 14.89. Found: C, 47.65; H, 4.5; N, 6.35; Fe, 6.50; I, 20.30; O, (by difference) 14.70%. All the Mössbauer spectra of the precipitates contained evidence of at least two iron(III) species, only one was analysed, as they were obviously all mixtures of two species. All spectra were collected at 78 K.

The Mössbauer spectra were recorded on an instrument previously described [38]. The source was ^{57}Co (25 mCi) in rhodium (Amersham International) at 20 °C. The spectrometer was operated in a saw-tooth mode and the spectra computer fitted. The spectrometer was calibrated with a 25 μM thick natural iron reference absorber. All isomer shifts are referred to this as zero shift.

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