Mössbauer Studies on Cytochrome b Models: Bis Ligated Complexes of Iron(III) Protoporphyrinate IX with Imidazole and Substituted Imidazoles†

Okhil K. Medhi and Jack Silver*
Department of Chemistry and Biological Chemistry, University of Essex, Wivenhoe Park, Colchester CO4 3SQ

Iron-57 Mössbauer spectra at 80 K have been recorded for a series of bis ligated complexes of iron(III) protoporphyrinate IX (3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionate) $[FeL]^+$ with imidazole (Him), 1-methylimidazole (1Me-im), and 2-methylimidazole (2Me-im). The spectra were recorded in frozen solutions of dimethyl sulphoxide (dmso), acetone, and 50% (v/v) ethanol–water and also in the presence of hydrogen-bonding agents such as trifluoroethanol or 1,10-phenanthroline in acetone. This is the first report of Mössbauer spectroscopic studies on the solvent dependence of a series of substituted imidazole complexes of [FeLCl]. Significant new results are obtained which are interpreted in the light of reported e.s.r., n.m.r., and electronic absorption data. A value of 2.43 mm s^{-1} for the quadrupole splitting (ΔE_Q) of the imidazolate complex [FeL(Him)(im)] is assigned to a parallel orientation of the planar axial ligands, whereas a value of ΔE_Q = 1.87 mm s^{-1} for the $[FeL(2Me\text{-}im)_2]^+$ is assigned to a perpendicular orientation of the imidazole planes. Large linewidths of about 0.6—0.98 mm s^{-1} are attributed to the presence of a statistical distribution of imidazole planes around the thermodynamically stable arrangement. The influences of hydrogen bonding on the Mössbauer parameters are discussed. The 2Me-im complex and the 1Me-im complex in dmso show slow spin–lattice relaxation at 80 K.

An understanding of the chemistry of iron porphyrin complexes containing imidazoles [Figure 1(*a*)] as axial ligands is of considerable interest as histidylimidazole is found co-ordinated to iron porphyrins in haem proteins.[1,2] Thus bis(imidazole) complexes of (protoporphyrinato IX) iron(III) chloride [FeLCl] (L = 3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionate) [Figure 1(*b*)] are appropriate models for the prosthetic group of cytochrome b_5[3] and also for a wide variety[4] of membrane-bound cytochromes b, including mitochondrial b_{566} and b_{562}, chloroplast b_6, and chloroplast b_{559}. Other related proteins that contain bis(histidine) co-ordination are cytochrome a of cytochrome oxidase[1,2] and cytochrome c_3.[5]

Cytochromes b show a wide range of physical properties as evidenced by their electrode potentials and e.s.r. *g* values,[4,6–8] though there is apparently little change in the iron co-ordination environment. It is generally believed that such differences in properties arising from the same co-ordination environment originate from a change in the relative orientation of the planes of the two imidazoles that are bound to the iron porphyrin moiety.[4,8] Other factors such as (1) 'strains' in bis(histidine) ligation[9] and (2) hydrogen bonding (and/or deprotonation of) histidylimidazole rings[10,11] may also be important. There is no doubt that the primary control of haem iron reactivity in haemproteins involves the steric and/or electronic influence of the ubiquitous histidylimidazole ligand.[12]

Bis(imidazole) complexes of natural iron porphyrins have been widely studied using electronic absorption,[13–16] e.s.r.,[4,6,9,16–18] n.m.r.,[19–22] and Mössbauer spectroscopy.[23–25] Electronic absorption spectroscopy has been used to study the dependence of axial ligand binding constants on the solvent[13,14] and also to study hydrogen-bonding effects.[15,16] E.s.r. studies on such model complexes have reproduced the unusual 'highly anisotropic' low-spin (h.a.l.s.) signals observed for proteins.[6,9,17] N.m.r. data provided evidence for extensive π-electron delocalisation in the metal–ligand bond,[19–21] and showed that the influence of deprotonation or hydrogen bonding of co-ordinated imidazole on the contact shifts is significant.[22] There have been relatively few Mössbauer

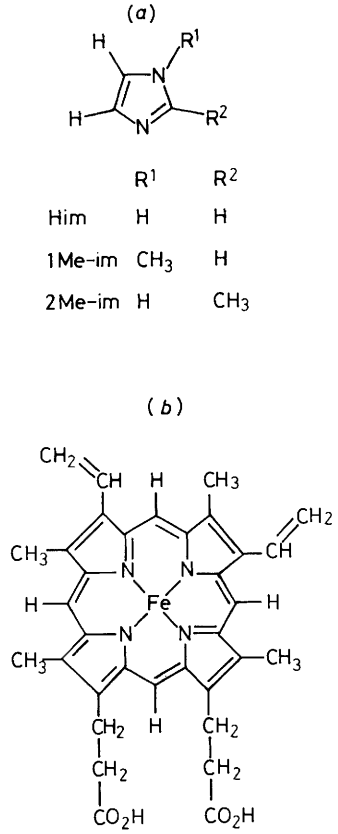

Figure 1. Structure of imidazole derivatives relevant to this work (*a*), and (*b*) structure of iron protoporphyrinate IX (haem)

† Protoporphyrinate IX = 3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionate.

Table 1. Iron-57 Mössbauer parameters at 80 K for (Protoporphyrinato IX)iron(III) bis(imidazole) complexes

Compound	Solvent	δ(Fe)/ mm s^{-1}	ΔE_Q/ mm s^{-1}	Γ*/ mm s^{-1}	Relative % area of absorption lines
[FeL(Him)(im)]	dmso + NBu_4OH	0.24(3)	2.43(3)	0.27(5), 0.25(4)	48.6(10.7), 51.4(10.1)
$[FeL(Him)_2]^+$	dmso	0.22(2)	2.38(2)	0.21(3), 0.26(4)	49.1(7.9), 50.9(8.6)
	Water–ethanol (1:1)	0.24(1)	2.35(1)	0.31(1), 0.32(1)	50.9(1.9), 49.1(1.9)
	Acetone	0.28(1)	2.34(1)	0.25(2), 0.26(2)	52.5(4.4), 47.5(4.4)
	Acetone + trifluoroethanol	0.24(2)	2.32(2)	0.25(2), 0.26(2)	52.9(5.2), 47.1(5.2)
	Acetone + 1,10-phenanthroline	0.22(7)	2.34(7)	0.39(8), 0.42(12)	56.1(13.6), 43.9(14.2)
$[FeL(1Me\text{-}im)_2]^+$	Water–ethanol (1:1)	0.26(1)	2.34(1)	0.16(1), 0.18(1)	52.2(1.7), 47.8(1.7)
	dmso	0.23(1)	2.24(1)	0.37(1), 0.49(2)	53.9(2.1), 46.1(2.5)
$[FeL(2Me\text{-}im)_2]^+$	Water–ethanol (1:1)	0.16(2)	1.87(2)	0.29(1), 0.59(3)	42.7(2.5), 57.3(3.8)

* Half-width at half-height.

spectroscopic studies[8,23–25] on bis(imidazole)porphyrinato-iron(III) complexes, and the accuracy of some of the data[25] and their interpretation have been questioned.[26,27]

The structural effects of unusual orientations of axial ligands or the spin state of bis(2-methylimidazole)(porphyrinato)-iron(III) species have been studied in perchlorate structures of the tpp[28] (*meso*-tetraphenylporphyrinate) and oep[29] (octaethylporphyrinate) complexes. The oep complex[29] is predominantly of $S = \frac{5}{2}$ character in the solid state but in solution is consistent with a thermal spin equilibrium ($S = \frac{1}{2}$ to $\frac{5}{2}$). This material has an h.a.l.s. spectrum in solution but not in the solid.[8,29] The tpp complex is low spin[28] and in the solid state exhibits an h.a.l.s. spectrum. It is characterised by having a porphyrin core that has S_4 ruffling and the planes of the axial ligands are mutually perpendicular. In addition, it has a quadrupole splitting (ΔE_Q) of 1.77 mm s^{-1}.[8]

In the light of recent developments in the chemistry of low-spin bis(imidazole)(porphyrinato)iron(III) complexes,[4,6–9,15–19,22] it seemed to us timely to reinvestigate and extend the earlier Mössbauer spectroscopic work on these complexes, particularly as this experimental technique provides unambiguous characterisation of the iron electronic structure.[26] The aims of the studies described here were to gather evidence to test the various hypotheses of steric and/or electronic influences of the imidazole ligand on the haem electronic structure. One question that is answered is: 'Does $[FeL(2Me\text{-}im)_2]^+$ have a low ΔE_Q value, as it displays a h.a.l.s. spectra?'[17] The studies reported here also consider (1) the effect of various hindered and non-hindered imidazoles and (2) solvent effects on hydrogen bonding and deprotonation of the axial ligands on the Mössbauer spectroscopic parameters of the resulting low-spin protoporphyrin IX iron(III) complexes $[FeL(R\text{-}im)_2]^+$, where R = H, 1-Me, or 2-Me [Figure 1(*a*)].

Experimental

All chemicals used in this work were purchased from Aldrich Chemical Co. and were used without further purification. Tetrabutylammonium hydroxide was a 1.0 mol dm^{-3} solution in methanol. The solvent, 50% (v/v) ethanol–water, was prepared from distilled, deionized water and reagent-grade 95% ethanol.

Since it is known that [FeLCl] undergoes extensive aggregation in water,[14] the choice of the solvent mixture and reaction conditions were dictated by the stability and solubility of the monomeric bis(ligated) complexes, which were known from the previous work using electronic absorption spectroscopy.[13–15] The solutions for the Mössbauer spectroscopic measurements were prepared by dissolving [FeLCl] in an appropriate solvent containing a thousand-fold excess of imidazole (Him), 1-methylimidazole (1Me-im), or 2-methylimidazole (2Me-im). The reason for using such a large excess of imidazole was to drive the equilibria towards the exclusive formation of the low-spin bis(imidazole) complexes in solution.[13–15] The formation of the bis complexes in the Mössbauer samples were verified by recording the electronic absorption spectra and comparing them with the reported results.

The Mössbauer spectra were recorded using an instrument and techniques previously described by us.[30] The visible spectra were recorded on a Perkin-Elmer Lambda 5 spectrophotometer.

Results

The Mössbauer data recorded in this work are presented in Table 1 and representative spectra are presented in Figures 3 and 4. Relevant literature data are also included in Table 1.

Our data in Table 1 agree quite well with those reported by Epstein *et al.*[23] for their solid complex. The data are also comparable with other low-spin iron(III) porphyrin complexes reported previously[8,24–26] and also with those for cytochrome b_5 and other low-spin ferrihaem proteins.[31,32] The isomer shift reported by Bullard *et al.*[25] is significantly smaller than that of ours and others.[23]

The isomer shifts and quadrupole splittings for the low-spin bis(imidazole) complexes are relatively independent of the nature of the solvents used unless hydroxide anions are added. However, the linewidths and relative intensities of the two spectral lines are quite sensitive to the nature of the solvent present. A marked solvent dependence of the Mössbauer parameters is observed when substituted imidazoles are used.

We have observed that Him and 1Me-im complexes in neat dimethyl sulphoxide (dmso) and acetone show evidence for the presence of small amounts of high-spin species. We have chosen to neglect these species and have fitted the experimental data by two Lorentzian lines arising from the low-spin species. It must be noted that on addition of hydrogen-bonding agents, such as trifluoroethanol or 1,10-phenanthroline, to the solutions the high-spin species were not observed. Moreover, in water–ethanol (1:1) the non-hindered imidazole (Him and 1Me-im) complexes show that only low-spin complexes are present, whereas for the hindered 2Me-im complex a high-spin species is present to a small extent.

Finally, the spectra obtained for the 2Me-im complex in 50% ethanol and for the 1M-im complex in dmso are particularly asymmetric. Such spectra for low-spin iron(III) complexes were previously observed by Lang *et al.*[33] for cyano haemoglobin at 77 K and also by us for lyophilized samples of cytochrome *c* polypeptides.[34,35]

Discussion

Quadrupole Splittings in Low-spin Iron(III) Porphyrins.—To put some of the experimental results obtained in this study into

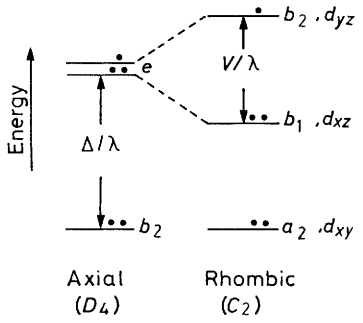

Figure 2. Ordering of one-electron energy states and ligand-field parameters for low-spin iron(III) porphyrins in axial (tetragonal) and rhombic symmetry perturbations. Here λ is the spin–orbit coupling constant; Δ/λ and V/λ are the magnitudes of the axial and rhombic distortions expressed in units of λ

perspective, it is necessary briefly first to consider aspects of the electronic structure of low-spin (porphyrinato)iron(III) complexes. A theoretical understanding of such systems was lucidly discussed by Palmer[36] in two excellent reviews. The important features of the theory relevant to this work are briefly summarised here.

The low-spin d^5 iron(III) ion in O_h symmetry has a t_{2g}^5 electronic configuration. In the bis(imidazole) complexes of FeL the degeneracy of the one-electron states is lifted by axial and rhombic perturbations to yield d_{xy} as the most stable followed by d_{xz} and d_{yz} in order of increasing energy[36] (Figure 2).

Lang and co-workers[33,37] extended this theory to calculate the hyperfine parameters such as the magnetic hyperfine field and the electric field gradient (e.f.g) at the nucleus. Golding[38] demonstrated that the ΔE_Q value is sensitive to the distortion parameters and gave a maximum value of $\Delta E_Q = 2.5$ mm s^{-1} for low-spin iron(III) complexes.

The e.f.g is the sum of q_{val} (the valence term) [arising from the imbalance in the electron distribution in the valence orbitals around the iron(III) ion] and q_{latt} (the lattice term) [arising from the distribution of the other charges in the neighbourhood of the iron(III) ion]. We expect q_{val} and q_{latt} to be of opposite sign, and $q_{val} \gg q_{latt}$ in the covalent compounds reported in this work.

In order to relate the e.f.g. to molecular structure and bonding, q_{val} may be expressed as the sum of q_{CF} and q_{MO}, where q_{CF} results from the population of one-electron states (Figure 2) within the assumptions of crystal-field theory, and q_{MO} originates from the donation and/or withdrawal of charge density due to covalency. Thus q_{val} results from an asymmetric distribution of electron density in both bonding and non-bonding orbitals (Figure 2). The relative changes in q_{val} in various situations may then be estimated qualitatively by using the Townes–Dailey approximation[39] and chemical intuition of σ donation and/or π donation by the ligands in the x,y plane and along the z axis (taken as the normal to the x,y porphyrin plane[36]).

In low-spin iron(III) systems the q_{CF} term is dominant and its contribution may obscure the relation of σ or π bonding contributions.[40] We suggest that in low-spin (porphyrinato)iron(III) complexes the symmetry of the charge distribution and hence q_{CF} is mainly controlled by the magnitude of the rhombic distortion parameter (V) in relation to the spin-orbit coupling (λ) in the complex. Three situations can then be distinguished.

(*a*) When $V \gg \lambda$ and $\Delta \gg \lambda$, the ground state is an orbital singlet (^2B) and the unpaired electron is localised in d_{yz} (Figure 2). This generates an unequal distribution of charge density in the x and y directions giving rise to a large q_{CF}. Such a situation arises when the two imidazole planes are parallel and both molecules interact with the iron d_{yz} orbital making it the highest-energy orbital (as found from the analysis of e.s.r. data[17,36]). Scheidt and co-workers[8,41,42] have pointed out that such a parallel orientation of imidazoles lying over the porphyrin nitrogen atoms is the thermodynamically most stable form. In the absence of any contribution from q_{MO} and q_{latt}, this situation corresponds to Golding's value of maximum ΔE_Q of 2.5 mm s^{-1} for the maximum distortion in a low-spin iron(III) complex.

(*b*) When $V < \lambda$ and $\Delta \gg \lambda$, the unpaired electron is delocalised over the d_{xz} and d_{yz} orbitals giving rise to an orbital doublet (^2E) ground state for the complex. This arises from an effective electronic axial symmetry; q_{CF} is obviously smaller than that in case (*a*). This case occurs when one of the imidazole ligands forms a π bond with the iron d_{yz} orbital and the other forms a π bond with the iron d_{xz} orbital. For maximum π overlap within these orbitals the imidazole planes should be orientated perpendicular to each other. Recently the crystal structure and e.s.r. studies of $[Fe(tpp)(py)_2]^+$ have been reported;[43] the two pyridine (py) molecules adopt a perpendicular geometry and the d_{xz} and d_{yz} orbitals are nearly degenerate. Analysis of the e.s.r. g values of the $[FeL(py)_2]^+$ complex show that the d_{xz} and d_{yz} orbitals in this molecule are quite close in energy.[17,44] This suggests that the two pyridine planes are aligned approximately perpendicular to each other as in the tpp derivative. The structure of the $[Fe(tpp)(2Me\text{-}im)_2]^+$ salt[28] ($\Delta E_Q = 1.77$ mm s^{-1}) shows that the axial ligand planes are aligned close to a perpendicular orientation and e.s.r. data again indicate[8,28] the near degeneracy of the d_{xz} and d_{yz} orbitals ($V = 0.9\ \lambda$). In addition, e.s.r. studies[17] on $[FeL(4Me\text{-}py)_2]Cl$ (4Me-py = 4-methylpyridine) and $[FeL(4NH_2\text{-}py)_2]Cl$ also indicate that $V \approx \lambda$ in these two compounds. Mössbauer data for the $[FeL(L')_2]^+$ species where L′ = py[23,44] ($\Delta E_Q = 1.88$ mm s^{-1}), 4 Me-py[25] ($\Delta E_Q = 1.97$ mm s^{-1}), or 4NH$_2$-py[25] ($\Delta E_Q = 1.93$ mm s^{-1}) show that ΔE_Q is around 1.9 mm s^{-1} for the perpendicular orientation of the axial ligand planes in $[FeL(L')_2]^+$ complexes.

(*c*) When $V < \lambda$ and $\Delta < \lambda$ then the energy states are close together. Electrostatic interactions or spin–orbit coupling may mix the ^2E and ^2B states leading to a more symmetric charge distribution around the iron nucleus. This possibility arises when there are two or more possible conformations of the ligand orientations. A comparison of e.s.r.[17,43,44] and Mössbauer[23,25,44,45] data indicates that this situation would generate a much smaller value of ΔE_Q than either of the two cases above. Inclusion of lattice and covalency contributions give ΔE_Q in the range 0.3—1.3 mm s^{-1} for cyanide-containing complexes.[44,45] We have recently found from n.m.r. studies that the temperature variation of the isotropic proton-shift data fits well with this model for bis(cyanide) and pyridine cyanide complexes of FeL encapsulated in a detergent micelle.[46]

It is therefore significant that in the Mössbauer data for the low-spin porphyrinatoiron(III) complexes described here and in the literature[8,23–25,44] the crystal-field term (q_{CF}) dominates ΔE_Q values. Hence any comments on secondary effects due to ligand σ and π bonding contributions to the e.f.g. (that are argued to arise from the relative basicity or π-donating abilities of a series of axial ligands) would be valid only if the ground state of the system is known not to change as the ligand basicity increases or decreases. For example, the differences in ΔE_Q between the bis(imidazole) (2.35 mm s^{-1}) and the bis(pyridine) (1.88 mm s^{-1}) complexes of FeL is most likely due to a change in q_{CF} arising from differing electronic occupation of d_{xz} and d_{yz} [cases (*a*) and (*b*) above], rather than from a change in the π-donating ability of these two ligands as proposed by Epstein *et al.*[23]

Orientation Effects of Substituted Imidazoles.—$[FeL(2Me\text{-}im)_2]^+$. The quadrupole splitting for this complex ($\Delta E_Q = 1.87$

mm s^{-1}) is the lowest in the series of compounds studied here. The value is similar to that of $[FeL(py)_2]Cl$ (ΔE_Q = 1.88 mm s^{-1}).[23] If ΔE_Q was simply a result of ligand basicity, this would be a surprising result [2Me-im, $pK_a(HB^+)$ = 7.56; py, $pK_a(HB^+)$ = 5.2]. It is much more likely that these ΔE_Q results are fortuitous and the 2Me-im and py ligands in fact use differing amounts of σ and π overlap in their bonding to FeL. Interestingly both these FeL complexes have h.a.l.s. e.s.r. spectra[9,17,42] with $g_{max.} > 3.0$.

Recent e.s.r. studies[8,43] on h.a.l.s. systems show that structures having perpendicular orientation of planar axial ligand have a line at $g_{max.} > 3.0$. This geometry results in a near degeneracy of d_{yz} and d_{xz} orbitals and therefore corresponds to case (*b*) discussed above. Thus the quadrupole splitting of 1.87 mm s^{-1} is assigned to such a structure.

The Mössbauer parameters for the non-hindered imidazoles (Him and 1Me-im) show that those with the largest rhombic distortion and rhombicity show the largest ΔE_Q (Tables 1 and 2).

$[FeL(1Me\text{-}im)_2]^+$. The quadrupole splitting of this complex is highly solvent dependent, ΔE_Q increasing by 0.1 mm s^{-1} in a water–ethanol (1:1) solution compared to a dmso solution. The linewidth of the Mössbauer lines in the former solvent are less than half their width in the latter. Moreover, this compound in a frozen solution of aqueous ethanol manifests a symmetric quadrupole doublet in contrast to an asymmetric doublet in dmso. The e.s.r. data in Table 2 show that in dmso the rhombic distortion and rhombicity are much less than that in aqueous alkaline solution.

The crystal structure of $[FeL(1Me\text{-}im)_2]CH_3OH{\cdot}H_2O$ is known;[47] the two axial ligands are nearly parallel (φ = −3 and 16° respectively, and θ = 19°). As a result of steric interactions associated with the differing orientations of the two axial imidazoles, the two iron–imidazole bond lengths were found to be non-equivalent. Both CH_3OH and H_2O molecules are hydrogen bonded to the propionate carboxylate groups of L and the complex may be best formulated as a hydroxide $[FeL(1Me\text{-}im)_2]^+OH^-$.[47]

From the preceeding facts it is probable that the 1Me-im complex has different conformations of the imidazole plane orientations in aqueous ethanolic and in dmso solutions. In aqueous ethanol the C_2H_5OH and H_2O molecules may form hydrogen bonds to the propionate groups (as in the crystal structure), and thus stabilise the bis adduct through charge neutralisation and solvation. It is known that in polar solvents the bis(imidazole) adducts are more stable and probably have stronger iron–imidazole bonds.[13–15] In a frozen solution the most stable form would be the one where the two imidazole planes are oriented parallel to each other.[41] Thus, a large value of ΔE_Q (symmetric quadrupole doublet), and a large rhombic distortion (Table 2) in aqueous ethanol, indicate that in this solvent the two 1Me-im planes are parallel to each other.

In dmso solution, the smaller value of ΔE_Q and a smaller rhombic distortion (Table 2) indicate that the two axial 1Me-im ligands are no longer coplanar. However, the ΔE_Q observed (2.24 mm s^{-1}) in dmso is much larger than that expected (*ca.* 1.9 mm s^{-1} as discussed earlier) for a perpendicular orientation of the planar ligands. Hence the orientation of the 1Me-im planes in dmso are non-parallel, though they do not approach a perpendicular alignment. Thus their orientation in dmso could be similar to that found in the crystal structure but possibly with somewhat larger values of φ and θ (defined in Ref. 42).

Any deviation from the stable parallel orientation leads to a small distribution range of imidazole planes since molecules with several combinations of φ and θ values may have equal energy. The observed increase in the linewidths in dmso (0.37, 0.49 mm s^{-1}) as compared to that in ethanol–water (1:1) (0.16, 0.18 mm s^{-1}) indicates the presence of more than one Mössbauer site in $[FeL(1Me\text{-}im)_2]^+$ in dmso.

Support for such a change in orientation of axial imidazole planes in dmso may be found in thermodynamic data on ligand binding[13,14] which infers that the iron–imidazole bonds in dmso are weak ($\beta_2 \approx 10^4$ dm^6 mol^{-2}) compared to those in water–ethanol (1:1) ($\beta_2 \approx 10^6$ dm^6 mol^{-2}). A weaker bond would also be expected to be present in sterically hindered 2Me-im. This would both allow some degree of freedom for rotation and could in part arise from such rotations. Steric interactions associated with the differing orientations of the two axial ligands are therefore related to long iron–imidazole bonds. Electronic factors due to solvent polarity and hydrogen bonds, in stabilising the bis(imidazole) adducts, also have considerable influences on the iron–imidazole bond and hence on the orientation of the axial ligand.

$[FeL(Him)L']^+$ (L′ = Him *or* im). The quadrupole splitting of $[FeL(Him)_2]^+$ is independent of the nature of the solvent (Table 1) (2.34 ± 0.02 mm s^{-1}). On the other hand, on deprotonation of one of the imidazole ligands to give [FeL(Him)(im)] ΔE_Q becomes 2.43 mm s^{-1}. This is an increase of 0.09 mm s^{-1} in ΔE_Q, similar to what was observed for the 1Me-im analogue in changing the solvent from dmso to aqueous ethanol. We found that the basicity and hydrogen bonding of axial ligands does not change the isomer shifts and quadrupole splittings as drastically as observed on deprotonation (see below). Thus deprotonation may well be accompanied by a change in orientation of the axial ligand planes.

If it is assumed that the stable structure of $[FeL(Him)_2]^+$ in ethanol–water (1:1), dmso, and acetone is similar to that of $[FeL(1Me\text{-}im)_2]CH_3OH{\cdot}H_2O$ in the crystal (with similar values of φ and θ),[47] then on deprotonation in alkali θ may approach 0°, *i.e.* the axial ligand planes become parallel. This structure would then possess a large rhombic distortion such as was found in the e.s.r. study[17] (*ca.* 2.5 λ in Table 2), and a large ΔE_Q value as observed in this work.

The differences in the Mössbauer linewidth data and the ΔE_Q values for Him and 1Me-im in dmso are significant and point to differences in the behaviour of these two ligands in dmso. The Him complex has narrower linewidths, a larger ΔE_Q, and thus exists in less orientational conformations than does the 1Me-im complex in dmso.

To summarise, [FeL(Him)(im)] and $[FeL(1Me\text{-}im)_2]^+OH^-$ in aqueous ethanol have their imidazole planes in parallel orientation, whereas in $[FeL(Him)_2]^+$ and $[FeL(1Me\text{-}im)_2]^+$ in dmso the imidazole planes are non-parallel. The complex $[FeL(2Me\text{-}im)_2]^+$ corresponds to a situation where the imidazole planes are in a near or exactly perpendicular orientation.

Effects of Hydrogen Bonding.—There are two differing positions of hydrogen bonding which are possible in the $[FeL(L')_2]^+$ complexes studied here. These are (1) hydrogen bonding of the propionic acid carboxylates with the solvent or counter anion (when L′ = 1Me-im as discussed above), and (2) hydrogen bonding of the N–H of axially co-ordinating imidazoles (when L′ = Him or 2Me-im) with solvents, with added hydrogen-bonding agents[15] (such as trifluoroethanol or 1,10-phenanthroline) or with excess of imidazole in solution. The influence of hydrogen bonding on the stability of the low-spin state is discussed in the following section. Here we discuss the effect of hydrogen bonding of axial ligands in favouring a non-parallel orientation of the imidazole planes and in influencing the basicity of axial ligands.

When hydrogen bonding to the axial ligands is possible, the linewidths of the Mössbauer spectra are large. This may be confirmed as follows.

(*i*) For the $[FeL(L')_2]^+$ complexes where L′ = Him or

Table 2. E.s.r.[a] and Mössbauer (ΔE_Q)[b] data and crystal-field-parameters for relevant $[FeL(L')_2]^+$ complexes

Compound	Medium	g_z	g_y	g_x	V/λ	Δ/λ	V/Δ	ΔE_Q/ mm s^{-1}
$[FeL(Him)_2]^+$	dmso[c]	3.02	2.24	1.51	1.77	3.54	0.50	2.35
[FeL(Him)(im)]	dmso[d] + OH^-	2.76	2.28	1.68	2.45	3.71	0.59	2.43
$[FeL(1Me\text{-}im)_2]^+$	dmso[e]	2.97	2.27	1.51	1.84	3.27	0.56	2.24
$[FeL(1Me\text{-}im)_2]^+OH^-$[f]	Water–ethanol (1:1)[g]	2.74	2.27	1.72	2.57	2.8	0.67	2.34
$[FeL(2Me\text{-}im)_2]^+$	*h*	3.48	2.36	1.05	1.15	2.26	0.50	1.87
Cytochrome b_5, liver, native	neutral[c]	3.03	2.23	1.43	1.68	3.23	0.52	2.27[i]
	alkaline[c]	2.76	2.28	1.68	2.45	3.71	0.66	—
Cytochrome b_{559}, high potential	(membrane)[j]	3.08	2.16	1.36	0.62	1.52	0.41	—
low potential	(membrane)[j]	2.94	2.26	1.50	1.08	1.86	0.58	—

[a] From refs. 4, 9, 16—18. [b] This work. [c] Ref. 18. [d] Refs. 16 and 18. [e] Ref. 9. [f] Formulation from ref. 47 [also see text for water–ethanol (1:1)]. [g] E.s.r. data for alkaline solution in ref. 4. [h] E.s.r. data in ref. 17 taken in dimethylformamide (dmf) solution. [i] Experimental details are not known; $\delta = 0.23 \pm 0.03$ mm s^{-1} and $\Delta E_Q = 2.27 \pm 0.03$ mm s^{-1} at 195 K reported by E. Müenck, *Methods Enzymol.*, 1978, **54**, 346. [j] Ref. 4.

1Me-im in water–ethanol (1:1) the large linewidths found for the former lead to the suggestion that several similar sites may be caused by hydrogen bonding, but only one site is found for L′ = 1Me-im (where no axial ligand hydrogen bonding can take place).

(*ii*) For L′ = Him in dmso, acetone, and water–ethanol (1:1), the linewidth is largest for the strongest hydrogen-bonding solvent.

(*iii*) Also for the case L′ = Him, addition of hydrogen-bonding agents such as 1,10-phenanthroline to the acetone solution increases the linewidth by increasing the number of sites present in the solution.

The fact that hydrogen-bonding solvents do not influence the isomer shift and apparently the quadrupole splitting does not cast doubt on the formation of hydrogen bonds with axial imidazole ligands. Such bonding has been well established in the complexes studied here.[15] In fact the obvious inference to be taken from these unchanged Mössbauer parameters is that there are two opposing contributions to the electronic environment around the iron(III) ion. For example, hydrogen bonding of co-ordinated imidazoles could not only change the basicity of the axial ligands,[15] but also favour non-parallel orientation of imidazole planes as found[48] in the crystal-structure study of $[Fe(tpp)(Him)_2]^+Cl^-$ ($\theta = 57°$). The nearly constant value of ΔE_Q in the $[FeL(L')_2]^+$ (L′ = Him) complexes in Table 1 may be due to opposing contributions of a change in ligand basicity *versus* a change in the imidazole plane orientation, both induced by hydrogen bonding of axial Him ligands.

Constraints imposed by hydrogen-bonding interactions of imidazole ligands and solvent are responsible for non-parallel orientation of axial planes, particularly if the iron–imidazole bond is weak. We suggest that in the bis(imidazole) complex the resultant non-parallel orientation leads to a statistical distribution of angular alignment of imidazole planes, within a small range of φ and θ values close to the thermodynamically favourable parallel orientation.[41] These molecules with differing conformations of imidazole plane orientation would be in a state of slow interconversion and give rise to similar Mössbauer parameters. Thus a broad envelope would be expected in the Mössbauer spectrum of such a distribution. This could explain the large linewidths observed when hydrogen bonding is possible as against the smaller values obtained when hydrogen bonding is not favoured. Such line broadening in the Mössbauer and e.s.r. spectra of iron porphyrin complexes has been reported previously.[24,31,32]

Spin State and Hydrogen Bonding.—The Mössbauer spectra reported in this investigation are quite sensitive to the polarity and hydrogen-bonding ability of the solvents used. In solvents such as dmso or acetone the complexes show the presence of high-spin impurities, whereas for the non-hindered imidazoles in water–ethanol (1:1) or in the presence of hydrogen-bonding agents in acetone predominantly low-spin spectra are observed.

The presence of high-spin forms was previously reported in dmso[13] and in acetone solution.[15] The imidazole binding constant is quite large in water–ethanol (1:1)[14] ($\beta_2 = 3.5 \times 10^6$ dm^6 mol^{-2}) as compared to that in dmso[13] ($\beta_2 = 7 \times 10^4$ dm^6 mol^{-2}) and acetone[15] ($\beta_2 = 2.5 \times 10^4$ dm^6 mol^{-2}).

The present Mössbauer data reflecting the influence of hydrogen-bonding agents on stabilisation of the low-spin iron(III) states can be rationalised in the light of the reported electronic absorption spectroscopic data. However, correlation of isomer shift with ligand basicity (and hence ligand-field strengths) is not straightforward. For example, though 1Me-im is a stronger base [$pK_a(HB^+) = 7.33$] than Him [$pK_a(HB^+) = 6.65$], the isomer shift of the latter in water–ethanol (1:1) is similar to that of the former. This could be due to the fact that hydrogen bonding makes co-ordinated Him a stronger base than free Him. In polar hydrogen-bonding solvents the product ion pair $[FeL(1Me\text{-}im)_2]^+X^-$ is stabilised giving rise to a stronger iron–imidazole bond.[13] This strengthening of metal–ligand bonds would stabilise the low-spin species and this is the observed spin state.

The Mössbauer spectrum of the 2Me-im complex shows the presence of a small amount of a high-spin species even in water–ethanol (1:1). Based on e.s.r. studies, Carter *et al.*[9] showed that the compound in dmso is present as a mixture of high- and low spin states. Though 2Me-im is the strongest base in the series [$pK_a(HB^+) = 7.56$] the presence of the high-spin state arises from steric interactions.[9] However, our observation that the compound is *predominantly* low spin in frozen water–ethanol (1:1) suggests that hydrogen bonding to axial imidazole ligand can dramatically influence the stability of the low-spin iron(III) porphyrins.

Slow Spin-Lattice Relaxation and Asymmetric Broadening.—It may be seen from Figures 3 and 4 that in the spectra of 2Me-im and 1Me-im complexes the two Mössbauer lines of the quadrupole doublets are of unequal intensity and that the higher-velocity lines are broadened compared to those of the low-velocity lines. However, the relative areas of the two Mössbauer lines (Table 1) are the same within the experimental errors. Such an observation is quite rare for a low-spin iron(III) system. There are several possible mechanisms for asymmetric line broadening in Mössbauer spectra, such as randomly oriented polycrystalline samples.[26] However, in a frozen solution of low-spin FeL complexes the only mechanism con-

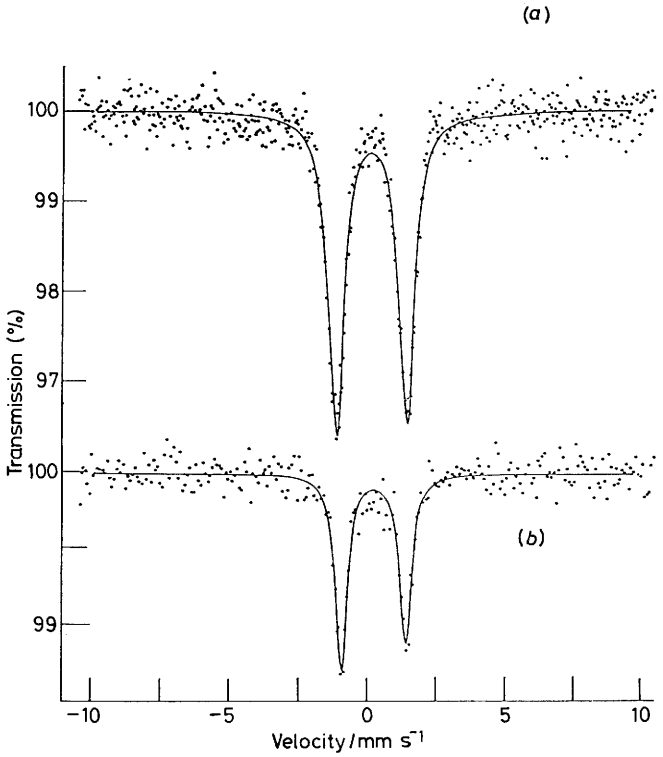

Figure 3. Mössbauer spectra (80 K) of $[FeL(Him)_2]^+$ in (*a*) ethanol–water (1:1) and (*b*) acetone

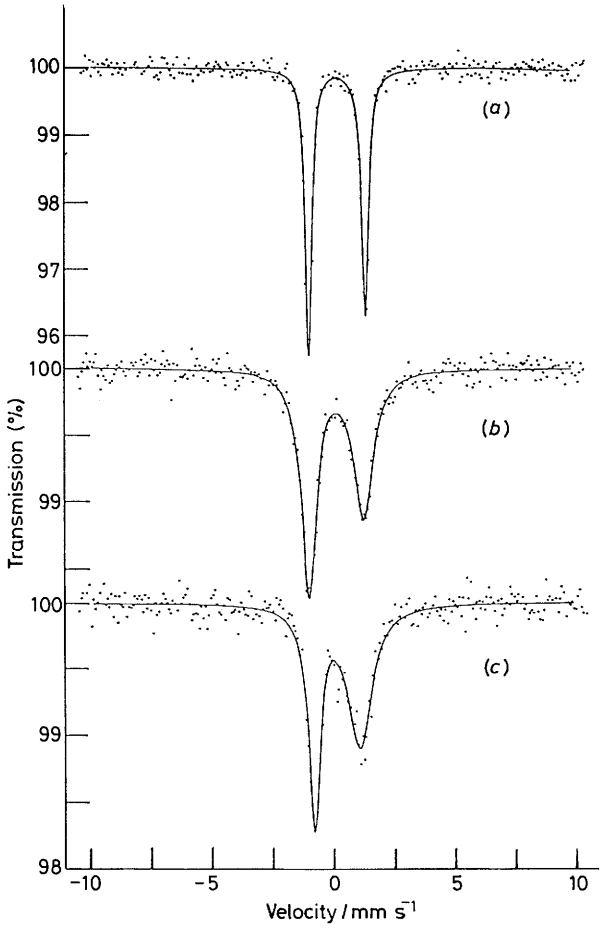

Figure 4. Mössbauer spectra (80 K) of $[FeL(1Me\text{-}im)_2]^+$ in (*a*) ethanol–water (1:1), (*b*) dimethyl sulphoxide, and (*c*) of $[FeL(2Me\text{-}im)_2]^+$ in ethanol–water (1:1)

sistent with the observation reported here is that the spin–lattice relaxation rates are slow compared to the nuclear precession frequencies.[33,37] Bradford and Marshall[49] used such a model successfully to fit the haemoglobin cyanide spectrum[33] at 77 K. The asymmetric line broadening in the Mössbauer spectrum of ferricytochrome c_2, cytochrome c, and dehydrated metmyoglobin was also attributed to slow spin-lattice relaxation.[32,50,51]

When the dominant mechanism is spin–lattice relaxation, that is coupling of the electron spin with the lattice phonon modes *via* spin–orbit interaction, we note that the Mössbauer spectra would be dominated by (1) the nature of the solvent, (2) the various possible hydrogen-bonding interactions with the porphyrin, and (3) by the exact nature of the electronic ground state. The crystal structure of the 1Me-im complex[47] shows that there is a water molecule hydrogen bonded to the two propionic acid groups of L and that the complex may be best formulated[47] as $[FeL(1Me\text{-}im)_2]^+OH^-$. The lattice of the 1Me-im complex in frozen dmso cannot provide any hydrogen-bonding possibilities as we have discussed earlier. These factors would have significant influence on the stability of the bis complexes, on the iron–imidazole bond lengths, and on the orientation of the 1Me-im planes in various solvents.

In complexes with a pure orbital singlet ground state there would be no orbital contribution to the magnetic moment, and hence any coupling of the electron spin with the lattice phonons would be negligibly small. Hence the slow relaxation in the 2Me-im complex and in the 1Me-im complex in dmso may result from a degeneracy of the orbital ground state. The doublet state has an effective hyperfine field (parallel to *z* axis) which will not time average to zero if the fluctuations are sufficiently slow.[50–53] This will lead to an onset of paramagnetic hyperfine splitting as found for some octahedral high-spin iron(II) complexes.[52,53] When the spin–lattice relaxation is the dominant mechanism, we expect the complexes to have either an orbital doublet (^2E) ground state or a ground state where there is a significant mixing of a ^2E state with an orbital singlet-state (^2B) by spin–orbit coupling or by thermal energy. We have pointed out earlier that the 2Me-im complex belongs to the first category by virtue of the perpendicular orientation of axial ligand planes and that the 1Me-im complex in dmso belongs to the latter category where the axial ligand planes are tilted from each other by a small angle.

Relevance to Cytochrome b.—It is noteworthy that the e.s.r. results for the complexes (Table 2) [FeL(Him)(im)] and $[FeL(1Me\text{-}im)_2]^+OH^-$ in aqueous ethanol are comparable to those for cytochrome b_5 in alkaline solution, whereas the e.s.r. results for the complexes $[FeL(L')_2]^+$ where L' = Him or 1Me-im in dmso are comparable to those for cytochrome b in a neutral medium.[4,9,16–18] This Mössbauer study demonstrates that these two situations (*viz.* aqueous ethanol *vs.* dmso) correspond to a change in orientation of the axial ligand planes. This change in orientation is associated with changes in the iron electronic structure in the porphyrin as observed in the data in Tables 1 and 2. Thus the Mössbauer data reported here support the hypothesis of Babcock *et al.*[4] that a change in the histidine (imidazole) plane orientation could give rise to the observed differences in the electrode potentials and e.s.r. *g*-values of the two forms of cytochrome b.

We found that the range of changes in ΔE_Q for non-hindered imidazole (Him and 1Me-im) complexes are much smaller than that expected for parallel to perpendicular orientation of ligand planes. The expected value of ΔE_Q for the perpendicular orientation of ligand planes is *ca.* 1.9 mm s^{-1} which was

obtained in the sterically hindered 2Me-im complex, $[FeL(2Me\text{-}im)_2]^+$, in aqueous ethanol. It is interesting to note that the e.s.r. results for the 2Me-im complex[17] are significantly different from those for both forms[18] of cytochrome b but are similar to those of cytochrome b_{562}, cytochrome c in alkali,[10] and certain mitochondrial b cytochromes.[9] Hence we suggest that in order to stabilise the perpendicular orientation of ligand planes considerable steric interaction between the porphyrin and the axial ligands is necessary.

Thus the size of change from a parallel to a non-parallel orientation of the axial ligand planes of the non-hindered imidazole model complexes (studied here by Mössbauer spectroscopy) is much smaller (*i.e.* never approaching perpendicular orientation) than the parallel to perpendicular change in the orientation of axial histidines proposed[4] for cytochromes b. The Mössbauer results for cytochrome b_5 (Table 2) are similar to those of $[FeL(1Me\text{-}im)_2]^+$ in dmso indicating a similarity of the imidazole plane orientation in the protein.

It may be pointed out that the crystal structure of a tetrahaem protein, cytochrome c_3 from *Desulphovibrio desulphuricans* strain Norway, has three haems that have their axial imidazoles in nearly parallel planes, while the fourth haem, which is the most remote from the aqueous medium, has its axial imidazoles perpendicular to each other.[5] The exposure of the haems to aqueous environments may be an important factor[54] in influencing the electronic structure of iron as found by us in the 1Me-im model, $[FeL(1Me\text{-}im)_2]^+$ in water–ethanol (1:1) and in dmso.

Conclusions

Considerations on the Mössbauer spectra of [FeLCl] complexes with imidazole and two of its substituted derivatives (recorded in various solvents and hydrogen-bonding agents) show that the orientation of planar axial ligands and various steric and/or electronic factors have considerable influence on the iron electronic structure of porphyrins. A value of ΔE_Q = 2.43 mm s^{-1} for the imidazolate complex is assigned to a parallel orientation of the planar axial ligands. A much lower value of ΔE_Q = 1.87 mm s^{-1} for the 2Me-im complex is found for the situation where the planar ligands are in a perpendicular orientation. Complexes where planar ligands are aligned perpendicular to each other may show an asymmetric quadrupole doublet where the higher-velocity line is comparatively broadened. This is attributed to slow spin–lattice relaxation in these complexes. Hydrogen bonding by various donors, external or internal to the porphyrin, shows considerable influence on the spin state of iron(III), particularly in the case of the 2Me-im complex. The large linewidths of about 0.6—0.98 mm s^{-1} obtained are attributed to the presence of a range of similar complexes varying in the distribution of angular alignment of their axial ligand planes around the thermodynamically most stable arrangement for a given substituted imidazole bound to [FeLCl].

Acknowledgements

We thank the Association of Commonwealth Universities, London, for support to one of us (O. K. M.). O. K. M. thanks the Department of Chemistry, Gauhati University, Gauhati, Assam for leave.

References

1 D. Dolphin (ed.), 'The Porphyrins,' Academic Press, New York, 1979, vols. 4 and 7.
2 A. B. P. Lever and H. B. Gray (eds.), 'Iron Porphyrins,' Part 2, Addison-Wesley, Reading, Massachusetts, 1983, parts 1 and 2.
3 G. N. La Mar, P. D. Burns, J. T. Jackson, K. M. Smith, K. C. Langry, and P. Strittmatter, *J. Biol. Chem.*, 1981, **256**, 6075.
4 G. T. Babcock, W. R. Widger, W. A. Cramer, W. A. Oertling, and J. Metz, *Biochemistry*, 1985, **24**, 3638.
5 M. Pierrot, R. Haser, M. Frey, F. Payan, and J. P. Astier, *J. Biol. Chem.*, 1982, **257**, 14341.
6 J. C. Salerno, *J. Biol. Chem.*, 1984, **249**, 2331.
7 A-H. Tsai and G. Palmer, *Biochim. Biophys. Acta*, 1983, **722**, 349.
8 F. A. Walker, B. H. Huynh, W. R. Scheidt, and S. R. Osvath, *J. Am. Chem. Soc.*, 1986, **108**, 5288.
9 K. R. Carter, A. Tsai, and G. Palmer, *FEBS Lett.*, 1981, **132**, 243.
10 D. L. Brautigan, B. A. Feinberg, B. M. Hoffman, E. Margoliash, J. Peisach, and W. E. Blumberg, *J. Biol. Chem.*, 1977, **252**, 574.
11 J. S. de Ropp, V. Thanbal, and G. N. La Mar, *J. Am. Chem. Soc.*, 1985, **107**, 8268.
12 M. F. Perutz and L. F. Ten Eyck, *Cold Spring Harbor Symp. Quant. Biol.*, 1971, **36**, 295.
13 R. F. Pasternack, B. S. Gillies, and J. R. Stahlbush, *J. Am. Chem. Soc.*, 1978, **100**, 2613.
14 B. B. Hasinoff, H. B. Dunford, and D. G. Horne, *Can. J. Chem.*, 1969, **47**, 3225.
15 M. Quing-jin, C. A. Tondreau, J. O. Edwards, and D. A. Sweigart, *J. Chem. Soc., Dalton Trans.*, 1985, 2269.
16 T. Yoshimura and T. Ozaki, *Arch. Biochem. Biophys.*, 1984, **230**, 466.
17 C. T. Migita and M. Iwaizumi, *J. Am. Chem. Soc.*, 1981, **103**, 4378.
18 J. Peisach and W. B. Mims, *Biochemistry*, 1977, **16**, 2795.
19 J. D. Satterlee and G. N. La Mar, *J. Am. Chem. Soc.*, 1976, **98**, 2804.
20 G. N. La Mar, J. S. Frye, and J. S. Satterlee, *Biochim. Biophys. Acta*, 1976, **428**, 78.
21 R. J. Kurland, R. G. Little, D. G. Davis, and C. Ho, *Biochemistry*, 1971, **10**, 2237.
22 V. P. Chacko and G. N. La Mar, *J. Am. Chem. Soc.*, 1982, **104**, 7002.
23 L. M. Epstein, D. K. Straub, and C. Maricondi, *Inorg. Chem.*, 1967, **6**, 1720.
24 D. K. Straub and W. M. Connor, *Ann. N.Y. Acad. Sci.*, 1973, **206**, 383.
25 L. Bullard, R. M. Panayappan, A. N. Thorpe, P. Hambright, and G. Ng, *Bioinorg. Chem.*, 1974, **3**, 161.
26 J. R. Sams and T. B. Tsin, in 'The Porphyrins,' ed. D. Dolphin, Academic Press, New York, 1979, vol. 4, ch. 9, pp. 425—478.
27 G. N. La Mar, in 'The Porphyrins,' ed. D. Dolphin, Academic Press, New York, 1979, vol. 4, ch. 2, pp. 61—157.
28 W. R. Scheidt, J. F. Kirner, I. L. Hoard, and C. A. Reed, *J. Am. Chem. Soc.*, 1987, **109**, 1963.
29 D. K. Geiger, Y. J. Lee, and W. R. Scheidt, *J. Am. Chem. Soc.*, 1984, **106**, 6339.
30 M. Y. Hamed, R. C. Hider, and J. Silver, *Inorg. Chim. Acta*, 1982, **66**, 13.
31 A. Dwivedi, W. A. Toscano, and P. G. Debrunner, *Biochim. Biophys. Acta*, 1979, **576**, 502.
32 B. H. Huynh, M. H. Emptage, and E. Münck, *Biochim. Biophys. Acta*, 1978, **534**, 295.
33 G. Lang and W. Marshall, *Proc. Phys. Soc. London*, 1966, **87**, 3.
34 J. Peterson, J. Silver, M. T. Wilson, and I. E. G. Morrison, *J. Inorg. Biochem.*, 1980, **13**, 75.
35 J. Peterson, M. M. M. Saleem, J. Silver, and M. T. Wilson, *J. Inorg. Biochem.*, 1983, **19**, 165.
36 G. Palmer, in 'The Porphyrins,' ed. D. Dolphin, Academic Press, New York, 1979, vol. 4, ch. 6, pp. 313—353; in 'Iron Porphyrins,' eds. A. B. P. Lever and H. B. Gray, Addison-Wesley, Reading, Massachusetts, 1983, part 2, ch. 2, pp. 43—88.
37. W. T. Oosterhuis and G. Lang, *Phys. Rev.*, 1969, **178**, 439.
38 R. M. Golding, in 'Applied Wave Mechanics,' Van Nostrand, Princeton, 1969, ch. 9, pp. 376—434.
39 B. P. Dailey and C. H. Townes, *J. Chem. Phys.*, 1955, **23**, 118.
40 G. R. Johnson and R. E. Shepherd, *Inorg. Chem.*, 1983, **22**, 3506.
41 W. R. Scheidt and D. M. Chipman, *J. Am. Chem. Soc.*, 1986, **198**, 1163.
42 W. R. Scheidt and Y. J. Lee, *Struct. Bonding (Berlin)*, 1987, **64**, 1.
43 D. Inniss, S. M. Soltis, and C. E. Strouse, *J. Am. Chem. Soc.*, 1988, **110**, 5644.
44. D. Rhynard, G. Lang, K. Spartalian, and T. Yonetani, *J. Chem. Phys.*, 1979, **71**, 3715.
45 B. Lukas and J. Silver, *Inorg. Chim. Acta*, 1986, **124**, 97.
46 O. K. Medhi, S. Mazumdar, and S. Mitra, *Inorg. Chem.*, 1989, **28**, 3243.

47 R. G. Little, K. R. Dymock, and J. Ibers, *J. Am. Chem. Soc.*, 1975, **97**, 4532.
48 D. M. Collins, R. Countryman, and J. L. Hoard, *J. Am. Chem. Soc.*, 1972, **94**, 2066.
49 E. Bradford and W. Marshall, *Proc. Phys. Soc. London*, 1966, **87**, 731.
50 G. Lang, D. Herbert, and T. Yonetani, *J. Chem. Phys.*, 1968, **49**, 944.
51 W. S. Caughey, W. Y. Fujimoto, A. J. Bearden, and T. H. Moss, *Biochemistry*, 1966, **5**, 1255.
52 J. R. Sams and T. B. Tsin, *Inorg. Chem.*, 1975, **14**, 1573.
53 J. R. Sams and T. B. Tsin, *J. Chem. Phys.*, 1975, **62**, 734.
54 E. Stellwagen, *Nature* (*London*), 1978, **275**, 73.

Received 17*th February* 1989; *Paper* 9/00756C