Hemes and Hemoproteins. Part 8.[1] Co-ordination of Amines and Amino Acids by the Iron(III) Porphyrin Microperoxidase-8

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Equilibrium constants K for the substitution of co-ordinated H_2O in the iron(III) porphyrin microperoxidase-8 by 20 amines and amino acids have been determined in 20% aqueous MeOH at 25 °C. Values of $\log K$ increase approximately linearly with the basicity (pK ranging from 5.3 to 10.7) of the primary amine, including the unsubstituted amino acid glycine, according to the relationship $\log K = 0.43\, pK - 0.5$; this is the first demonstration of such a linear relationship for any iron-(II) or -(III) porphyrin. The values are decreased by substitution (*i.e.* branching) on either the co-ordinated N_α or neighbouring C_β atoms; comparison with published data shows a sensitivity to β substitution in the co-ordination of amines by the d^5 iron(III), d^6 cobalt(III) and d^8 nickel(II) ions, but not by the larger d^{10} silver(I) and $HgMe^+$ ions. The values may be increased through π–π interaction of the donor–acceptor type between the conjugated porphyrin ring and phenyl [phenylalanine or $NH_2(CH_2Ph)$] or indolyl (tryptophan) substituents. The compounds NH_2NH_2 and NH_2OH both show an enhanced value of $\log K$ which can be ascribed to the so-called α effect; this appears to be the first reported example of this effect in metal–ligand bonding.

The wide range of nitrogen-containing bases (especially heterocycles and aliphatic amines) available as potential ligands provides the means of separating some of the many factors (electronic, steric, *etc.*) which determine the magnitude of the equilibrium constant $K = [M\text{–}B]/[M\text{–}OH_2][B]$ for substitution of co-ordinated H_2O by the base (here denoted by B) according to the generalised reaction (1). Basicity or inductive

$$M\text{–}OH_2 + B \rightleftharpoons M\text{–}B + H_2O \qquad (1)$$

effects have tended to be studied with substituted pyridines, steric effects with aliphatic amines. It is well known[2] that, within a related series of B but excluding those likely to exhibit marked steric hindrance, the values of $\log K$ may increase with the basicity (pK) of the amine according to the relationship (2) to

$$\log K = a \cdot pK + b \qquad (2)$$

provide a 'baseline' of simple inductive or basicity effects against which to assess other effects, and that steric hindrance reduces the values of $\log K$ below this baseline. Such relationships were first established for the Ag^+ ion in aqueous solution with a limited range of pyridines and non-heterocyclic amines (here termed 'true' amines).[3] Most subsequent work of this type with monodentate ligands has been carried out with pyridines, often in non-aqueous solvents; interpretation of the results has frequently been complicated by the possibility of the bases acting as π acceptors as well as σ donors, as in the case of iron(II) porphyrins.[4,5] Brown *et al.*[6] were the first to use primary amines with a wider range of pK (5.7–10.8) suitable for studying basicity effects, while Walker[7] has used a range of substituted imidazoles (pK 3.6–7.1). No such studies have, however, yet been reported for any iron(III) or cobalt(III) porphyrins or cobalt(III) corrinoids.

In spite of their relevance to haemoproteins such as cytochrome f[8] and the alkaline form of cyt c,[9] where one axial ligand is probably provided by the amine side-chain of Lys, surprisingly little has been reported on complexes of any protein-free iron(III) porphyrin with NH_3 (see preceding paper) or alkylamines.[10–12] Their study is, however, often complicated by competition from formation of the dimeric μ-oxo complex at the high effective pH required to deprotonate the amines and by ready reduction of the Fe^{III} by the amine; the mechanism of this reduction has been studied.[11] Until recently it was generally assumed that, with the single known exception of piperidine, such complexes with aliphatic amines could not be formed.[12] The co-ordination of NH_3 and glycine (Gly) by microperoxidase-8 (MP-8) was reported in 1960 and a value of K determined for Gly by indirect (electrochemical) methods.[13] The first directly measured equilibrium constant was reported in 1992 for the iron(III) haematoporphyrin with ethanolamine in MeOH.[14] There is a pressing need, from the point of view of both co-ordination and bioinorganic chemistry, to open up the field of amine complexes of iron(III) porphyrins.

Complications due to π bonding are, of course, absent in the case of the 'true' amines and they are reduced or even eliminated by use of the higher-valent Fe^{III} and Co^{III}, while possible correlations between $\log K$ and pK (which refer to aqueous solutions) are optimised by determining values of K in aqueous solution. We have therefore initiated a systematic study of the co-ordination in aqueous solution of nitrogen-containing bases, with particular emphasis on the true amines, by the iron(III) porphyrin microperoxidase-8 and, in parallel studies, by several cobalt(III) corrinoids. The water-soluble and monomeric MP-8 exists as the aqua–His complex over the pH range from 4.4 (protonation and displacement of His) to 8.9 (conversion into the hydroxo complex)[1,15] and resembles cobalt(III) corrinoids such as aquacobalamin in that the metal ions are kinetically labile towards ligand substitution in the axial positions, but one axial ligand (the heterocyclic base) is held with a reasonably high binding constant because it forms part of the side-chain; they both offer an excellent opportunity for determining and comparing values of the single-step binding constants K for a wide range of ligands. The cobalt(III) corrinoids also offer the further advantage of easily varying the *trans* ligand through a graded series of increasing σ-donor power (from H_2O through CN^- to CH_3^-),[16] allowing one to explore the interplay between varying the basicity of the ligand (through varying the substituent) and varying the Lewis acidity of the cobalt ion (through varying the *trans* ligand). We have already studied steric effects and the *trans* effect in the co-ordination of aliphatic amines by cobalt(III) corrinoids.[17]

We have now selected bases which include those where the donor atom may be sp^3- (true amines and amino acids) or sp^2-

Table 1 Equilibrium constants (K) for the substitution of co-ordinated H_2O in MP-8 by amines and amino acids

	pK^a of free base	log K^b/ $dm^3\ mol^{-1}$
1 $NH_2(CH_2CN)$	5.3	1.7
2 $NH_2(CH_2CF_3)$	5.7	1.3
3 $NH_2(CH_2CH_2CN)$	7.7	2.9
4 $NH_2(CH_2CH_2Br)$	8.49	3.2
5 NH_3	9.25	3.34[c]
6 NH_2Me	10.62	4.0
7 $NHMe_2$	10.64	2.7
8 NMe_3	9.76	*ca.* 0
9 NH_2Et	10.63	3.65
10 NH_2Pr^i	10.63	1.5
11 NH_2Bu^t	10.55	<0.5
12 NH_2Bu	10.7	4.4
13 $NH_2CH_2CO_2^-$ (Gly)	9.6	3.46
14 $NH_2CHMeCO_2^-$ (Ala)	9.7	2.89
15 Val	9.6	3.73
16 Leu	9.6	3.99
17 Phe	9.1	4.76
18 Trp	9.4	5.64
19 $NH_2(CH_2Ph)$	9.34	4.65
20 NH_2Ph	4.58	2.67[c]
21 NH_2NH_2	8.12	3.8
22 NH_2OH	5.96	2.7

[a] Values of pK taken from refs. 18 (amines) and 19 (amino acids). [b] All values of log K ± 0.1 or better except for NH_2Pr^i (±0.2), NMe_3 and NH_2Bu^t. [c] Values from ref. 1.

hybridised (*e.g.* pyridines and imidazoles); aniline represents an intermediate situation (see Discussion). We have already[1] investigated the pH dependence of the co-ordination of the parent NH_3, imidazole, pyridine (py) and aniline by MP-8 in order to obtain values of K and to explore the occurrence of related equilibria (*e.g.* ionisation of the co-ordinated His of the side-chain). In this paper we report the determination of values of K for 20 amines and amino acids (see Table 1) with MP-8; results with the amino acids have already been presented in a preliminary communication.[20] Amines have been selected to investigate the effects of (*a*) changing basicity (bases 1–6 in Table 1, with pK ranging from 5.3 to 10.7), thereby providing the 'baseline' against which to assess the varying contributions of (*b*) the steric effects of substitution on the co-ordinated N_α (NH_3, NH_2Me, $NHMe_2$, NMe_3) and C_β atoms (NH_2Me, NH_2Et, NH_2Pr^i, $NH_2Bu^t{}_3$, Gly, Ala), (*c*) any additional hydrophobic [NH_2Me, NH_2Bu, Ala, Val, Leu (leucine)] or donor–acceptor interaction [Ala, Phe, Trp, NH_2Me, $NH_2(CH_2Ph)$] between the substituents and the conjugated porphyrin ring, and (*d*) the so-called α effect (NH_2NH_2, NH_2OH).[21] The structures of many haemoproteins reveal π–π interaction between the porphyrin ring and an aromatic side-chain of Phe or Trp; a well known example is the invariant Phe/Trp in the peroxidase enzymes.[22] We have therefore used the co-ordination of amino acids *via* their NH_2 group in order to anchor the relevant side-chains (*e.g.* of Phe and Trp) near the porphyrin ring and to characterise the electronic effects (changes in the UV/VIS spectra) and energetics (changes in the binding constant) of these π–π interactions.

In their 1962 review of the kinetic reactivity of nucleophiles (including ligands in metal complexes) Edwards and Pearson[21] were able to explain all the observed orders of reactivity on the basis of three factors, *viz.* basicity, polarisability and the 'α effect', the term given to the excess of reactivity (above that expected from basicity alone) exhibited by reagents where the donor atom is attached directly to an electronegative atom bearing one or more lone pairs of electrons as in RO_2^-, ClO^-, NH_2NH_2, NH_2OH and their derivatives. Other examples such as the 1,2-diazine pyridazine were discovered later.[23] Though usually considered as a kinetic phenomenon (*e.g.* as a nucleophile in the catalysed hydrolysis of esters), the α effect can also be observed as a thermodynamic phenomenon (*e.g.* in equilibrium constants for hydrogen-bond formation).[24] Many such potentially 'α effective' ligands are of interest to bioinorganic chemists in general (O_2, O_2^-, H_2O_2, HO_2^-; NH_2OH in NO_2^- reduction) but Edwards and Pearson[21] were unable to find any examples of an α effect in published rates of ligand substitutions. In 1986 Taube[25] suggested that some of the anomalies observed for co-ordinated O_2^- 'may be traceable to the α effect exerted in equilibrium behaviour' and noted that 'little systematic work has been done with ligands of this kind'. We have been unable to find any test or search for the occurrence of the α effect in metal complexes since that date, though in 1983 Olabe and Gentil[26] had noted a three-fold slower rate of loss of NH_2NH_2 compared to the more basic $NH_2CH_2CH_2NH_2$ from the complexes $[Fe(CN)_5B]^{2-}$; they suggested this might indicate a special stabilisation of the Fe–NH_2NH_2 bond through operation of the α effect, but did not investigate this further. We are particularly interested in proving (or disproving) the occurrence of the α effect in co-ordination to the iron(III)-containing MP-8 by testing whether hydrazine and hydroxylamine (and later pyridazine) show an enhanced binding constant K compared to that expected from the baseline; the result would be relevant to the co-ordination of HO_2^- (another 'α effective' nucleophile which cannot be studied directly because of rapid destruction of the porphyrin ring) in the first step of the peroxidase reaction.[27]

Experimental

Materials.—Microperoxidase-8 was prepared from cytochrome c (Sigma, type III) as described.[28] Reagents were obtained as follows: trifluoroethylamine from Fluka; $NH_2CH_2CN{\cdot}HCl$ and β-aminopropionitrile monofumarate salt from Sigma; $N_2H_4{\cdot}H_2O$ from BDH; $NH_2OH{\cdot}HCl$ and other amines from Aldrich; all amino acids from Sigma with a second sample of Ala from BDH. Dimethylamine was purified by recrystallising the HCl salt from $CHCl_3$;[29] all other reagents were used as received.

Methods.—The UV/VIS spectra and measurements of optical densities at a fixed wavelength (397 nm) for the spectrophotometric titrations were carried out on a Philips Analytical SP 1800 or Philips PU 8740 spectrophotometer. Except where otherwise stated, all studies were carried out with cells of 1 cm pathlength, thermostatted at 25 °C and containing *ca.* 5 μmol dm^{-3} solutions of MP-8 in 20% (v/v) aqueous MeOH; for quantitative work the solutions also contained NaH_2PO_4–Na_2HPO_4 buffers (BDH) of pH 6.5–9.0 and I = 0.08 mol dm^{-3}. Solutions of the reagents were prepared by titrating the free base with HCl or $HClO_4$ or the various acid salts with NaOH to the appropriate pH before adding the buffer. No differences were noted between NaCl and $NaClO_4$ to suggest, for example, co-ordination of Cl^-. The pH measurements were made with a Hanna HI B417 pH meter and appropriate glass electrode.

Results

All equilibrium constants for the substitution of co-ordinated H_2O in MP-8 by amines and amino acids were studied at 25 °C in 20% (v/v) aqueous MeOH in order to suppress dimerisation of the starting MP-8. The amines and acids studied, together with the pK values of the free bases[18,19] and data for NH_3 and NH_2Ph from the preceding paper,[1] are given in Table 1.

Preliminary qualitative experiments scanning the spectrum over the range 300–600 nm showed that all the products exhibited similar spectra (*cf.* Fig. 2 of ref. 1) with a sharp Soret band *ca.* 404 nm (but lower than for MP-8 itself), with a noticeable shoulder at *ca.* 350 nm and a much lower band around 520 nm, characteristic of a low-spin iron(III) MP-8 complex.[15,30] Equilibrium was established rapidly, reasonable to good isosbestic points were observed at *ca.* 330 and 400 nm and, where studied, the reaction could be reversed by acidification. No side-reaction was observed under the conditions used, even with the potentially strong reducing agents NH_2NH_2 and NH_2OH.

The main problem concerned the choice of pH for the quantitative titration. The octapeptide is present in the form with His and H_2O as the axial ligands from pH 4.4 (protonation and displacement of His to give the diaqua complex) to 8.9 (formation of the His-Fe–OH^- complex).[1,15] Equilibrium constants should, if possible, be determined at pH < 10 to avoid complications due to reversible proton loss from the co-ordinated His and the terminal Cys-NH_3^+ of the side-chain.[1] Amines with p$K \leqslant 8.5$ offer few problems and values of K were obtained at pH 6.0. The higher the pK of the base, the more K_{obs} will be depressed and the error increased by competition from protonation of the base and/or conversion into the hydroxo complex. The amino acids (pK 9.1–9.7) were studied at pH 8.75 and the more basic amines (pK 9.8–10.7) at pH 9.0; for those with low binding constants (in particular nos. 7, 8, 10 and 11) the ionic strength could not be held constant.

Analysis of the changes in optical density at 397 nm (Soret band of the initial MP-8) established the stoichiometry of 1 B per Fe and the experimental data were used to derive values of K_{obs}. Appropriate corrections were made both for (*i*) protonation of the base (when the pH used was less than the pK of the base) and (*ii*) conversion of the aqua- into the hydroxo-complex (when the pH used was greater than the pK for formation of the hydroxo-complex) to give the true K. It was assumed that the pK values were not significantly affected by the presence of 20% MeOH (*cf.* ref. 31 for the amino acids). At least two experiments were carried out for each amine and at least three for each amino acid. A second source of Ala was used to confirm an unexpectedly low value of K (no significant difference detected). A test was made for the possible role of impurities in the case of $NHMe_2$, where the value might be affected by the presence of NH_2Me and/or NMe_3 with very different values of log K (see Table 1), by comparing purified and 'as-received' samples; no significant differences were detected. From the simple and self-consistent pattern of log K observed it was concluded that no serious problems were caused by impurities and all the other bases were used as 'as-received'. The value of log K obtained for NH_2Pr^i is less accurate than those for other amines due to the appearance of turbidity at higher concentrations of base.

The possibility that any of the amine-containing products with typical spectra (as indicated above) could be dimers was tested and excluded in the case of NH_2NH_2, which could in principle use each NH_2 group to co-ordinate to a different Fe and hence stabilise the dimer. The co-ordination of NH_2NH_2 was therefore studied with three concentrations of Fe (each in duplicate), using 17.6, 3.0 and 0.8 μmol dm^{-3} in 0.2, 1 and 4 cm pathlength cells respectively. In the first case (highest concentration) the spectrum of the product differed in showing a higher shoulder at *ca.* 360 nm: this suggests some aggregation. The second and third concentrations, however, gave identical final spectra and identical values of log K (*viz.* 3.8 ± 0.1), indicating no dimerisation. We assume that all the other amine-containing products with similar spectra are also monomeric.

The values of log K determined are listed in Table 1. All the products, including that from cyclohexylamine,[20] exhibit a Soret band at 403.5 ± 0.5 nm except, as already noted, those with NH_2Ph (406),[1] $NH_2(CH_2Ph)$ (405.5), Phe (406) and Trp (406 nm).[20]

Discussion

The values of log K for the co-ordination of the amines B determined here, together with those for NH_3 and NH_2Ph reported in the preceding paper,[1] are listed in Table 1. Under the conditions used all the reactions observed correspond to the simple, reversible and rapid co-ordination of 1 B per Fe. The initial aqua complex of MP-8 is high spin, while all the products exhibit similar UV/VIS spectra corresponding to low-spin complexes.[15,30] The possibility that the product is a dimer was tested and excluded only in the case of the potentially bridging ligand NH_2NH_2; from the simple pattern observed (Fig. 1) we assume all the products are monomers. The values of log K are plotted against the pK values for the bases in Fig. 1. The various factors which determine the magnitude of log K are discussed in turn.

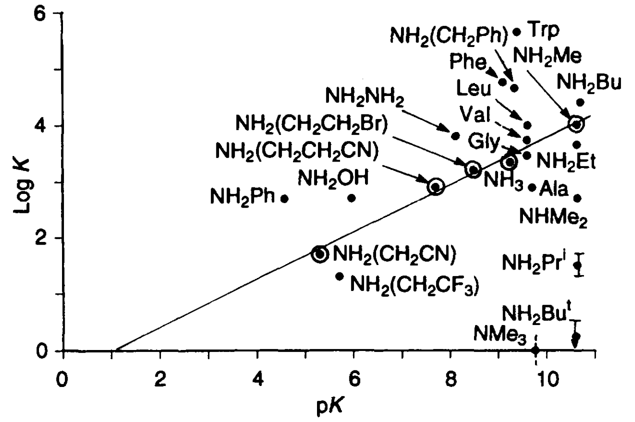

Fig. 1 Plot of log K (for co-ordination) *vs.* pK (for protonation of the free base) for amines and amino acids. Data from Table 1. The solid line corresponds to log $K = 0.43$ p$K - 0.5$. The points corresponding to the five primary amines (nos. 1 and 3–6) used to establish this 'baseline' are circled

Basicity.—Five primary aliphatic amines (nos. 1 and 3–6) were used to establish the 'baseline' of basicity effects; the solid line corresponds to log $K = 0.43$ p$K - 0.5$, *i.e.* to the relationship (2) with $a = 0.43$ and $b = -0.5$. The compound $NH_2(CH_2CF_3)$ falls significantly (by *ca.* 0.5) below the line as drawn; because of the large bulk of the CF_3 group and possible minor steric hindrance (see below) together with the possibility of unusual interactions between the C–F bonds and water, it seems reasonable to discount this point in constructing the linear relationship (2). Our results show that, in contrast to previous work on iron(II) porphyrins with pyridines,[4,5] the expected linear relationship (2) is exhibited by iron(III) porphyrins with true amines; we shall show later that a linear relationship is also found for iron(III) porphyrins with pyridines. Possible interpretations of the values of a and b in equation (2) will be discussed later, together with results for pyridines and imidazoles and for cobalt(III) corrinoids. Our results also suggest that, for other higher (*e.g.* tri-) valent metal ions where the high stability of the hydroxo complex makes it difficult or impossible to study the binding of amines with a high pK such as NH_3, relevant results may be obtained by the use of amines with a lower pK to reduce the competition from HO^- and to construct a 'baseline' which can then be used to extrapolate to amines such as NH_3 with a higher pK.

The solid line of Fig. 1 provides the 'baseline' for the expected dependence of log K on pK alone, against which to assess the values of log K observed for other amines in terms of steric and other factors. Since Gly lies on this line within experimental error, we conclude that the terminal CO_2^- has no significant effect on log K and that the amino acids can, subject to the change in conformation on co-ordination (see below), be compared directly with the baseline. One complication relates to possible changes in the state of hybridisation of (*a*) the lone pair on N in the free base, (*b*) the N–H bond in the protonated form and (*c*) the N–Fe bond in the co-ordinated form. Differences between (*a*) and (*b*) are incorporated into the values of pK and those between (*a*) and (*c*) into the values of log K. The first two are assumed to approximate to sp^3 hybridisation, though aniline is known to be an exception (see below), while little is known about the third. X-Ray studies of cobalt(III) complexes have shown Co–N–C bond angles of 121, 122, 123, 124 and 124.5° in $[Co(NH_2Me)_5Cl][NO_3]_2$[32] and a Co–C–C bond angle of 122° in $[CoEt(Hdmg)_2(py)]$ (Hdmg = dimethylglyoximate),[33] *i.e.* the formally tetrahedrally co-ordinated N and C atoms have bond angles characteristic of trigonal sp^2 rather than sp^3 hybridisation. There appear to be no analogous data for Fe–N–C bond angles in low-spin iron(III) complexes and one cannot exclude the possibility that the co-ordination of amines by the iron(III)-containing MP-8 will also involve considerable rehybridisation of the lone pair; correlations of log *K* with p*K* will therefore remain valid only for those bases where the degree and energy of any rehybridisation on co-ordination are roughly comparable. The case of aniline is more complex. Recent experimental (X-ray and microwave) and theoretical work on the structure of aniline and its ring-substituted derivatives in their unprotonated form (see summaries in refs. 34 and 35) reveals a subtle dependence of the distortion/rehybridisation of both the N atom and its neighbouring C atom both on the pattern of hydrogen bonding to and from the N atom and on the ring substituents; the sums of the bond angles around N vary from 360 (*i.e.* trigonal sp^2) down to 338° but never reach 328° (*i.e.* tetrahedral sp^3). The possible effects of differences in rehybridisation of the N atom, associated with changes in the degree of overlap with the π orbitals of the benzene ring, must therefore be borne in mind when comparing anilines with the true amines.

Steric Effects.—Comparison of the results for NH_3 and the alkylamines, where the p*K* values do not vary greatly, shows that the value of log *K* is hardly affected by the successive addition of Me groups in a linear chain ($NH_3 \approx NH_2Me \approx NH_2Et$) but is significantly reduced by the introduction of branching or lateral Me groups onto either the co-ordinated N_α ($NH_2Me > NHMe_2 > NMe_3$) or the neighbouring C_β atoms ($NH_2Et > NH_2Pr^i > NH_2Bu^t$). This probably also explains the fall in log *K* from Gly to Ala. It should be noted that interpreting any observed effects of alkyl substitution is not as straightforward as often assumed because they represent the resultant of different changes in inductive, polarisability and solvation as well as steric effects; it does, however, appear that any observed effects of β-substitution are more likely to represent clear-cut cases of steric effects than are the effects of α substitution.[17] The effects of β- as well as α-substitution have been studied with Ag^+, $HgMe^+$, cobalt(III) corrinoids (all determined directly by equilibrium studies) and the aquated nickel(II) ion (values of log *K* determined indirectly by kinetic methods); see summary of data in ref. 17. The addition of our directly determined values of log *K* for the iron(III)-containing MP-8 serves to emphasise the interesting fact that the d^5 iron(III), d^6 cobalt(III) and d^8 nickel(II) ions are all sensitive to β- as well as α-substitution, while the larger d^{10} Ag^+ and $HgMe^+$ are not. Whether this lack of sensitivity to β substitution in the last two is due to their larger size and/or disappearance of the filled d^{10} shell into the core cannot yet be decided. Changes in the hybridisation of the co-ordinated lone pair on N_α (see above) could provide a mechanism for transmitting effects between the d shell and the β position in addition to direct interaction.

Hydrophobic and Donor–Acceptor Interactions.—Aniline, benzylamine, Phe and Trp all show both enhanced values of log *K* (by 1.3, 1.1, 1.4 and 2.1 respectively) above the baseline (see Fig. 1) and a shift in the Soret band from 403.5 ± 0.5 (as observed with all other amines, including cyclohexylamine)[20] to 405.5–406 nm. We have ascribed this to π–π interaction of the donor–acceptor type between the benzene or indole rings and some part of the conjugated porphyrin system.[20] It is interesting that similar effects on log *K* are observed whether the phenyl group is attached to N_α, C_β or C_γ; one might have expected a different energy of π–π interaction in the case of aniline, and the observed similarity may reflect a balancing of several factors (see above). Crystal structure determinations have established the occurrence of such π–π interactions between several metalloporphyrins and even the simple benzene, toluene and xylene in the solid state (for a summary see ref. 36), but not apparently for any indole derivative. Our results appear to provide the first direct (UV/VIS) evidence for a charge-transfer contribution to such interactions. The increase in log *K* by 1.1–1.4 and 2.1 gives values of *ca.* 7 and *ca.* 12 kJ mol^{-1} for the apparent energies of interaction of the benzene and indole rings respectively with the porphyrin ring; for the amino acids these values should be corrected for the change in conformation (see below) and be referred to Ala, rather than Gly, as the 'baseline'.

Useful comparisons can be made with values of log *K* reported for the co-ordination of amino acids with other metalloporphyrins (where hydrophobic and donor–acceptor interactions can be expected) and with $[Fe(CN)_5(OH_2)]^{3-}$ and $[Fe(CN)_5(OH_2)]^{2-}$ (where they are impossible). Mikros *et al.*[37] have recently studied aqueous solutions of water-soluble cobalt(III) porphyrins possessing two axially co-ordinated amino acids and used NMR spectroscopy to show that all the co-ordinated acids adopt a similar conformation which differs from the preferred geometry of the free acids but allows the side-chains to interact with the porphyrin ring by hydrophobic interaction (as in Leu) or by 'stacking' with π–π interaction (as in Phe, Tyr and Trp). They calculated energies of interaction of 2.5, 6.4, 6.9 and 7.1 kJ mol^{-1} for Leu, Phe, Tyr and Trp respectively, which give the order Leu < Phe < Trp and are comparable in magnitude to our values. Phenylalanine does not, however, appear to show a significantly greater interaction than Leu with a zinc porphyrin in aqueous solution (*cf.* log *K*: Leu, 2.3; Phe, 2.5; Trp, 3.7).[38] If genuine, this difference in behaviour could reflect the greater ease of reduction, and hence greater energy of the charge-transfer interactions, with Fe^{III} and Co^{III} compared to the Zn^{II}; an intense absorption band corresponding to charge transfer from the phenyl group to Co^{III} has been identified for the complex $[Co(Hdmg)_2(NH_2Ph)_2]^+$ at 355 nm,[39] and the intense band of $[Co(CN)_5(CH_2Ph)]^{3-}$ at 296 nm[40] is probably analogous. It seems likely that the increased values of log *K* (see Table 1) shown by Val and Leu above that of Ala (by 0.8 and 1.1 respectively), perhaps even the smaller increase shown by NH_2Bu over NH_2Me (by 0.4), may reflect additional hydrophobic interaction between the alkyl side-chains and the porphyrin ring.

The following values of log *K* have been determined for the co-ordination of NH_3 and various amino acids by the $[Fe(CN)_5(OH_2)]^{2-}$ ion in aqueous solution: NH_3, 4.8; Gly, 4.2; Val, 3.8; Leu, 3.7; Phe, 3.5; Trp, 3.7.[41] A similar pattern is also observed for the $[Fe(CN)_5(OH_2)]^{3-}$ ion: NH_3, 4.3; Gly, 4.0; Val, 3.1; Leu, 3.0; Phe, 3.2; Trp, 3.25.[41] The order NH_3 > Gly presumably reflects Coulombic repulsion between the negatively charged amino acid carboxylate and pentacyanide units and may even indicate that the $Fe(CN)_5$ unit is more sensitive than the iron(III) porphyrin to introduction of the first substituent on N_α. The order Trp ≈ Phe ≈ Leu (and all other amino acids) reflects the expected absence of any hydrophobic or donor–acceptor interactions. It would obviously be very instructive to obtain further data on the iron pentacyanide complexes to compare with the pattern for the iron porphyrins.

In spite of considerable recent interest in such π–π interactions, 'their ability to modulate some of the electronic and reactivity features of metalloporphyrins remains relatively undefined'.[36] We have pointed out[20] that such donor–acceptor interactions could increase the effective polarisability of the porphyrin system and that, in the specific case of the peroxidase enzymes which all possess an invariant Phe/Trp adjacent to the porphyrin ring, this could have two effects relevant to the mechanism of action as well as simply helping to anchor the key pentapeptide loop containing the invariant His and Arg near the distal co-ordination site of the Fe. The increased polarisability could serve, first, to stabilise the highly oxidising iron(IV) and porphyrin radical cation (the so-called Compound I) and, secondly, to facilitate any nearby movement of charge in the protein environment of low relative permittivity (in particular the swinging of the flexible side-chain of the invariant Arg) through an enhanced condenser effect.

The α *Effect.*—Hydrazine (NH_2NH_2) and hydroxylamine (NH_2OH), both founder members of the class of compounds known to exhibit the α effect,[21] show a value of log *K ca.* 1 greater than expected on the basis of their p*K* values (see Fig. 1); *cf.* the similar increases (over that expected from the appropriate 'baseline') which have been reported in log *K* for catalysed ester hydrolysis (1.0 with pyridazine[23] and 1–2 with hydrazine[42]) and in log *K* for hydrogen bonding (*ca.* 0.6 with pyridazine,[24] no data available for hydrazine or hydroxylamine). A more local explanation such as stabilisation of the co-ordinated base by an unusually favourable hydrogen bond to the peptide side-chain can be eliminated by our findings that the 1,2-diazine pyridazine shows a significantly enhanced log *K* above the baseline established with substituted pyridines, which is not shown by the 1,3-diazine (pyrimidine) and 1,4-diazine (pyrazine),[43] and that enhanced binding constants are also found for these compounds with cobalt(III) corrinoids,[44] *i.e.* there is some common denominator. We conclude that for both NH_2NH_2 and NH_2OH with Fe^{III} the value of log *K* is enhanced by operation of the so-called α effect[21] due to the presence of a second electronegative atom with one or more lone pairs of electrons in the β position. It would appear (see Introduction) that our results on NH_2NH_2 and NH_2OH with MP-8 provide the first established examples of the operation of the α effect in metal complexes. The possible origin of this effect, on which there is presently no agreement, will be discussed in the next paper.[43]

Conclusion

The results presented in this and the preceding paper[1] and in the preliminary communication[20] include the first equilibrium constants reported for the simple co-ordination by any iron(III) porphyrin (with or without a protein) of NH_3, alkylamines, amino acids or, with the single exception noted in the Introduction, of any other non-heterocyclic amines. They demonstrate that, at least for MP-8 in aqueous solution, the adducts are easy to prepare and study, with no complications due to side-reactions such as reduction of the Fe^{III}. The data produce a very simple and readily interpreted pattern (see Fig. 1), in which values of log *K* increase linearly with the p*K* of the base according to equation (2), when basicity is the sole or major effect, but may be either decreased by steric factors or increased by the α effect or by favourable (*e.g.* hydrophobic or donor–acceptor) interactions between the ligand side-chains and the porphyrin ring. They provide the first demonstration of the occurrence of the linear relationship (2) amongst either iron-(II) or -(III) porphyrins, the first demonstation of the occurrence of the α effect in any transition-metal complexes, and the background against which to assess the results of parallel studies on the co-ordination of pyridines and imidazoles which have the potential for π bonding.

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