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A Six-coordinate High Spin Protoporphyrin IX Iron(III) Complex as a Model for Ferric Haemproteins: Mössbauer Spectrum of Bisaquo(protoporphyrinato IX)iron(III) Encapsulated in Aqueous Detergent Micelles

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Abstract

The Mössbauer spectrum is reported for a six-coordinate high spin iron(III) protoporphyrin complex, $[(PPIX)Fe(H_2O)_2]^+$, which is monodispersed in an aqueous frozen solution of sodium dodecyl sulphate micelles. The isomer shift of 0.46 mm^{-1} and the quadrupole splitting of 1.19 mm^{-1} are similar to those of aquo met myoglobin. These results are compared with the literature results on other high spin six-coordinated models and with those of some proteins. This is the first report of a Mössbauer study on a haem encapsulated in a detergent micelle.

Introduction

In high spin ferric haemproteins the iron(III) ion is always found as a six-coordinate monomeric species. However, in solids and in most solvents (protoporphyrinato IX)iron(III), $[(PPIX)Fe(III)]^+$ (the most ubiquitous haem found in animals and plants) complexes are five-coordinate species [1, 2]. In such high spin species the iron(III) ion is nearly always found outside the plane of the porphyrin ring [1]. Before the recent work of Scheidt *et al.* [1, 3–7] this led to the misheld belief that high spin states are associated with displacements of the iron from the porphyrin ring plane. Indeed it was thought that high spin iron(III) ions were too large to fit in the porphyrin plane and that spin change caused by movement or interchange of axial ligands might be important in protein movement for ferric haemproteins, as they are for ferrous haemoglobin. The work of Scheidt and others [3–7] on ferric complexes prepared from (tetraphenylporphyrinato)iron(III) $[(TPP)Fe(III)]^+$ [3–6] and from (octaethylporphyrinato)iron(III) $[(OEP)Fe(III)]^+$ [7] has shown that six-coordinate high spin iron(III) complexes can be prepared in which the iron(III) ions remain in the porphyrin plane. These high spin iron(III) ions are accommodated inside the N_4 porphyrin core through a radial expansion generated by the population of the $d_{x^2-y^2}$ orbital, and a significant non-bonding repulsion of the axial ligand by the porphyrinato core [1, 3, 6].

In order to produce a better model for the six-coordinate high spin met haemproteins, we found it necessary to study a system based on natural porphyrins in aqueous solutions.

We recently discussed [2, 8] the electronic spectra of $[(PPIX)Fe(III)]^+$ in the pH range 6 – 14 and in micelles (sodium dodecyl sulphate, SDS). We found evidence for a six-coordinate $[(PPIX)Fe(III)(H_2O)_2]^+$ complex in micelles at low pH. The great similarity between the visible spectrum of the $[(PPIX)Fe(III)(H_2O)_2]^+$ complex in the micelles, and model $[(TPP)Fe(III)(H_2O)_2]^+$ species verified the former as a six-coordinate high spin complex [8]. This is the only monomeric complex in this spin state to be found in a soluble form in the absence of a protein. Recent magnetic resonance studies [9] on $[(PPIX)Fe(III)(H_2O)_2]^+$ have shown that it is monomeric, hexacoordinate with a ^6A ground state in frozen solution in aqueous SDS micelles.

We report in this work the Mössbauer spectrum of $[(PPIX)Fe(III)(H_2O)_2]^+$ in SDS and compare it to other high spin six-coordinate ferric porphyrin complexes.

Experimental

^{57}Fe enriched [(PPIX)FeCl] was prepared according to the method of Caughey *et al.* [10]. 90% enriched ^{57}Fe was purchased from Harwell stable isotopes division. The compound was prepared and characterised by reported procedures [9, 11], by mixing an alkaline solution of haemin chloride (Sigma) with freshly prepared 5% sodium dodecyl

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sulphate (SDS obtained from BDH) micelles. The pink complex obtained in the micelles at pH 2.6 was verified to be the bisaquo(protoporphyrinato IX) iron(III) cation by its visible spectrum [9]. The pH was measured using a Philips (PW-9409) digital pH meter using the procedure reported earlier [9, 11] and the visible spectrum was recorded in 1 cm^2 cells using a Beckman DU7 spectrophotometer. The Mössbauer spectrum was recorded using an instrument previously described [12].

Results and Discussion

The Mössbauer spectrum of a frozen solution of $[(PPIX)^{57}Fe(III)(H_2O)_2]^+$ in SDS is shown in Fig. 1 and the data are presented in Table 1 along with relevant literature data.

The asymmetric quadrupole doublet is characteristic of high spin iron(III) [16]. Such broad asymmetric lines are typical of slow electronic spin–spin relaxation which has been well explained in the literature by Blume [17]. Since the high velocity line is broad, the sign of V_{zz} is likely to be positive as in other high spin iron(III) porphyrins [16, 18, 19]. The positive sign of V_{zz} arises from strong in plane (porphyrin plane) and weak axial (H_2O) electrostatic fields, with the lattice term dominating the EFG.

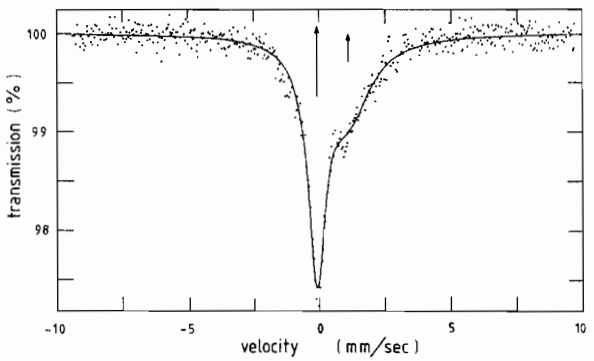

Fig. 1. ^{57}Fe Mössbauer spectrum of $[(PPIX)Fe(H_2O)]^+$ in sodium dodecyl sulphate micelles in frozen solution at 78 K.

It might have been expected that for $[(PPIX)Fe(III)(H_2O)_2]^+$ molecules isolated in SDS micelles a reduction in the relaxation rate would have been seen in the Mössbauer spectrum, generated by increasing the interspin separation. As we have discussed previously [2] in connection with the mechanism of rapid freezing of solution it must be appreciated that some segregation of the Mössbauer nucleus may occur (in this case micelles may stack together) in one amorphous phase. 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 atoms increases the spin–spin interactions and the frequency of spin relaxation. This results in the disappearance of the magnetic hyperfine structure. Our spectrum shows no such hyperfine structure. Attempts to reduce the relaxation rate by increasing the spin–spin separation have been reported [14, 20, 21]. It is worth noting that such a hyperfine structure was only found for frozen haemin solutions at very low temperatures (4.2 K) with very good counting statistics. Even at 4.2 K Lang *et al.* [20] found a major component of their spectrum was similar to that of crystalline haemin. This they attribute to iron(III) sites in which the electron spins are relaxing rapidly. A similar explanation would be offered for our spectrum, based on micelle aggregations, without the need to invoke rapid micelle breakdown on freezing. It must be pointed out that the early work in organic solvents was likely to have been complicated by the presence of aggregated species which are known to exist even at millimolar concentrations and at low temperatures [22].

The Mössbauer data for the $[(PPIX)Fe(III)(H_2O_2]^+$ species (see Table 1) are similar to those of ferrihaem

TABLE 1. ^{57}Fe Mössbauer parameters for high spin six-coordinate iron(III) porphyrin entities

Complex	Temperature (K)	δ (mm s^{-1})[a]	Δ (mm s^{-1})	Reference
$[(PPIX)Fe(H_2O)_2]^+$	78	0.46	1.19	this work
$[(TPP)Fe(H_2O)_2]^+$	78	0.33	1.69	3
	278	0.41	1.53	3
$[(TPP)Fe(dmso)_2]^+$	4.2	0.45	1.22	4
$[(TPP)Fe(EtOH)_2]^+$	4.2	0.42	1.89	6b
Aquo met myoglobin	4.2	0.40	1.3	13
Fluoro met myoglobin	4.2	0.40	0.8	13
Fluoro met haemoglobin	4.2	0.40	0.7	13
Haem in dmso[b]	4.2	0.38	1.10–1.16	14

[a]Isomer shifts are referred to natural iron foil at 298 K as zero shift. [b]Based on NMR studies this compound is considered to be a six-coordinate high spin $[(PPIX)Fe(dmso)_2]^+$ cation [15].

proteins, particularly with those of aquo met myoglobin [13]. The parameters for the diaquo species of protoporphyrin IX are significantly different to those for $[(TPP)Fe(H_2O)_2]^+$. This is indicative of the porphyrin basicity and indicates that electron delocalisation from iron to porphyrin plays a dominant role in determining the isomer shifts and quadrupole splittings (Δs).

From Table 1 it is obvious that the Δs of the TPPFe(III) complexes are larger than those of $[(PPIX)Fe(III)(H_2O)_2]^+$ and also those of the proteins. Since the sign of the EFG and magnitude of q_{val} are likely to be the same in all high spin six-coordinate $s = \frac{5}{2}$ systems [16, 20, 21], then the Δs may be directly related to the lattice contribution and covalency effects. This would be in agreement with the calculations for five-coordinate high spin porphyrinato iron(III) ($s = \frac{5}{2}$) complexes of Harris [23–25] and would also be in keeping with our findings for such systems [26].

Within the $[(TPP)Fe(L)_2]^+$ complexes (L = H_2O, DMSO or EtOH) [2, 3, 6b] and the proteins the Δs are clearly sensitive to the nature of the axial ligands and Δ increases as the Fe–L bond length increases [1]. Again this is in agreement with the results of the calculations of Harris [23–25] and is expected from our recent work [26]. It is at first surprising that the Δ of $[(PPIX)Fe(III)(H_2O)_2]^+$ is similar to that of aquo met myoglobin, in the latter the (PPIX)Fe(III) moiety has one H_2O and one histidine residue as axial ligands. It might be expected that the histidine would be a strong field ligand, but from the facts that the aquo met myoglobin is a high spin species and that its Δ is similar to $[(PPIX)Fe(III)(H_2O)_2]^+$ it clearly does not act as a strong field ligand in the protein with the iron in this oxidation state. The most likely explanation for this is that the protein backbone restricts the ability of the histidine to approach the iron atom as closely as it would if it were free, and thus weakens its ligating ability.

Lang *et al.* [20] also found that the Mössbauer spectrum of haemin in tetrahydrofuran (thf) was similar to that of aquo met haemoglobin though the axial ligands are different in the two compounds. Their explanation that there is little interaction with the axial ligands and that the dominant effect is spin transfer to the porphyrin nitrogen atom was presented when there was no model data for comparison. Now in the light of more data in such compounds it is clear that there is a significant axial ligand contribution.

It appears from the data in the table that the Δs are less sensitive to displacement of the iron atom from the porphyrin plane, as both aquo met and fluoro met myoglobin are reported [27] to have the iron atom displaced from the porphyrin plane by 0.3 Å.

In $[(PPIX)Fe(III)(H_2O)_2]^+$, the axially symmetric ESR spectrum and the presence of a large contact interaction [9] suggests that the iron atom is in the plane of the porphyrin as in the case of $[(TPP)Fe(III)L_2]^+$ complexes [3, 4]. However, no full correlation could be found between the Mössbauer data and stereochemistry in these three compounds, although it has been suggested for $[(TPP)Fe(EtOH)_2]^+$. The quadrupole splittings may be consistent with mixing an amount of $s = \frac{3}{2}$ character in these predominantly $s = \frac{5}{2}$ (high spin) complexes [1, 6b]. It can be seen from the discussion presented here that the axial ligands do influence the iron–porphyrin π-delocalisation and metal ligand charge transfer in these high spin six-coordinate iron(III) complexes. It may be mentioned here that the porphyrin–iron(III) charge transfer bands at *c.* 500 and 630 nm are quite comparable between the bisaquo haemin complex [9] and aquo met myoglobin [28], e.g. the intensity ratio A504/A632 is 2.7 in $Mb(H_2O)$ and 3.0 in the diaquo haemin complex. (The iron orbitals involved are $d_{xz, yz}$ in the proteins [28] and the CT transitions are quite sensitive to axial ligands.) Further work is needed on an extended series of such high spin six-coordinate(porphyrinato)iron(III) complexes of known stereochemistry in order to fully correlate the Mössbauer parameters with structural features.

Finally we now consider the nature of the $[(PPIX)Fe(III)(H_2O)_2]^+$ complex. Obviously the Fe(III) centre is positively charged, but what is not apparent is the nature of the compensating negative charge. This must either be (i) a negative ion, either OH^- or Cl^- ion paired to the complex or (ii) it could possibly be that one of the two propionate side chains on the PPIX molecule is deprotonated, and that this is bent back over the porphyrin plane and hydrogen bonds to an axial water molecule.

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