A Mössbauer Study on the (Protoporphyrinato IX)iron(II) Complexes of Imidazole and Substituted Imidazoles as Axial Ligands in Frozen Aqueous Solutions

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(Received May 24, 1989)

Abstract

A Mössbauer study on (protoporphyrinato IX)-iron(II) complexes of the type [(PPIX)Fe(II)(ImR)$_n$] where ImR = imidazole (ImH), 1-methylimidazole (1-MeIm) or 2-methylimidazole (2-MeImH) indicates that the ImH and 1 MeIm complexes are low spin $S = 0$ and monomeric in 1:1 ethanol:water whereas the 2 MeImH complex in this solvent is present as a five-coordinated high spin ($S = 2$) aggregate. The quadrupole splitting (ΔE_Q) of the bis(1-MeIm) compound was found to be similar to that of reduced cytochrome b_5. In a frozen aqueous solution of a detergent, ethyltrimethylammonium bromide (CTAB), the five-coordinated complex [(PPIX)Fe(II)-(2-MeImH)] was obtained as a monomeric high spin compound with a ΔE_Q similar to that of deoxy myoglobin. A low spin bis(2-MeImH) complex was also found in the frozen (78 K) solution of the detergent with $\Delta E_Q = 1.26$ which is similar to that found for the low spin [(PPIXFe(II)] complexes with sterically hindered amines. Analysis of the isomer shifts and the quadrupole splittings of the low spin bis(ImR) complexes indicate that the dominant mechanism in the covalent bonding is the σ donation of imidazole → iron and the observed trend of σ basicity of the coordinated ligands found is: 2-MeImH < 1-MeIm < ImH. Hydrogen bonding of coordinated imidazoles and steric hindrance of axial ligands are shown to influence the trend in basicity of the free ligands.

Introduction

Iron porphyrin complexes have provided useful models for haem proteins. Studies on such complexes as (protoporphyrinato IX)iron(II) [(PPIX)Fe(II)] under physiological conditions are of importance in understanding structure–function relationships in haem proteins [1–3]. Correlation of the spin state of the iron with its surrounding stereochemistry is the principal aim of such studies [4, 5]. In addition an understanding of the chemistry of [(PPIX)Fe(II)] is of practical importance in the preparation of synthetic oxygen carriers [6–9], in cancer research [10, 11] and in the possible catalytic use for oxidation, hydroxylation and oxidative degeneration of many organic complexes (based on modelling the detoxifying chemistry of the cytochromes P450 found in living systems) [12, 13].

One of the major problems encountered in obtaining stable [(PPIX)Fe(II)] complexes is that these species have a tendency to aggregate in aqueous solution [14–17]. Such aggregated species in the presence of oxygen ultimately lead to irreversible oxidation products [6–9] and prevent the stabilisation of molecular oxygen complexes. Additional constraints in constructing suitable models for the natural oxygen carrying proteins are (i) the difficulty in synthesising five-coordinate geometries for [(PPIX)Fe(II)] in free solution (such model complexes are rare) [5, 16–19] and (ii) obtaining [(PPIX)Fe(II)] in a non-polar hydrophobic environment as in a haemprotein pocket so as to prevent auto-oxidation of Fe(II) ions [6–9].

Recently we have found two approaches to the study of [(PPIX)Fe(II)] that circumvent these difficulties. Firstly, following the work of Keillen [20] we found that monomeric low spin six-coordinated porphyrins in the presence of strong ligands such as imidazoles or pyridines can be prepared in alkaline solution mixtures of 1:1 ethanol: water. Secondly, [(PPIX)Fe(II)] can be prepared in the monomeric form in aqueous solutions if encapsulated in the hydrophobic environment of detergent micelles [21, 22]. We have shown [21] that a five-coordinated compound [(PPIX)Fe(II)(2-MeImH)] can be obtained as a monomer in 5% cetyltrimethylammonium bromide (CTAB) micelles, the visible spectrum of which is similar to that of deoxy myoglobin [21].

Mössbauer spectroscopy has proved to be a very good probe of the electronic environment around the

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0020-1693/89/$3.50

iron in haems, particularly in the study of ferrous porphyrins which are difficult to study by other spectroscopic techniques such as ESR. In our hands preparation of ferrous porphyrins using a rapid freezing technique in a nitrogen atmosphere has proved successful in preventing auto-oxidations. During our studies [16–19, 23, 24] of the aqueous chemistry of [(PPIX)Fe(II)] we have characterised its complexes in a wide variety of ligand environments and have found that the Mössbauer parameters are sensitive to both small changes in the iron electronic environments and to the tendency of [(PPIX)Fe(II)] to aggregate.

We report herein Mössbauer spectroscopic studies on complexes of the six-coordinate type [(PPIX)Fe(II)(ImR)$_2$], where ImR = imidazole (ImH), 1-methylimidazole (1-MeIm) or 2-methylimidazole (2-MeImH) in 1:1 ethanol:water and in 5% CTAB in water. We also report the Mössbauer spectrum of [(PPIX)Fe(II)(2-MeImH)] in both water and in 5% CTAB (in water).

Experimental

All chemicals were purchased from Aldrich Chemical Co. and were used without further purification.

The complexes of the type [(PPIX)Fe(II)(ImR)$_2$] where R = H or 1-Me were prepared from the corresponding bis(imidazole) ferric protoporphyrin IX complexes [25] in 1:1 ethanol:water by reduction with solid sodium dithionite in alkaline solution (pH = 10.0). The 2-MeImH complexes were prepared by using a procedure which involved dithionite reduction [17] of the ferric porphyrin complexes [26, 27] of the type [(PPIX)Fe(H$_2$O)(OH)] in 5% aqueous CTAB at pH 10.0. About 10 mM solutions were encapsulated in nylon cells under nitrogen atmosphere and rapidly quench frozen, then quickly transferred to the cryostat (78 K). All precautions, such as handling in an inert atmosphere and the presence of excess of dithionite, were taken so as to eliminate any possible exposure of the samples to molecular oxygen.

The Mössbauer spectra were recorded using an instrument previously described [28]. About 500 000 to 10^6 counts per channel were collected and the data computer fitted. The source was ^{57}Co (25 mCi) in Rh (Radiochemical Centre, Amersham) at 20 °C. The isomer shifts reported here are relative to iron foil.

Results and Discussion

The Mössbauer parameters obtained from the frozen solutions are presented in Table 1 and representative spectra are shown in Fig. 1. In Table 1 we have also shown for comparison the spectral data of reduced [29] cytochrome b_5 and deoxymyoglobin [30].

Of the complexes studied here the Mössbauer data were previously known only for [(PPIX)Fe(II)(ImH)$_2$] which was studied in frozen water by Epstein *et al.* [31]. Our results in 1:1 ethanol:water for this compound agree quite well with their results [31]. The bis(imidazole) complexes are Fe(II) low spin ($S = 0$) and the results agree quite well with those reported [29] for ferrous cytochrome b_5.

The behaviour of the frozen solutions containing [(PPIX)Fe(II)] and 2-MeImH are surprisingly different. In the frozen solution of 1:1 ethanol:water the Mössbauer spectrum clearly indicates the presence of only one iron electronic environment, a high spin ($S = 2$) five-coordinate species [16–19, 32]. In the frozen detergent solution the Mössbauer parameters of two distinct iron(II) electronic envivonments are

TABLE 1. Mössbauer spectral data (78 K) of the adducts of [(PPIX)Fe(II)] with various imidazoles

Compound	Solvent	δ (mm s^{-1})	ΔE_Q (mm s^{-1})	Γ (mm s^{-1})
[(PPIX)Fe(II)(ImH)$_2$]	1:1 EtOH:H$_2$O	0.45(1)	0.97(1)	0.14(1)
	H$_2$O [a]	0.42	0.95	
[(PPIX)Fe(II)(1-MeIm)$_2$]	1:1 EtOH:H$_2$O	0.47(1)	1.03(1)	0.15(1)
[(PPIX)Fe(II)(2-MeImH)]$_n$	1:1 EtOH:H$_2$O	0.95(2)	2.12(3)	0.15(2)
[(PPIX)Fe(II)(2-MeImH)] [b]	5% CTAB	0.82(3)	2.2(4)	0.15(3)
[(PPIX)Fe(II)(2-MeImH)$_2$] [b]	5% CTAB	0.51(2)	1.26(3)	0.21(3)
Cytochrome b_5, reduced [c]		0.43(2)	1.04(3)	
Deoxy myoglobin [d]		0.91	2.17	
Deoxy haemoglobin [d]		0.9	2.2 (90%)	
		0.46	1.07 (10%)	

[a]Ref. 31. [b]Both these data were obtained from the same sample which contained 34% of monoligated adduct and 66% of the bisligated adduct (percentages were estimated from area absorbance of the Mössbauer spectrum). [c]Ref. 29. [d]Ref. 30.

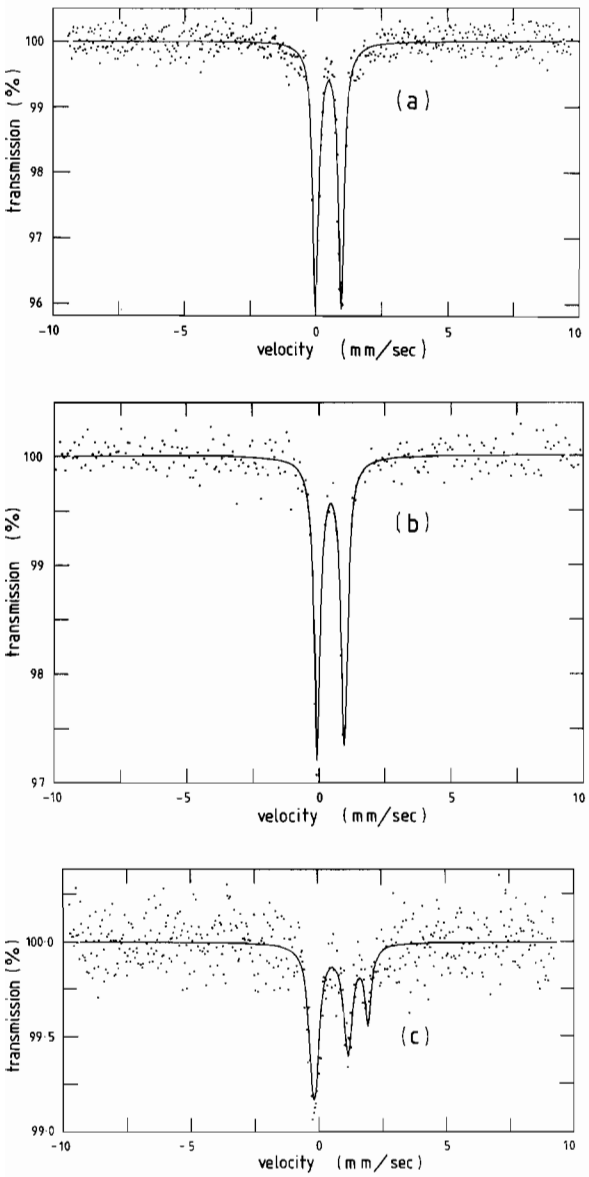

Fig. 1. Frozen solution Mössbauer spectra at 78 K of: (a) [(PPIX)Fe(II)(ImH)$_2$] in 1:1 ethanol:water, (b) [(PPIX)Fe(II)(1-MeIm)$_2$ in 1:1 ethanol:water, (c) [(PPIX)Fe(II)(2-MeImH) and [(PPIX)Fe(II)(2-MeImH)$_2$] in 5% CTAB, at pH 10.0.

observed, the first is that of a high spin ($S = 2$) five-coordinate species (34% of the absorption spectrum) and the other (66%) is that of a low spin ($S = 0$) six-coordinate complex (similar to those of other bis-haemochromes [18, 31, 32]). The quadrupole splitting (ΔE_Q) of the 2-MeImH adducts in the detergent solution are very similar to those found for deoxy haemoglobin and deoxy myoglobin [30].

As the Mössbauer data of the low spin complex are similar to those of non-hindered bishaemochromes [18, 31–33], we suggest that this species is a bis(2-MeImH) complex, [(PPIX)Fe(II)(2-MeImH)$_2$]. The fact that such a low spin complex with sterically hindered imidazole can be obtained is interesting. The visible spectrum and proton NMR studies on the 2-MeImH adduct indicate [21] that at room temperature a monomeric five-coordinate high spin ($S = 2$) complex like that in deoxymyoglobin is exclusively present in the aqueous detergent solution. Hence the formation of the six-coordinate low spin [(PPIX)Fe(II)(2-MeImH)$_2$] complex is attributed to stronger ligand binding at a low temperature (78 K) within the detergent. Brault and Rougee [34] previously reported that the binding of ethers to haems was favoured at 77 K. We now must offer an explanation as to why a similar bis(2-MeIm) is not formed in frozen 1:1 ethanol:water solution. The obvious clues are that either the detergent plays an active (or passive role), or alternatively the five-coordinate complex formed in 1:1 ethanol water is not the same as that in the detergent and is more stable at low temperatures. Evidence in favour of the latter explanation is found in the differing Mössbauer parameters (Table 1) of the two five-coordinate species.

We have previously shown that the square planar complex [(PPIX)Fe(II)] can be prepared in very dilute conditions in frozen aqueous solution using [(PPIX)^{57}Fe(II)] (90% enriched with ^{57}Fe) [16], however, in more concentrated solutions the Mössbauer data for this species change [17] and the ΔE_Q is concentration dependent. We interpreted the change in the Mössbauer parameters as arising from π–π interactions between neighbouring [(PPIX)Fe-(II)] complexes [17]. More recently we have shown that the Mössbauer parameters of the ^{57}Fe enriched species in dilute solution [16] are identical to that of unenriched [(PPIX)Fe(II)] encapsulated in detergent micelles [22]. Thus aggregation of porphyrins causes interactions that change the Mössbauer parameters.

We suggest that the five-coordinate species in frozen aqueous solution is not monomeric. We have previously pointed out that equilibria can change on freezing [35], and although 1:1 ethanol:water solutions may facilitate monomeric [(PPIX)Fe(II)] species at room temperature [21], on freezing these species may form dimers or more extensive oligomers and other aggregated species. As such species will have π–π interactions between the porphyrin rings they will be able to resist attack by excess 2-MeImH as the temperature falls (they will become more thermodynamically favourable) and thus the biligated [(PPIX)Fe(II)(2-MeImH)$_2$] will not be able to form. The five-coordinate species present in frozen aqueous solution is thus best formulated as [(PPIX)Fe(II)-(2-MeImH)]$_n$ (where $n = 2$ or more).

In the detergent however, monomeric five-coordinate [(PPIX)Fe(II)(2-MeImH)] does exist and

excess 2-MeImH in the solution is able to force this species to form a bisligated haemochrome on freezing, as there is only one [(PPIX)Fe(II)(2-MeImH)] unit in each micelle. It should be pointed out that the monodispersion at room temperature gives a species similar to deoxymyoglobin [21], and that the low spin bis adduct of this sterically hindered imidazole forms only at low temperatures where the axial binding of a sixth ligand is favoured.

The results for $[(PPIX)Fe(2\text{-}MeImH)]_n$ in the presence ($n = 1$) and absence ($n > 1$) of CTAB show the importance of the hydrophobic environment around a haem in influencing and restricting the iron electronic structure. This is important in view of the fact that haems in the haemoproteins are commonly found in hydrophobic pockets.

It should be pointed out that a small amount of a low spin complex with Mössbauer parameters [30] similar to the low spin bis(imidazole) complexes reported here was found in the Mössbauer spectrum of deoxyhaemoglobin at 4.2 K.

The low spin $S = 0$ Fe(II) system (d^6) with its filled shell in octahedral symmetry should have no valence contribution to the electric field gradient (EFG). Hence the quadrupole splitting in these complexes should be directly related to the covalency in the metal–ligand bond. The most important bonding interactions in the low spin Fe(II) compounds are (i) σ bonding from ligand to hybrid d^2sp^3 orbitals of Fe(II), and (ii) π bonding from filled Fe(II) d_{xy}, d_{xz} and d_{yz} orbitals to the empty π^* orbital of the ligand. In a detailed study on low spin Fe(II) complexes Bancroft *et al.* [36] found that the isomer shifts and quadrupole splittings are additive and may be expressed in terms of partial isomer shift (*pis*) and partial quadrupole splitting (*pqs*)

$$pis = -k(\sigma + \pi)$$

$$pqs = -q_{\text{latt}} + C(\pi' - \sigma')$$

where k and C are proportionality constants, σ is the σ donating ability of the ligands and π is the π accepting ability of the ligands, and q_{latt} is the field gradient due to external charges.

The trend in isomer shifts of the bis(imidazole) complexes in Table 1 indicate the $\sigma + \pi$ bonding increases in the order 2-MeImH < 1-MeImH < ImH implying that ImH is the strongest σ donor and the strongest π acceptor. Figure 2 shows the correlation of quadrupole splitting with decreasing isomer shift which indicates that the dominant mechanism of covalency is σ donation from the imidazole ligands and that the trend in σ basicity of coordinated imidazoles is 2-MeImH < 1-MeImH < ImH. This result is contrary to that suggested in an earlier study by Epstein *et al.* [31].

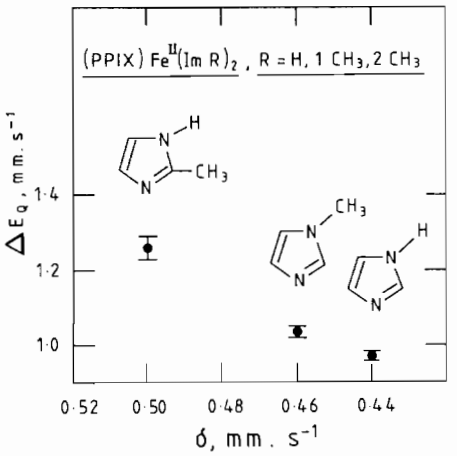

Fig. 2. A plot of ΔE_Q vs. δ for the complexes of the type $[(PPIX)Fe(II)(L)_2]_n$ where L = ImH, 1-MeIm, 2-MeImH.

They pointed out that iron → imidazole π bonding was the dominant mechanism of covalency. However, as imidazole is a weak π acceptor to Fe(II), our observation is in agreement with the suggestion of Sundberg and Martin [37] that the σ donor properties of imidazoles are more important than their π accepting ability.

In the above analysis we have assumed that the sign of V_{zz} is positive and does not change in the series of low spin complexes studied. This is reasonable from the known literature [32, 38–40]. It is interesting to note that the trend in σ basicity of imidazoles found in this study is the reverse of the trend in the pK_a values of the free ligands [pK_a-(BH^+): ImH (6.65) < 1-MeIm (7.33) < 2-MeImH (7.56)]. This implies that additional factors such as solvent hydrogen bonding to the N–H of coordinated ImH, and the steric hindrance of the methyl group in 2-MeImH play significant roles in reversing this trend in the basicity of the axial ligands. For example, hydrogen bonding of solvent water or ethanol or even free ImH to the coordinated ImH can change it to a stronger σ donor than free ImH or 1-MeIm. In contrast the steric hindrance of coordinated 2-MeImH would weaken the Fe–2-MeImH bond and thereby make such 2-MeImH coordination to the Fe(II) in the haem weaker in σ donation than might have been expected. Hydrogen bonding to coordinated imidazole is well known and such bonding to proximal histidine in a haemoprotein was suggested to have considerable biological significance, particularly in modulating oxygen affinity [41]. The fact that ΔE_Q does not correlate with free ligand basicity was previously reported by Straub and Connor [33] in their study on low spin Fe(II) porphyrin bis(amine) complexes. They suggested that steric effects of the six-membered amines [33] may be of significance in influencing the quadrupole splittings.

Conclusions

The Mössbauer spectroscopic study has shown that the bis(imidazole) complexes [(PPIX)Fe(II)] in 1:1 ethanol:water are monomeric low spin complexes with the isomer shift and quadrupole splittings similar to those found for the reduced cytochrome b_5 and other low spin ferrous haemoproteins. The 2-MeImH ligand binds to generate a mono adduct and is an aggregate ($n > 2$) in aqueous or aqueous ethanolic solution, whereas on encapsulating in a detergent micelle the aggregation is avoided and a monomeric five-coordinated high spin ($S = 2$) [(PPIX)Fe(II)(2-MeImH)] complex is obtained that has Mössbauer parameters similar to those of deoxymyoglobin. At 78 K a low spin bis(2-MeImH) adduct is also obtained in the detergent micelle indicating stronger metal–imidazole interactions at lower temperatures. The trend of basicity of the free imidazole ligands changes dramatically when coordinated to the metal and the Mössbauer data indicate that the dominant mechanism of covalency in the Fe–imidazole bond is σ donation from imidazole to iron(II). Hydrogen bonding to axially coordinated imidazoles and the steric effect of hindered imidazoles may play a significant role in altering the basicity of coordinated ligands, thereby strongly influencing the quadrupole splittings in low spin Fe(II) porphyrins.

The results described here are of significance in understanding the behaviour of a haem in haemoprotein since they demonstrate the role of hydrophobic environments around the haem in influencing and restricting the stereochemistry and electronic structure of ferrous iron. The results give credence to the suggestion that hydrogen bonding to the proximal histidyl imidazole can modulate the basicity of the axial histidines in many haemoproteins.

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