Mössbauer Spectroscopic Evidence for Molecular Complex Formation between Histidine and μ-oxo-bis(protoporphyrinato IX)iron(III)

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Abstract

Compelling Mössbauer spectroscopic evidence is presented here for the formation of molecular complexes of μ-oxo-bis(protoporphyrinato IX)iron(III) ([(PPIXFe)$_2$O]) with histidine. The quadrupole splitting of [(PPIXFe)$_2$O] ($\Delta E_Q = 0.56 \pm 0.01$ mm s^{-1}) is modified considerably in the molecular complexes to give $\Delta E_Q = 0.43 \pm 0.01$ mm s^{-1} in the histidine complex. Histamine and *N*-α-acetyl histidine also form similar molecular complexes. Mössbauer studies and a molecular scale model suggest that the donor–acceptor complex is formed via π–π charge transfer interaction between the imidazole of histidine with one of the pyrrole rings of the porphyrin. The role of the amino group in the histidine side chain is to form a NH$\cdots^-$OOCR hydrogen bond with the propionic carboxylate group of protoporphyrin IX. This aligns the imidazole ring of histidine parallel to the pyrrole ring of the porphyrin. It is suggested that the apoprotein conformation in a haem protein may provide such a favourable alignment of an aromatic amino acid residue in the protein pocket so as to favour such a π–π interaction. This is to our knowledge the first Mössbauer spectroscopic study on a molecular π complex involving a porphyrin moiety.

Introduction

Structural studies on haemproteins [1–3] indicate that certain aromatic amino acid residues such as histidine, phenylalanine or tyrosine, are often orientated parallel to the porphyrin π system. These residues are close enough to the latter to enable extensive overlap of their π-orbitals with those of the porphyrins [4–7]. If these interactions significantly alter the electron density on the porphyrin ring, they could also indirectly influence the electron density at the iron centre and thus influence its properties and reactivity. The mechanism by which such residues could modify the electronic structure would include (i) charge transfer, (ii) van der Waals forces and/or (iii) electrostatic interactions or (iv) a combination of (i), (ii) and (iii). As these aromatic amino acid molecules are ubiquitous in haemproteins, such molecular interactions have been suggested as a possible mechanism of control of haem reactivity [8–11].

The probable formation of a donor acceptor 1:1 adduct of histidine with a haematin dimer has been reported [9]. However, because of the weak nature of these interactions, the adduct formation has little influence on the visible spectrum of haematin [9]. Thus very little is known about how the distribution of electron density on the porphyrin ring or at the iron centre was modified in these complexes.

Mössbauer spectroscopy has proved to be a useful technique to examine the iron electronic environments in protoporphyrin IX iron (PPIXFe) complexes [12–20]. We therefore thought it worthwhile to study the formation of PPIXFe(III) complexes with histidine, histamine and *N*-α-acetyl histidine at alkaline pH, such conditions being similar to those where a 1:1 adduct of histidine with the haematin dimer was found to occur [9], and also similar to those reported for two molecules of either aniline or *N*-methyl pyridinium bonding to the haematin dimer [11].

Experimental

μ-Oxo-bis(protoporphyrinato IX)iron(III) (haematin), histidine, histamine and *N*-α-acetyl histidine were purchased from Aldrich Chemical Company and were used without further purification. The structure of the ligands is shown in Fig. 1. The histidine–haematin π complexes were prepared in 20% vol./vol. ethanol:water following the reported procedure [9]. The adducts of histamine and *N*-α-acetyl histidine were prepared using identical conditions. All results are compared with that of haematin in the same solvent system.

The pH of the solutions were recorded using a standard digital pH meter (Philips PW9420) and the

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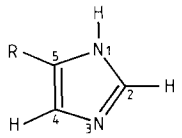

compound	R
Histidine	$-CH_2-CH(NH_2)-CO_2^-$
Histamine	$-CH_2CH_2NH_2$
N-α-Acetyl Histidine	$-CH_2-CH(CH_3CONH)-CO_2^-$

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

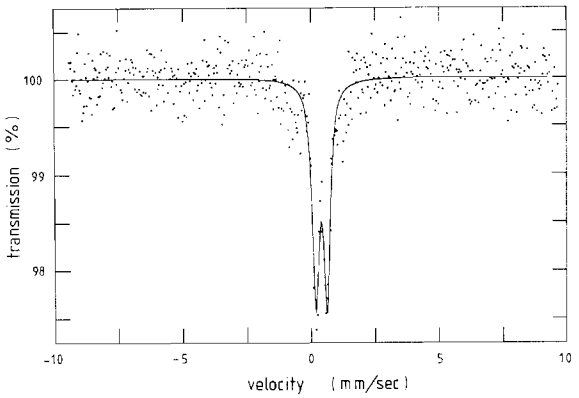

Fig. 2. Mössbauer spectrum of the histidine–[(PPIXFe)$_2$O] complex; [haematin] = 20 mM, T = 80 K, pH = 10.1, solvent: 20% (vol./vol.) ethanol–water.

pH values reported are uncorrected (for the mixed solvent) at room temperature. The visible spectra were recorded in a Perkin-Elmer Lambda 5 spectrophotometer. The Mössbauer spectra were recorded using an instrument and techniques previously described [13–17]. The isomer shifts are reported with reference to metallic iron foil at room temperature as zero shift.

Results and Discussion

The Mössbauer data are presented in Table 1 and a typical spectrum is given in Fig. 2.

In our previous studies on PPIXFe(III) solutions at high pH we found that [(PPIXFe)$_2$O] was the only species present and that its Mössbauer spectrum was a symmetric doublet with a quadrupole splitting (Δ) of 0.58(2) mm s^{-1} [13]. In this work in a 20% (vol./vol.) ethanol:water frozen solution at pH 10.1 (uncorrected) in the absence of other ligands a similar Mössbauer spectrum was observed with the same Δ. However, in the presence of histidine (Fig. 1) a smaller Δ was observed, though the Mössbauer spectrum was still symmetric. Such symmetric spectra, arising from antiferromagnetic coupling via the μ-oxo-bridges have been well explained for μ-oxo-bridged high spin (porphyrinato) iron(III) complexes [19], and the fact that the spectra reported here (in the presence of aromatic nitrogen ligands) have the same characteristics, suggests they still contain a μ-oxo-bridge.

To attempt to investigate the origin of the change in Δ on 'binding' histidine, two similar ligand molecules were chosen. The first of these, histamine, which contains only an amine side chain, was chosen to see if the carboxylate on histidine plays an active role. The second ligand was *N*-α-acetyl histidine and was chosen to investigate the effect of more steric bulk at the amine nitrogen. The frozen solution Mössbauer spectra of the resulting complexes of both these ligands with [(PPIXFe)$_2$O] have similar Δ values to that of histidine itself. This leads us to suggest that the side chain on the ligand plays no significant role in influencing the electron density changes on the iron(III) atoms due to the imidazole–pyrrole π–π interaction. This clearly must indicate that these ligands do not bind at the iron(III) centre

TABLE 1. Mössbauer data of haematin–histidine molecular complexes at 80 K

Adducts	Isomer shift (w and t Fe in mm s^{-1})[a]	Linewidth (mm s^{-1})[a]	Quadrupole splitting (mm s^{-1})[a]
[(PPIXFe)$_2$O][b]	0.39	0.19	0.56
[(PPIXFe)$_2$O] (solid)[b]	0.40	0.17	0.58
(PPIXFe)$_2$O–histidine[b]	0.38	0.14	0.43
(PPIXFe)$_2$O–histamine[b]	0.41	0.20	0.46
(PPIXFe)$_2$O–*N*-α-acetyl histidine[b]	0.41	0.19	0.46

[a]Error in measurement ±0.01 mm s^{-1}. [b]Frozen solution in 20% (vol./vol.) ethanol:water at pH (uncorrected) 10.1.

through amino or carboxylate groups. We have also found that the amino group of glycine does not bind to the Fe(III) in haematin.

To investigate whether the change in Δ could be explained by invoking a contribution arising from charge neutralization of the propionate groups on PPIX by the NH_3^+ group of histidine we recorded the spectrum of PPIXFe(III) in the presence of glycine. We found that under identical conditions glycine (an amino acid that contains no aromatic residues) has no influence on the Mössbauer spectrum of $[(PPIXFe)_2O]$. We therefore suggest that the changes in the Mössbauer parameters of $[(PPIXFe)_2O]$ in the presence of histidine arise from a charge transfer interaction due to π–π interactions between them.

The charge transfer is most likely to be between a pyrrole ring on the side of the porphyrin opposite to the μ-oxo-bridge and the imidazole ring of the histidine. However, the fact that Baldwin *et al.* [9] found little electronic spectral evidence for the complex suggests that this charge transfer is relatively weak at room temperature (the Mössbauer spectrum at the iron(III) centre is very sensitive to small changes in electron density and it is therefore detected at 80 K where the donor–acceptor interactions are likely to be stronger).

The question to then pose is: Do other interactions aid complex formation?

The answer to this question is probably yes, the role of the amino group could be to form a hydrogen bond with the PPIX propionate group (–N–N···$^-$OOCPor) so as to align the imidazole ring parallel to the porphyrin plane. Thus allowing π–π overlap between the imidazole ring and a pyrrole ring of the porphyrin (see model, 'Discussion' below). We note that Adams *et al.* [11] suggested a similar interaction between their pyridinium ion's positive nitrogen and the negatively charged propionic acid group. Moreover, these workers [11] found that methylation (esterification) of the propionate group deters the formation of such complexes. We have also found that 1-methylimidazole (without a side chain) does not form a donor–acceptor complex with haematin.

Assuming both the electrostatic hydrogen-bonding interaction (between the protonated ligand amino nitrogen and the negatively charged propionate group) and the π–π charge transfer interaction (between the imidazole ring and a pyrrole ring on the porphyrin), a scale model of a part of a histidine: $[(PPIXFe)_2O]$ complex can be constructed. The model indicates the imidazole ring of the histidine may be overlayed on one of the porphyrin pyrrole rings that contains an electron withdrawing vinyl substituent. At a pyrrole–imidazole ring distance of *c.* 3.0 Å the non-bonded repulsions of the other atoms of the porphyrin and histidine appear to be minimal. The NH_2 group of histidine is shown hydrogen-bonded to one of the propionate carboxylates with an N–H–O of *c.* 2.8. We note that the carboxylate group of the histidine can lie close to the iron(III) ion (Fig. 3). This model demonstrates that in addition to the π–π charge transfer interaction, there are electrostatic interactions that will contribute to the stability of this donor acceptor complex. We note that the complex may in fact be more than a 1:1 histidine $[(PPIXFe)_2O]$ ratio as for instance that found for aniline [11].

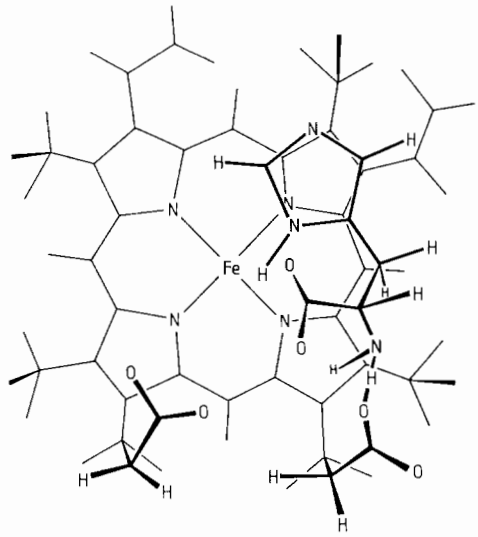

Fig. 3. Schematic diagram showing one (PPIXFe) ring of a $[(PPIXFe)_2O]$ histidine complex, showing proposed interaction between the histidine and the PPIX moiety. Note that the Fe atom sits below and out of the porphyrin plane.

Conclusions

The Mössbauer data and the scale model are compatible with a donor–acceptor histidine–$[(PPIXFe)_2O]$ complex in which the histidine ring binds to the porphyrin ring via a π–π charge transfer interaction. The role of the propionic acid group in the model complex is to mimic the role of the protein side chains in aligning the imidazole ring of histidine parallel to the pyrrole ring of the haem.

The implications of such a structure for haemproteins are interesting. The possibility of a strong donor–acceptor interaction between the haem and a nearby aromatic amino acid residue in the haem pocket of a protein is largely dependent on the conformation of the apoprotein which could bring about a favourable parallel orientation. Such a change in conformation may be possible during a R to T state transition in a haemprotein. Such a possible π–π interaction in one of the conformational states of the protein would change its reactivity to, for instance, oxygen binding or electron transfer [2, 8]. Conversely, a π–π charge transfer between the haem and an

aromatic amino acid may modify the dynamics of nearby amino acid residues so as to bring about a conformational change of the apoprotein leading to cooperative interactions.

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