Mössbauer Spectroscopic Studies on the Bis(histidine) Adducts of (Protoporphyrinato IX)Iron(II)

OKHIL K. MEDHI* and JACK SILVER**

Department of Chemistry and Biological Chemistry, University of Essex, Wivenhoe Park, Colchester CO4 3SQ (U.K.)

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Abstract

Mössbauer spectra in frozen aqueous ethanolic solution of complexes of the type [(PPIX)Fe(II)-(HisR)$_2$] are reported where HisR = histidine, *N*-α-acetyl histidine, pilocarpate or histamine. At alkaline pH 12.0, the complexes are found to be monomeric and low spin with quadrupole splittings (ΔE_Q) similar to that of reduced cytochrome b_5. The ΔE_Q values are typical of bis(imidazole) coordination to (protoporphyrinato IX)iron(II) (ΔE_Q = 0.9–1.04 mm s^{-1}). The results indicate that hydrogen bonding to the N–H of the coordinated imidazole considerably decreases the ΔE_Q values.

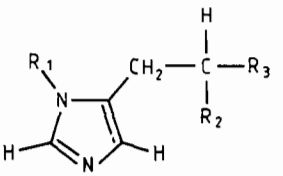
Introduction

The chemistry of (protoporphyrinato IX)iron(II), [(PPIX)Fe(II)], commonly known as 'haem', is of considerable significance since the haem group is to be found in all haemproteins. Understanding the structure–function relationships of the haem in proteins [1–3], particularly elucidation of spin state–stereochemistry relationships [4, 5], is therefore important. Iron(II) porphyrins are also of some practical importance in the preparation of synthetic oxygen carriers [6–8] and in the possible use as catalysts for oxidation and hydroxylation of organic substrates [6–10].

Haems have a strong tendency to undergo aggregation in aqueous solutions [11]. This constitutes a major problem in the study of haems, since the tendency for dimerization leads to irreversible oxidation [6, 8] or iron(II). However, following the work of Keilin [12] we found that in strong alkaline solution (see below) of ethanol–water mixed solvent the monomeric low spin six-coordinated complexes of [(PPIX)Fe(II)] can be prepared in the presence of ligands such as imidazole, pyridine and histidine. This paper presents a Mössbauer study on the bis adducts of [(PPIX)Fe(II)] with histidine and its substituted derivatives.

Mössbauer spectroscopy is a versatile technique in the study of ferrous porphyrins which are difficult to study by other methods. We have shown earlier [13–18] that preparation of frozen solution samples of [(PPIX)Fe(II)] using a rapid freezing technique in a nitrogen atmosphere prevents auto-oxidation of the iron(II). In our continuing studies of the aqueous chemistry of [(PPIX)Fe(II)] in the presence of a variety of ligand environments [13–18], we have found that the Mössbauer parameters of the haem are sensitive to small changes in the iron electronic environment such as aggregation [13, 15, 19] and a change in pH [13–18].

In this paper we report Mössbauer spectra of complexes of the type [(PPIX)Fe(HisR)$_2$], where HisR = histidine, N-α-acetyl histidine, pilocarpate or histamine (Fig. 1).



Compound	R_1	R_2	R_3
Histidine (His)	H	$-NH_2$	$-COO^-$
Acetyl histidine	H	$-NHCOCH_3$	$-COO^-$
Histamine	H	$-NH_2$	H
Pilocarpate	CH_3	$-CH_2OH$	$-CHCOO^-$ (with C_2H_5)

Fig. 1. Structure of histidine and its derivatives relevant to this work.

Experimental

All chemicals used in this work were purchased from Aldrich Chemical Co. and were used without further purification. ^{57}Fe enriched iron(III) protoporphyrinato IX chloride was prepared according to the method of Caughey *et al.* [20]. The bis

*On leave from Department of Chemistry, Gauhati University, Gauhati 781014, Assam, India.

**Author to whom correspondence should be addressed.

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ligated complexes of [(PPIX)Fe(II)] are obtained from dithionite reduction of monomeric [(PPIX)Fe(III)(HisR)$_2$]$^+$ complexes in ethanol:water mixed solvent reported previously by Baldwin *et al.* [21–23]. A typical sample preparation consists of reducing 10 mM solutions of the ferric complexes in the presence of excess of histidine by solid sodium dithionite at pH 12.0. The solutions were encapsulated in nylon cells under an N_2 atmosphere and rapidly quench frozen in liquid N_2, then quickly transferred to the cryostat (80 K). All precautions, such as handling in inert atmosphere and presence of excess dithionite, are taken so as to eliminate any possible exposure of the sample to molecular oxygen.

The Mössbauer spectra were recorded using an instrument previously described [24]. About 500 000 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 at room temperature.

Results and Discussion

The Mössbauer parameters obtained are presented in Table 1 and a representative spectrum shown in Fig. 2.

The Mössbauer spectrum of [(PPIX)Fe(II)] in the presence of histidine in frozen ethanol:water (50% vol./vol.) solution is quite sensitive to changes in pH. At pH 8.0 the spectrum (δ = 0.6 mm s^{-1}, ΔE_Q = 1.7 mm s^{-1}) is similar to that found [15] for aggregated haems [(PPIX)Fe(II)]$_n$. At pH 12.0 the spectrum resembles that expected [25, 26] for bis(imidazole) coordination to [PPIX)Fe(II)]. This result is in agreement with that reported by Keilin [12] for room temperature solution studies.

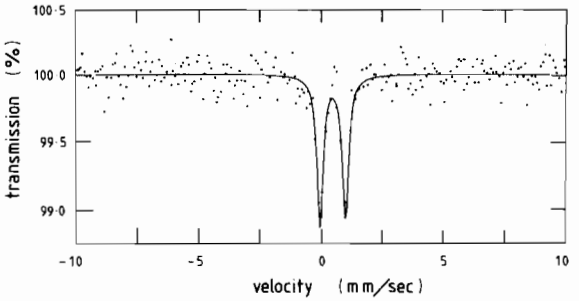
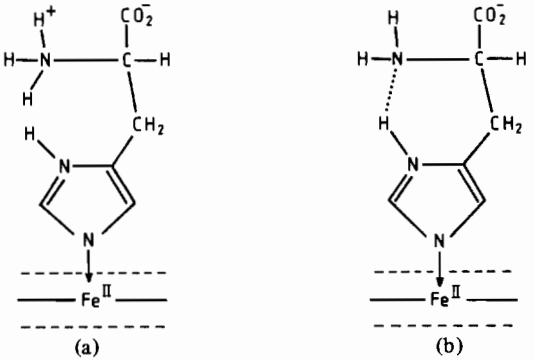


Fig. 2. Frozen solution Mössbauer spectrum (80 K) of [PPIX)Fe(II)(pilocarpate)$_2$] at pH 12.0.

In the bis adducts of [(PPIX)Fe(II)] with substituted histidines the isomer shifts and quadrupole splitting of the low spin complexes are found to be quite insensitive to the nature of the substituent (Table 1). The quadrupole could be due to:

(1) A lattice effect (q lattice would be negative and lower the ΔE_Q) based on charges around the iron centre changing.

(2) Internal H bonding of NH_3^+ (a) versus NH_2 (b) with NH of the imidazole ring may influence ΔE_Q:



(a) would only form a weak or no H bond to the NH of the imidazole ring, since solvation of NH_3^+

TABLE 1. Mössbauer spectra of the adducts of [(PPIX)Fe(II)] with histidine and its substituted derivatives at 80 K

Compound	pH	Isomer shift (mm s^{-1})	Quadrupole splitting (mm s^{-1})	Linewidth[a] (mm s^{-1})
[(PPIX(Fe(II)(His)$_2$]	8.0[b]	0.61(1)	1.70(2)	0.15(2)
	10.0[b]	0.63(1)	1.73(2)	0.20(2)
		0.42(2)	1.02(3)	0.16(2)
	12.0	0.42(2)	0.88(4)	0.41(3)
[(PPIX)Fe(II)(*N*-α-acetyl histidine)$_2$]	12.0[b]	0.64(2)	1.74(3)	0.19(3)
		0.44(2)	1.04(2)	0.18(2)
[(PPIX)Fe(II)(pilocarpate)$_2$]	12.0	0.46(1)	1.04(2)	0.16(2)
[(PPIX)Fe(II)(histamine)$_2$]	12.0	0.48(2)	1.04(3)	0.25(2)

[a]Half width at half height. [b]The site with ΔE_Q = 1.7 mm s^{-1} corresponds to aggregated [(PPIX)Fe(II)]$_n$ (see ref. 15).

would tend to break any H bonding. Hence the ΔE_Q of 1.02(3) at pH 10 is similar to 1.04(2) for the pilocarpate (at pH 12.0) which cannot form an H bond. On the other hand, (b) would form a strong H bond. At pH 10.0 two iron(II) electronic spectra are observed, the first is that of $[(PPIX)Fe(II)]_n$ [15] and the second is that of a low spin haemochrome. Interestingly, this site has a larger ΔE_Q than that at pH 12.0, though both are in the range expected for low spin haemochromes [17, 25]. We are therefore faced with a dilemma why should the ΔE_Q of a bis(histidine) haemochrome $[(PPIX)Fe(His)_2]$ be pH dependent? The obvious answer is that at pH 10.0 the NH_2 on the histidine is protonated (and the species present is $[(PPIX)Fe(HisH)_2]^{2+}$), and as at room temperature this has a pK_a of around 12.0 [21, 23]. Then in frozen solutions (frozen at pH 12.0) this will be deprotonated (and the species present is $[(PPIX)Fe(His)_2]$). This change of protonation influences the ΔE_Q of the complex as it is transmitted via electronic effects of some kind to the Fe atom by the histidine molecule.

Such a change in bonding to the NH of the imidazole ring would be expected to cause a pronounced difference to the ΔE_Q. Although we have suggested the species present at pH 12.0 is $[(PPIX)Fe(His)_2]$, it could equally be $[(PPIX)Fe(His)(HisH)]^+$. The fact that histamine and *N*-α acetyl histidine show larger ΔE_Q values at pH 12.0 may be due to a differing pK_a for the NH_2 of the former compound and steric hindrance preventing H bonding in the latter compound.

The ΔE_Q values of these adducts are similar to those found for $[(PPIX)Fe(II)(1\text{-}MeIm)_2]$ (ΔE_Q = 1.03(1) mm s^{-1}) [25] and are clearly different from that of the bis(histidine) or bis(imidazole) analogues (ΔE_Q *c.* 0.9 mm s^{-1}) [26]. It is interesting to note that the Mössbauer parameters of reduced cytochrome b_5 (δ = 0.43(2) mm s^{-1}, ΔE_Q = 1.04(3) mm s^{-1}) [27] are similar to the *N*-methyl imidazole and the analogous pilocarpate adduct (Fig. 2).

We have earlier pointed out [26] that hydrogen bonding of the N–H of imidazoles greatly influences quadrupole splitting of the bis(imidazole) complexes of [(PPIX)Fe(II)]. Such hydrogen bonding influences the basicity of the imidazole and hence alters the extent of metal ligand σ bonding. Histidine coordinated to [(PPIX)Fe(II)] can form hydrogen bonds with solvent or excess histidine molecules, in addition, it can form an internal hydrogen bond with the carboxylate or the amine moiety of the side chain.

The fact that the quadrupole splitting of ferrous cytochrome b_5 is similar to the *N*-methyl imidazole and pilocarpate may indicate that the histidines bound in the hydrophobic pocket of cytochrome b_5 do not form any hydrogen bonds with any external or internal proton donors. In the absence of a protecting hydrophobic environment the Mössbauer parameters of coordinated histidine ligands are influenced by hydrogen bonding to these axial ligands.

Conclusions

Mössbauer studies on the bis adducts of [(PPIX)Fe(II)] with histidine, *N*-α-acetyl histidine, pilocarpate and histamine show that monomeric low spin complexes are obtained at high pH. The quadrupole splitting in these complexes indicate bis(imidazole) coordination as in reduced cytochrome b_5. The results indicate that hydrogen bonding of the N–H of imidazole considerably influences the quadrupole splitting.

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