Studies on the binding of nitrogenous bases to protoporphyrin IX iron(II) in aqueous solution at high pH values Part I. Pyridine and imidazole ligands

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Abstract

The binding constants between protoporphyrin IX iron(II) and a series of pyridine ligands and imidazole have been determined by titration of the metal complex with these ligands. The adduct formation was monitored through optical spectroscopy. Parallel Mössbauer spectroscopic experiments were conducted to monitor the electronic environment around the central iron atom in these adducts. Both spectroscopic methods indicated that, with the exception of pyridine-*N*-oxide, all nitrogen bases yielded low spin octahedral complexes. The magnitude of the overall binding constants (β_2 values) are discussed and related to (i) the pK_a values of the free ligands and (ii) to the quadrupole splitting, ΔE_Q, of the haem iron. The β_2 and ΔE_Q values are also discussed in terms of the structure of the ligand.

Introduction

Haem proteins occur in a large number of biological systems. The haem groups perform roles in oxygen transport (haemoglobin) and storage (myoglobin), in electron transport (the cytochromes) and in the elimination of unwanted and toxic compounds (cytochrome P450). The chemical properties of the iron in the haem are modulated both by the porphyrin and by the nature of the axial ligands [1]. The manner in which the immediate environment of the metal is influenced by electron delocalization on the macrocycle and the nature of axial ligation in iron porphyrin complexes has been much discussed [2, 3].

Falk *et al.* [4] studied the binding of axial ligands to iron(II) haems in an attempt to distinguish between σ- and π-effects. The axial ligands chosen were 4-amino pyridine, pyridine and 4-cyanopyridine, and the porphyrins were mesoporphyrin, deuteroporphyrin, protoporphyrin IX and diacetyl deuteroporphyrin. They observed no evidence for stepwise addition of the ligands thus

$$\mathrm{Fe(PPIX)} + 2\,\mathrm{L} \overset{\beta_2}{\rightleftharpoons} [\mathrm{Fe(PPIX)L_2}] \qquad (1)$$

is the dominant reaction where β_2 is defined from eqn. (2)

$$\mathrm{Fe(PPIX)} + \mathrm{L} \overset{K_1}{\rightleftharpoons} [\mathrm{Fe(PPIX)L}]$$

$$[\mathrm{Fe(PPIX)L}] + \mathrm{L} \overset{K_2}{\rightleftharpoons} [\mathrm{Fe(PPIX)L_2} \qquad (2)$$

$K_2 \gg K_1$; $\beta_2 = K_1 K_2$

Diacetyldeuteroporphyrin iron(II) (which amongst the porphyrins was the weakest base [4]), was found to exhibit enhanced binding constants with increasing basicity of the pyridine ligand, a condition where σ-effects predominate. For mesohaem the stability constants were found to increase with increasing π-acceptor capacity of the ligand. Deutero and protohaems correspond to an intermediate situation. From the results of their studies they suggested that:

(i) the metal–pyridine bond strength increased by enhancing the ability of the metal to accept σ-electron density;

(ii) the metal–pyridine bond strength is decreased by reducing the donation of π-electrons to the axial ligand.

Cole *et al.* [5] presented more extensive data including $\Delta H°$ and $\Delta S°$ determinations on the dimethyl ester of the same porphyrin system in non-aqueous media. No simple correlation was observed ($\Delta H°$ versus pK_a plots were non-linear), and their

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data were explained in terms of the effects noted by Falk *et al.* [4]. The expected entropy loss on binding two ligand molecules was thought to be modified by effects of solvation and π-bonding.

Brault and Rougee [6] obtained very different results from some identical systems and suggested that the disagreement resulted from different methods of reduction used for the preparation of the iron(II) porphyrin complex from the iron(III) porphyrin. A particular problem they indicated was the possibility of the reducing agent binding to the haem. Brault and Rougee [6] observed slightly higher affinities of haem for imidazole when compared with that of pyridine (and that the binding for both ligands occurs in two steps) (as in eqn. (2)). Thus they suggested that in non-coordinating solvents the haem binds only one molecule of nitrogenous ligand (such as pyridine or imidazole) with high affinity. However, in most cases a second ligand molecule is subsequently bound. Their evidence for this was that the set of spectra produced on addition of pyridine did not show isosbestic points in the Soret region. Similar behaviour was observed for imidazole binding to the haem and it was suggested that the binding occurred in two overlapping steps (as eqn. (2)).

Other notable work on the binding of pyridine to PPIX(Fe(II)) has discussed aggregation effects [7].

In contrast to Brault and Rougee [6], Epstein and co-workers [8] suggested, based on their investigation using Mössbauer spectroscopy, that pyridine binds to iron(II) porphyrins with greater affinity than imidazole.

Several other reports have attempted to correlate Mössbauer data of hexa-coordinate low spin iron(II) porphyrin complexes with the electronic properties of the fifth and sixth axial ligands [9]. It was assumed that the nature of the iron–porphyrin bonds is unaffected by changes in the axial ligands, however, it has recently been shown that the bonding situation is rather complicated in some cases [9].

In this paper we report the preparation and characterization of a number of low spin protoporphyrin IX iron(II) (PPIXFe(II)) complexes with a number of pyridine ligands and with imidazole. This investigation was aimed at examining σ and π-bonding effects as well as steric effects in the bonding of axial ligands. Stability constants were calculated from electronic absorption spectra and Mössbauer spectra were obtained on frozen solutions of the complexes.

Protoporphyrin IX was the porphyrin selected for this study because it is the most widespread porphyrin found in natural protein, and from the work of others [7, 9] and our own data [10–14] it appears that different porphyrins contribute different amounts of electron density to the iron centre depending on the nature and number of substituent groups on the periphery of the porphyrins. The main drawbacks to this approach are as follows

(i) PPIXFe(II) is insoluble in most non-aqueous media (our experiments had to be carried out in water where OH^- and H_2O could also act as axial ligands). Other workers have used non-naturally occurring haems in non-aqueous systems to limit other coordinating ligands interfering but, as discussed, their results suffer from the fact that their porphyrins are not identical to PPIX [6].

(ii) PPIXFe(II) is insoluble in water below pH 6.9.

(iii) Aggregation of PPIXFe(II) in aqueous solution is a complication. (We have previously studied PPIXFe(II) in the pH range 7 to > 14 [10–14].)

For these reasons our investigations were carried out at high pH where we have previously shown a significant proportion of the PPIXFe(II) is monomeric in the absence of nitrogenous bases.

These monomeric forms are five-coordinated species in which either a water or an OH^- constitutes the fifth ligand. Under these conditions, a proportion of the haem is also present as a four-coordinate species which is aggregated. Mössbauer data [11, 13, 14] indicate, however, that the proportion of this aggregated species decreases rapidly with porphyrin concentration. Under the condition of our titration experiments, therefore, ~ 10 μM porphyrin, the large majority of the porphyrin Fe(II) is in the monomeric form.

Results and discussion

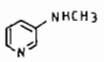
In all, nine ligands were studied. Their electronic absorption spectra in the visible region are presented in Table 1.

Visible spectra

From the known iron(II) porphyrin crystal structures, a useful approximation can be stated "the porphyrin ring is essentially planar and it has D_{4h} symmetry" [15]. The spectral bands in the visible region result from extensive delocalization of π-electrons on the porphyrin. A typical low spin PPIXFe(II)L_2 (L = ligand) spectrum is characterized by three bands. They are the Q and B (Soret) bands, respectively ordered from longer to shorter wavelength as shown in Fig. 1. The Q (0, 0) band arises from mixing bands II and IV of the free-base porphyrin spectrum, and the Q (1, 0) band results from mixing bands I and III of the free-base porphyrin spectrum following Gouterman [15].

The electrophilic side chains (the vinyl, formyl, etc. groups of the protoporphyrins) have an effect on

TABLE 1. The electronic absorption spectra of the complexes PPIXFe(II) (L_2) or (L), (where L = nitrogenous ligands) at pH 12, λ_{max} show Soret, β and α bands.

Complex no.	Nitrogenous ligands	Structure	Soret band (nm)	Band maxima	
				β band (nm)	α band (nm)
1	imidazole		421	526	555
2	5-chloro-1-methylimidazole		421	525	558
3	pyridine		419	525	556
4	4-methylpyridine		419	530	559
5	3,4-dimethylpyridine		418	530	559
6	4-chloropyridine		416	529	558
7	3-aminomethylpyridine		415	527	556
8	pyridine-*N*-oxide		418		556
9	isoquinoline		417	526	557

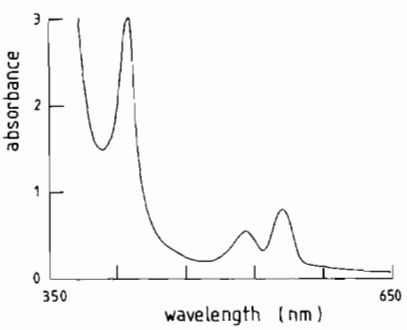


Fig. 1. The electronic absorption spectrum of PPIXFe(II)(imidazole)$_2$ at pH 12 in aqueous solution. The Q bands are at 526 and 555 nm, the B (Soret) band is at 421 nm. The band below 390 nm is due to sodium dithionite

the haem spectrum. A shift of the Soret band has been observed [16]. The spectrum of the haemochrome is sharp and much better resolved when compared with that of PPIXFe(II) in alkaline solution. It was thought that this sharpening resulted from the change of the dimeric to the monomeric species [17–19]. It is more likely that it results from a polymer to monomer breakdown [11].

It has been observed in this work (Table 1) and by others [20] that the Soret band of porphyrin Fe(II) complexes, coordinated to unsaturated ligands (π-bonded systems) moves towards shorter wavelengths. The movement of this Soret band has been explained as resulting from the fact that as the π-electron density of the metal t_{2g} orbital moves towards the periphery of the planar porphyrin nucleus, absorption occurs at longer wavelength. With unsaturated ligands, however, the metal t_{2g} orbitals (d_{yz}, d_{xz}) are involved in π-bonding with these ligands and thus lead to a decrease in the overlap of metal t_{2g} orbitals with the π-orbitals of the porphyrin ring via the N atoms; this results in the shift of the Soret band to shorter wavelength [15, 20, 21]. This blue shift of the Soret band is due to mixing of the empty eg(π^*) orbitals of the porphyrin with the filled 3dπ orbitals of the metals. This raises the energy of the eg(π^*) orbitals causing the energy gap between this and the top filled ring orbitals, $a_{2u}(\pi)$, $a1_u(\pi)$ to increase,

thus accounting for these hypso spectra [15]. Since the lowest energy empty orbitals (π^*) for most of these unsaturated ligands are far above the porphyrin (π^*) orbitals, the absorption spectrum of these ligand complexes with PPIXFe(II) can be characterized by relatively pure porphyrin π–π^* transitions of the low spin ferrous complex [21].

In this work we have established that two of the unsaturated ligands have spectra where the Soret band appears at longer wavelength, viz. 5-chloro-1-methylimidazole and imidazole. We suggest for imidazole that the reason for this is that it has better σ-donating abilities than the other aromatic ligands.

Pyridine-*N*-oxide is a weak ligand (see stability constant, Table 2). The spectrum of the complex of this ligand with PPIXFe(II) is that of a typical five-coordinate high spin complex (Fig. 2). This spectrum is different from PPIXFe(II) in alkaline media in the absence of the ligand [11, 12] (Fig. 3). It is most likely that in this weak ligand bonding to iron takes place via the oxygen atom. In contrast, however, this ligand appears to form a six-coordinate octahedral complex on lowering the temperature to 78 K; this is discussed in the section on Mössbauer spectroscopy.

Spectrophotometric titrations

A typical spectrophotometric titration of the PPIXFe(II) (reduced by dithionite and in the presence of dithionite) with imidazole is presented in Fig. 4. Isosbestic points can be seen at 402, 462 and 568 nm. Such spectral changes occur in all the ligands studied. The equilibrium constants for the Fe(II)PPIX moiety with these nitrogenous ligands have been measured. The Hill coefficient (h), the ligand concentration at 50% saturation, and pK_a values of the free ligand involved are given in Table 2.

The Hill coefficients (h) were found from Hill plots and took values between 1 and 2.5. Values of h close to unity indicate independent binding of ligands in a stepwise manner. Values of h greater than unity indicate cooperative binding of ligands. Thus for a system in which two ligands may bind to a central iron atom a value of h of 2 indicates that the ligands bind in such a fashion that throughout the titration no complex with a single ligand bound exists. The value of h cannot exceed the number of incoming ligand molecules. Therefore values of h greater than 2 indicate cooperative binding of ligands not only to the 2 coordination positions of the iron but also to either the porphyrin ring [22–24] or, through hydrogen bonding, to already bound ligands (i.e. ligand self association [25]). Hill coefficients may also be affected by the nature of the solvent; this is illustrated by the effect of water (as a polar solvent) upon the equilibrium constant, and h values found by McLees and Caughey [26] on complexes of Ni porphyrin with piperidine. The addition of ethanol, though it did not appear to have a great effect on the log K values, significantly lowered the value of h. It appears that polar solvents may participate via solute–solvent interaction lowering the apparent value of h [26].

The results we obtained for the β values in Table 2 are all lower than those for the analogous iron(II) porphyrin complexes (with, for example, imidazole and pyridine) in organic solvents. The reasons for this are, the effects of polar solvent and the aggregation state of the PPIXFe(II) molecules in solution and hence on the equilibrium constant (K_{eq}) values [27]. We have previously studied [11] PPIXFe(II) solutions in the pH range 7 to 14 and established that three species predominate; one, a polymer is present over the entire range and the others (monomers) are present at higher pH values. Before our work there was a great deal of controversy into the nature of the species present [28], and this we have previously discussed [11]. The presence of the polymer in polar solvents will certainly depress the β

TABLE 2. Hill coefficient, ligand concentration at 50% saturation, stability constant of the haemochromes and the pK_a of free nitrogenous ligand involved

Complex no.	Nitrogenous ligands	Hill coefficient, h	50% saturation (M)	Stability constant (log β_2 M^{-2})	pK_a
1	imidazole	1.75	0.01	4.0	6.95
2	5-chloro-1-methylimadazole	2.0	5.6×10^{-3}	4.5	4.75
3	pyridine	2.5	2×10^{-3}	5.4	5.23
4	4-methylpyridine	2.0	1.2×10^{-3}	5.9	5.98
5	3,4-dimethylpyridine	1.8	3.4×10^{-4}	6.9	6.46
6	4-chloropyridine	2.20	7.4×10^{-4}	6.3	3.83
7	3-aminomethylpyridine	2.10	1.4×10^{-4}	7.7	8.04
8	pyridine-*N*-oxide	1.01	0.05	2.6	0.89
9	isoquinoline	2.0	7.0×10^{-4}	6.31	5.14

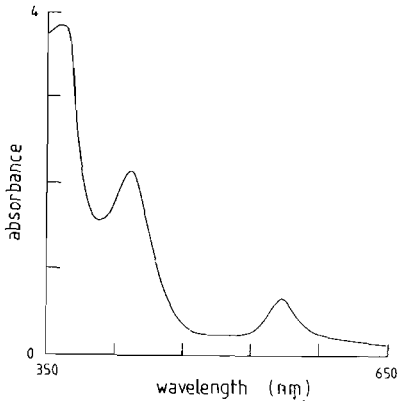


Fig. 2. The electronic absorption spectrum of PPIXFe(II)(pyridine-*N*-oxide) at pH 12 in aqueous solution (see Table 1). The band below 390 nm is due to sodium dithionite.

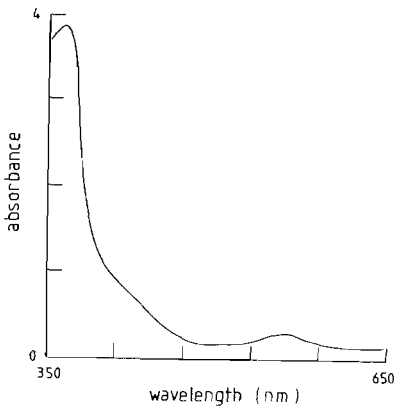


Fig. 3. The electronic absorption spectrum of PPIXFe(II) in aqueous solution at pH 12 (see Table 1). The band below 390 nm is due to sodium dithionite.

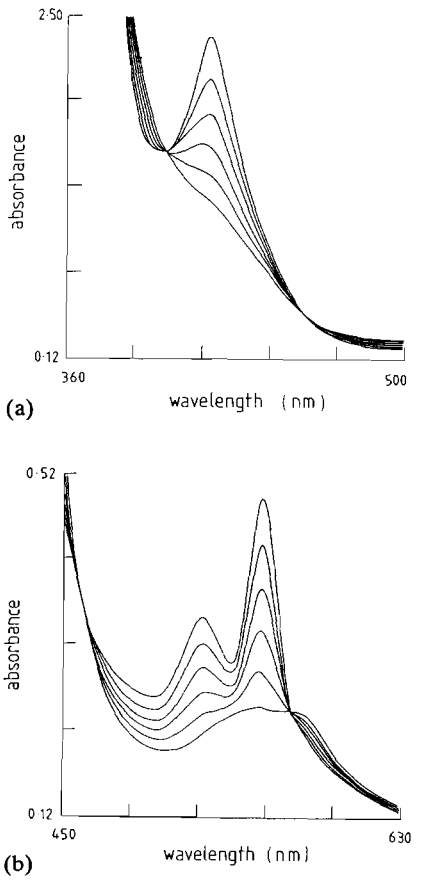


Fig. 4. Spectrophotometric titration of PPIXFe(II) (2×10^{-5} M) with imidazole. (a) The Soret band region of the titration. The band below 390 nm is due to sodium dithionite. (b) The visible region of the titration. Serial addition of aliquots of imidazole solution (0.5 M) leads to increase in absorbance in both spectral regions.

values. A third effect that has been suggested to depress the values of K_{eq} is the presence in aqueous solution of OH^- and H_2O moieties which themselves will bind [11, 12, 29] to the PPIXFe(II) (yet another solute–solvent interaction). We have found that the two monomeric PPIXFe(II) species at high pH contain OH^- and H_2O ligands [11–13].

Quinn *et al.* [30] found that there was a linear relationship between the equilibrium constant and pK_a, for the hydrogen-bonded imidazoles (ImH) and that such relationships are absent for non-hydrogen-bonded imidazole derivatives. Their pK_a values have been determined in aqueous solution, where ImH...OH_2 rather than ImH...ImH is the predominant mode of hydrogen bonding. They found that their values of K_{eq} as determined in non-hydrogen-bonding solvents are apparently influenced more by the nature of the hydrogen bonding to coordinated imidazole than by the nature of any substituent groups on the ligand.

Mössbauer spectroscopy

To aid in the understanding of the electronic environments around Fe(II) centres, and how these are affected by the binding of ligands, Mössbauer spectra were collected on frozen solutions at 78 K. The spectra all consisted of sharp doublets and the parameters are presented in Table 3. The range found for the isomer shifts of the unsaturated ligands was 0.43–0.48 mm s^{-1} and the quadrupole splitting range was 0.96–1.23 mm s^{-1}. These values agree well with previously reported data [31].

It can be observed, both in this work and that reported previously [31], that there is little difference in the isomer shifts of these complexes, all are close to each other with a slight trend to smaller values for the imidazole rings and larger for the pyridines. However, significant differences are observed in the quadrupole splittings (ΔE_Q). For example, for the

TABLE 3. ^{57}Fe Mössbauer spectral parameters for the haemochrome frozen solutions at 78 K

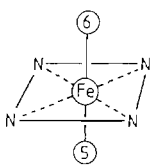
Complex no.	Nitrogenous ligand	δ (mm s^{-1})	ΔE_Q (mm s^{-1})	Γ (mm s^{-1})[a]
1	imidazole	0.43(1)	0.96(1)	0.19(1)
2	5-chloro-1-methylimidazole	0.43(1)	0.97(2)	0.17(1)
3	pyridine	0.45(1)	1.21(1)	0.18(1)
4	4-methylpyridine	0.48(1)	1.17(1)	0.17(1)
5	3,4-dimethylpyridine	0.46(1)	1.15(1)	0.15(1)
6	4-chloropyridine	0.46(1)	1.23(1)	0.24(1)
7	3-amino-methylpyridine	0.45(1)	1.14(1)	0.17(1)
8	pyridine-*N*-oxide	0.45(1)	1.19(1)	0.18(1)
9	isoquinoline	0.44(1)	1.11(1)	0.13(1)

[a]Half width at half height.

five membered unsaturated ring (imidazole) a small Δ is observed, whereas for the six membered pyridine rings larger splittings are observed.

All the known low-spin ferrous porphyrin structures are hexacoordinate and in every case where magnetic perturbation measurements have been made, V_{zz} is positive and the asymmetry parameter, η, is nearly or exactly zero. It has been accepted that the major contribution to V_{zz} in these diamagnetic complexes comes from an imbalance in electron densities in the iron $d_{x^2-y^2}$ and d_{z^2} orbitals, and the positive signs observed show that the covalent bonding to the planar porphyrin is stronger than that to the axial ligands [9, 32, 33].

If the geometry of the Fe(II) complexes are considered as below



the iron is coordinated by a square of pyrrole nitrogens, and by other groups at the fifth and sixth positions of the octahedron. If the axial ligands are weaker σ-donors than the nitrogen atoms of the porphyrin σ-bonding in the complex will generate a positive field gradient at the iron nucleus. If the lattice contribution is ignored, the V_{zz} is given approximately by eqn. (3) [34], where the n values are effective populations of the appropriate 3d orbitals. As the d_{xy} orbital is non-bonding, then n_{xy} will be essentially constant

$$V_{zz} = K[n_{x^2-y^2} - n_{z^2} + n_{xy} - \tfrac{1}{2}(n_{xz} + n_{yz})] \quad (3)$$

Obviously, since $V_{zz} > 0$, stronger back π-donation (which would decrease n_{xz} and n_{yz}) and weaker forward σ-donation (which would decrease n_{z^2}) would both serve to increase V_{zz}. The $3d_{x^2-y^2}$ orbital is the bonding orbital involved and has more metal-orbital character than the bonding orbital containing $3d_{z^2}$. The size of ΔE_q caused by this mechanism will be mainly dependent on the difference in bonding properties of the axial and equatorial ligands. As the σ-donor strength of the axial ligands is increased, the magnitude of the field generated by σ-bonding will decrease. In addition, increasing the σ-donor strength of the axial ligands generates greater delocalization of the electrons in the 3d t_{2g} orbitals of the metal. Electrons occupying these orbitals make a negative contribution to the field gradient [32]. Any low-lying π^* orbitals on the axial ligands, will then be able to interact with the filled 3d t_{2g} orbitals (d_{xz}, d_{yz}) of the metal. The electrons in these orbitals which, as stated, contribute negatively to the field gradient will now be delocalized into the ligand π^* orbitals, thereby causing an increase in the size of the field gradient at the iron nucleus [33].

We will first consider the pyridine type ligands. The pK_a and ΔE_Q value data of these ligands are presented in Table 4. The quadrupole splittings can be seen to be ordered and increasing with decreasing pK_a except for isoquinoline which is out of its expected position. It appears that isoquinoline has a much greater π-acceptor capacity than pyridine; this has been discussed by others [36] and is due to the greater delocalization of its π-electrons. Alkyl substituents release charge into the ring, and one might expect all alkyl pyridines to have smaller tendencies than pyridine to accept back coordinated charge. The apparent π-acceptor capacity might be therefore expected to decrease from pyridine through monosubstituted to disubstituted derivatives. Back coordination from metal to pyridine antibonding π^* orbitals should localize charge mainly on the 2, 4 and 6 positions on the ring; 3-alkyl substituents which direct charge to the same position will have the effect of inhibiting M–L π-bonding. The presence of Cl on the ring will tend to build up negative

TABLE 4. Relationship between selected properties of free pyridine ligand and the ^{57}Fe Mössbauer quadrupole splitting (ΔE_Q) of its low spin iron(II) complex

Complex no.	Pyridine and its substituents	pK_a	Order of σ-donor	Order of π-acceptor	ΔE_Q (mm s^{-1})
6	4-chloro-pyridine	3.83			1.23
9	isoquinoline	5.14	↓ increases	↑ increases	1.11
3	pyridine	5.23			1.21
4	4-methylpyridine	5.98			1.17
5	3,4-dimethylpyridine	6.46			1.15
7	3-aminomethylpyridine	8.04			1.14

charge and deplete electron density on the ring. This leads to 4-chloropyridine becoming a stronger π-acceptor [37, 38]. It can be safely assumed in this series of compounds that steric effects arising from the coordination of the pyridines to PPIXFe(II) are equivalent. Therefore, aside from isoquinoline (discussed above), the increased quadrupole splittings in this series are inversely related to the pK_a values. We note that in a similar study [39] less clear trends were observed, however if three of the ligands out of the eight used in that study (pyridine, 4-methylpyridine and 3,5-dimethylpyridine) were ignored, the rest order in a similar manner to those reported in this work.

There is good evidence that iron(II) in haemochromes acts as an electron donor and that pyridine acts as a π-acceptor [31]. Cole *et al.* [5] have presented evidence suggesting that σ-bonding is predominant in 4-aminopyridine and 4-methylpyridine. Pyridine itself shows intermediate bonding.

In pyridine-*N*-oxide the nitrogen of a pyridine ring is bound to an oxygen atom and the oxygen is the site of ligation to the haem iron. This ligand has a pK_a of 0.89, which is much smaller than the pyridines discussed above. It is interesting that the Δ value found for this ligand when bound to PPIXFe(II) is 1.19 mm s^{-1} and is similar to that of the other haemochromes discussed in this work. This ligand will not have the same steric restriction on approaching the iron at the haem centre that the normal pyridines encounter as the oxygen is one atom away from the pyridine ring. The Mössbauer spectrum of this complex is clearly that of a hexacoordinate low spin iron(II) species. Considering both the Mössbauer and optical spectra it is apparent that at least one of the axial coordinating ligands is pyridine-*N*-oxide. The identity of the sixth ligand is unclear but may be either pyridine-*N*-oxide or alternatively H_2O or OH^-.

The PPIXFe(II) complexes of imidazole (pK_a 6.95) and 5-chloro-1-methylimidazole (pK_a 4.75) have similar Δ values. This is expected as in the known haem structure [FeTPP(1-MeIm)$_2$] (containing two imidazoles) [40], the imidazoles are close to the haem (2.01 Å compared with pyridine 2.1 Å in [FeTPP(CO)(py)] [41], 2.037(2) Å in [FeTPP(py)$_2$] [42] and 2.039(1) Å in [FeTPP(py)$_2$]2py [43]) showing that the five-membered rings can get closer to the haem than six-membered rings. In addition, imidazole is a stronger base than pyridine and thus might be expected to cause a smaller ΔE_Q (it will be a better σ-donor than pyridine). It is interesting to consider the structure of 5-chloro-1-methylimidazole; this ligand is not sterically hindered and shows very similar properties to imidazole. The chloro and methyl inductive effects must balance in such a way that the total bonding, at the ligating nitrogen atom, is virtually the same as that of imidazole itself (even though the pK_a values are different).

Conclusions

The visible spectra of these PPIXFe(II)L$_2$ complexes have been characterized and provide insight into the electron density distribution between σ- and π-bonding in the z direction. Low-spin octahedral complexes are indicated in all cases except for pyridine-*N*-oxide.

The general conclusions that may be drawn from the data presented above are best appreciated by examination of Figs. 5 and 6. In these the overall binding constant, β_2 is related either to a property of the free ligand, here the pK_a value (Fig. 5), or to a property of the iron in the haem, here the quadrupole splitting, ΔE_Q (Fig. 6).

In Fig. 6 the pyridine compounds show an approximate linear correlation. The trend observed is that the higher the pK_a of the pyridine the higher is the binding constant. In other words increasing the affinity of a compound for protons increases its affinity for iron, presumably because each bears a positive charge. The 5-chloro-1-methylimidazole complex lies furthest from the line.

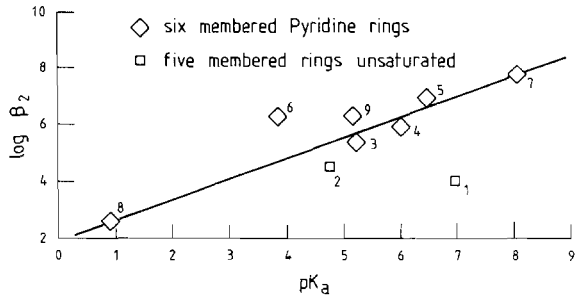


Fig. 5. A plot of log β_2 values against the pK_a values of the free ligands. The numbers refer to compounds listed in Tables 1 and 2.

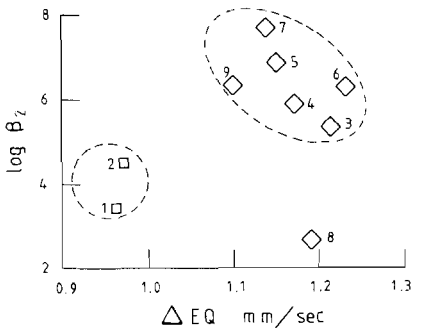


Fig. 6. A plot of log β_2 against quadrupole splitting for the haemochromes. The numbers refer to compounds listed in Tables 1 and 2. The key for the symbols as that included in Fig. 5. Compound **8** bonds to iron through oxygen.

Figure 6 shows the relationship between log β_2 and the quadrupole splitting (ΔE_Q). The ΔE_Q in this context gives an estimate of the electron imbalance between the four nitrogens of the porphyrin on the one hand and the two nitrogens of the axial ligands on the other. The major factor which affects ΔE_Q is the distance of the axial nitrogen ligands to the iron, this in turn will reflect the orbital composition of the bond (and bond strength) and also steric interactions.

The five-membered imidazole rings show the smallest orbital imbalance. Therefore they can (i) get close to the iron, and (ii) accept some iron 3d electron density into their π^* orbitals. As a result they have reasonable binding constants.

The six-membered rings experience greater steric hindrance than the five-membered rings and although they are better π-acceptors, which elevates their binding constants, they cannot approach the iron so closely. Thus they have greater electron imbalance than the five-membered rings and hence exhibit greater ΔE_Qs.

We have recently reported Mössbauer studies on PPIXFe(II) complexes of imidazole and substituted imidazole in 1:1 EtOH:H_2O frozen solutions [44]. In this study we established that the dominant mechanism on the covalent bonding is the σ-donation of imidazole → iron and the observed trend of σ-basicity of the coordinated ligands found was 2-MeImH < I-MeIm < ImH.

A striking feature of all the nitrogen ligands (except **8** which bonds through oxygen) irrespective of the value of log β_2 is that the Hill coefficient is close to two. This implies that during the course of a titration the single-liganded complex is poorly populated. In other words the binding of a *second* ligand is enhanced by the binding of the first. The reasons for this behaviour are not obvious. We may nevertheless offer a tentative explanation. The initial electron donation from the nitrogen ligand to the iron must be via its lone pair of electrons. There are only three possible orbitals on the iron which can accept these, they are the $3d_{z^2}$, the 4s and the 4pz. Of these the $3d_{z^2}$ and 4s are symmetrical. Donation into these two orbitals would also effect the bonding in the iron(II) porphyrin plane (Fe to porphyrin nitrogen atoms), as both of these orbitals are able to overlap with the orbital in the porphyrin plane. We can only suggest that this perturbation reorganizes the bonding in the plane of the porphyrin such that the iron needs a sixth ligand to satisfy its electron requirements. Recently a paper has appeared which considers the binding of imidazole to metalloporphyrins [45]; this included the somewhat surprising finding that the metal pπ orbitals bind to the imidazole nitrogen pπ orbitals. This conclusion which explained the stereochemistry of the known crystal structures would also be in keeping with the elevated values of log β_2 found for the aromatic compounds in this work (see Table 2). The finding of cooperative nitrogen binding ($h > 1$) reported here may have implications in the biological role of nitrogen ligands in haem proteins. Nitrogen is almost invariably one of the axial ligands and in many cases, for example in electron transfer proteins, two axial nitrogen ligands are present.

Experimental

Electronic absorption spectra were obtained using a DU-7 spectrophotometer (Beckman).

Haematin was purchased from Sigma and used without further purification. All the nitrogenous ligands were purchased from Aldrich. Haematin was dissolved in NaOH (0.1 M) and diluted to the desired concentration ($\sim 10^{-5}$ M) with NaOH to give a solution of final pH = 12. The haematin was reduced to PPIXFe(II) with a slight excess of solid sodium dithionite.

Spectrophotometric titrations (at 293 K) were carried out anaerobically by serial addition of degassed solutions of the various ligands. Small volumes (~ 20 μl) of a stock solution (either neat compound or suitably diluted solution) of ligand were serially added to ~ 3 ml of PPIXFe(II) solution, the precise volume being determined by weight assuming a solution density of unity. Spectra were recorded some 3 min after each addition to allow equilibrium to be established. The spectroscopic data were analysed by transforming the ligand binding curve utilizing a Hill plot from which both the Hill coefficient and the log β_2 values could be obtained. The values of these parameters quoted are the average of three experiments. For comparative purposes the titration data for pyridine-*N*-oxide is treated in the same way as for the other ligands even though the stoichiometry at 293 K is apparently lower. Clearly at low temperatures the complex is six coordinate (see text).

Mössbauer spectra were recorded on concentrated frozen solutions at 78 K. The Mössbauer spectrometer and experimental details have been previously described [46].

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