Hemes and Hemoproteins. Part 9.[1] Co-ordination of Pyridines and Diazines by the Iron(III) Porphyrin Microperoxidase-8†

Mohamed S. A. Hamza and John M. Pratt*
Department of Chemistry, University of Surrey, Guildford GU2 5XH, UK

Equilibrium constants K for the substitution of co-ordinated H_2O in the iron(III) porphyrin microperoxidase-8 by substituted pyridines and the three diazines have been determined in 20% aqueous MeOH at 25 °C. Values of log K for the co-ordination of the 1,3-diazine pyrimidine and the 1,4-diazine pyrazine as well as the 4-substituted pyridines increase approximately linearly with the pK of the base according to the relationship log K = 0.36 pK + 0.8. The value of log K observed for the 1,2-diazine pyridazine is significantly greater (by *ca.* 0.7) than that expected from basicity alone, which is ascribed to operation of the α effect. It is suggested that this effect, which reflects the breakdown in correlation between basicity towards the proton and towards other Lewis acids, originates from the existence of two opposing factors involving the vicinal atoms (*viz.* lone pair–lone pair repulsion and the inductive effect) which have a different dependence on the degree of negative charge removed by the Lewis acid.

We have initiated[1] a systematic study of the co-ordination in aqueous solution by the monomeric iron(III) porphyrin microperoxidase-8 (MP-8),[1-4] and in parallel studies by cobalt(III) corrinoids, of several classes of nitrogen-containing bases by determining the equilibrium constants K = [Fe–B]/[Fe–OH_2][B] for the substitution of co-ordinated H_2O by the base B. In Part 8[1] we examined the co-ordination of 20 aliphatic amines and amino acids and of NH_2NH_2 and NH_2OH. In this paper the co-ordination of six-membered pyridines (primarily for basicity effects) and diazines (primarily for the α effect) is examined. All values of K reported here have been determined by UV/VIS spectrophotometry at pH 6.5 or 7.5.

The α effect is the term given by Edwards and Pearson[5] in 1962 to the excess of reactivity (*e.g.* in organic nucleophilic substitution reactions) above that expected from basicity alone and exhibited by reagents where the donor atom is attached directly to an electronegative atom with one or more lone pairs of electrons as in HO_2^-, ClO^-, NH_2NH_2, NH_2OH and their derivatives. The 1,2-diazine pyridazine (pydz) and its benzo analogues cinnoline and phthalazine also show a reactivity greater (log K higher by 0.6–1) than that expected by extrapolation of the 'baseline' established for pyridines,[6,7] while the 1,3-diazine pyrimidine and the 1,4-diazine pyrazine deviate little, if at all, from the baseline. Though usually considered as a kinetic phenomenon, the α effect can also be observed as a thermodynamic phenomenon in equilibrium constants for hydrogen-bond formation (*e.g.* pydz)[8] and, as shown in Part 8, for co-ordination (*e.g.* NH_2NH_2, NH_2OH);[1] *cf.* also the associated shifts in the O–H stretching frequency.[9] Pyridazine is particularly important for understanding the origin of the α effect (see Discussion) because it is a neutral base and, unlike the better known hydrazine and hydroxylamine, it has a rigid conformation, the gas-phase proton affinities of pydz and the other diazines have been determined, and the differences in gas- and aqueous-phase basicities of the diazines analysed and explained.[10] There has, however, been little interest in the use of pydz as a ligand.[11-13]

We have already reported the equilibrium constant for co-ordination of pyridine (py) by MP-8.[2] The 4-substituted pyridines (with pK ranging from 1.9 to 9.8, see Table 1) provide the means for studying the effects of basicity while keeping steric factors constant; see, for example, refs. 15–18. Basicity effects have been studied with several iron(II) porphyrins[19,20] but, somewhat surprisingly, not with iron(III) porphyrins; the complexity of the results was attributed to the combined effects of changes in both σ-donor and π-acceptor properties of the py ring. It has been shown[21] that pyridines as well as alkylpyridinium cations can form donor–acceptor adducts through π–π interaction with the porphyrin ring of the dimeric iron(III) haematin and we have suggested[1] that such adduct formation could further complicate the basicity effects observed with iron(II) porphyrins. Steric effects can be highlighted, while minimising changes in basicity, by comparing 2- with 4-substituted pyridines which usually have very similar pK values.

The main aims of this study are to determine the equilibrium constants K for the substitution of co-ordinated H_2O in MP-8 by the five substituted pyridines and three diazines in Table 1 in order to establish (*a*) the 'baseline' of basicity effects (using py and its 4-substituted derivatives), (*b*) whether pyrimidine and pyrazine behave like pyridines of comparable pK, and (*c*) whether pydz shows an enhanced value of K, above that expected by analogy with the pyridines or the other diazines,

Table 1 Equilibrium constants (K) for the substitution of co-ordinated H_2O in MP-8 by substituted pyridines and diazines

Ligand	Free base pK[a]	Product log(K/dm^3 mol^{-1})[b]	Product λ_{max}[c]/nm
1 4CN-py	1.9	1.4	403
2 py	5.17	2.65	404
		2.73[d]	403.5[d]
3 4Me-py	6.02	2.8	405
4 4H_2N-py	9.11	4.17	406
5 4Me_2N-py	9.76	4.58	407
6 2Me-py	5.97	≤ +1	
7 Pyridazine	2.33	2.25	404.5
8 Pyrimidine	1.10	1.3	403
9 Pyrazine	0.37	1.1	403

[a] Values of pK taken from ref. 14 except for 4Me_2N-py from ref. 15. [b] All values of log K ± 0.1 or better except for pyrazine (±0.2). [c] Values ± 0.3 nm. [d] Value from ref. 2.

† *Non-SI unit employed:* cal = 4.184 J.

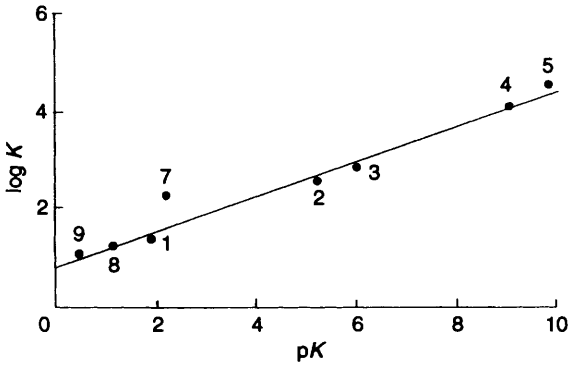

Fig. 1 Plot of log K (for co-ordination) *vs.* pK (for protonation of the free base) for 4-substituted pyridines and the three diazines. Data and numbering of ligands from Table 1. The solid line corresponds to log K = 0.36 pK + 0.8

which could be ascribed to the α effect. We also wish (*d*) to compare the effects of steric hindrance in the pyridine series (using 2-methylpyridine) and the sp^3 amines (*cf.* the sterically comparable NH_2Bu^t).[1]

Experimental

Materials.—The octapeptide MP-8 was prepared from cytochrome c (Sigma, Type III) as described.[22] All reagents except for 4-dimethylaminopyridine (Fluka) and 2-methylpyridine (Lancaster) were obtained from Aldrich. 4-Cyanopyridine was purified by recrystallisation from a CH_2Cl_2–Et_2O mixture[23] and pydz by distillation (in both cases to remove impurities which interfered with the spectrophotometric titration), also 2-methylpyridine by distillation; all other reagents were used as received.

Methods.—The UV/VIS spectra, spectrophotometric titrations and the few kinetic studies (all followed at 397 nm) were carried out on a Philips PU 8740 or 8720 spectrophotometer and, except where otherwise stated, in cells of 1 cm pathlength thermostatted at 25 °C and containing *ca.* 5 μmol dm^{-3} MP-8 in 20% (v/v) aqueous MeOH buffered at pH 6.5 or 7.5 with NaH_2PO_4–Na_2HPO_4 (BDH) and I = 0.2 mol dm^{-3}. Where necessary (*e.g.* to check the buffers, to confirm no change in pH at high concentration of base), pH measurements were made with a Hanna HI BI417 pH meter and appropriate glass electrode.

Results

The heterocyclic bases studied, together with their published pK values, are listed in Table 1. Preliminary experiments, scanning the spectrum over the range 300–600 nm, indicated that all the heterocyclic bases probably (for 2-methylpyridine see below) behaved in a similar simple fashion. The initial MP-8 with a Soret band at 397 nm was converted with reasonable to good isosbestic points at *ca.* 400 and 330–340 nm into a species with a slightly lower Soret band in the range 403–407 nm (*cf.* Fig. 2 of ref. 2); equilibration was essentially instantaneous.

Quantitative determination of the equilibrium constant K was carried out by spectrophotometric titration at pH 7.5 (py, $4H_2N$-, $4Me_2N$- and 2Me-py) or 6.5 (all others), following changes in absorbance at 397 nm. At least two experiments were performed for each base. Analysis of the data (*cf.* ref. 2) confirmed the stoichiometry of 1 B per Fe and the experimental results were used, with correction for protonation of the base in the case of $4H_2N$- and $4Me_2N$-py (where pK > pH of the experiment), to derive values of K. The monomeric nature of the product was checked only in the case of pyrazine (pyz), where a dinuclear complex was most likely to be formed through co-ordination of both N atoms, by studying the spectrum produced and determining the value of K over a 20-fold range of MP-8 concentration in cells of 2 mm, 1 and 4 cm pathlength. The same spectrum and value of K was observed with the standard and lower iron concentration, *i.e.* K remains independent of iron concentration and the product is therefore monomeric; at the higher concentration, however, the presence of a more prominent shoulder at *ca.* 360 nm suggested the onset of some dimerisation or further aggregation. It is assumed that the other products are also monomeric. The values of log K obtained and the wavelengths of the Soret band of the products are listed in Table 1.

The addition of 'as-received' 2-methylpyridine (2Me-py) caused a rapid change in spectrum characterised by a marked broadening and fall in the intensity of the Soret band without any obvious change in the wavelength; this is typical of the formation of aggregated forms of MP-8 (see refs. 24 and 25). This aggregation was avoided by the use of redistilled 2Me-py. The impurity causing the aggregation has not been identified. The sterically hindered 2Me-py shows a low but finite binding constant towards MP-8. The addition of up to 1 mol dm^{-3} 2Me-py at pH 7.5 causes an increasing shift of the Soret band to longer wavelength and a fall in its intensity. The spectrum in 1 mol dm^{-3} base (λ_{max} at 400 nm) is typical of that observed for *ca.* 50% formation of analogous products with the other pyridines. If the final spectrum were similar to that of other pyridines with $\lambda_{max} \geqslant 403$ nm this would give log $K = 0$; because steric hindrance may alter the final spectrum, it is probably safer to conclude that log $K \leqslant +1$.

Discussion

With the possible exception of 2Me-py (see above) all the bases listed in Table 1 reacted rapidly and with reasonable to good isosbestic points to give products with a Soret band at 403–407 nm, as already found for complexes with NH_3, other amines and imidazole (see Fig. 2 of ref. 2). All the constants K listed in Table 1 correspond to the simple co-ordination of 1 B per Fe and, although checked directly only in the case of pyz which could use both N atoms to co-ordinate to two different Fe atoms and stabilise a dinuclear complex, it is assumed that all products are monomers.

Basicity Effects.—Fig. 1 shows a plot of log K *vs.* pK for all the six-membered heterocyclic bases listed in Table 1 except 2Me-py, *i.e.* ignoring steric effects. It is assumed that the presence of 20% MeOH used in determining the log K values does not invalidate any correlation with the pK values determined in purely aqueous solution. The results yield a remarkably simple pattern, in which all bases except pydz show a reasonably linear relationship between log K and pK; for the present we ignore the possibility of a slight but systematic upward curvature as being within the combined experimental errors of pK and log K. The relationship (1) is, therefore, obeyed

$$\log K = a \cdot pK + b \qquad (1)$$

by pyridines with iron(III) porphyrins, in contrast to the results previously reported for iron(II) porphyrins.[19,20] This is perhaps not surprising since complications due to π-back bonding and adduct formation will be far less with iron(III) porphyrins. The best values of a and b in equation (1) depend very much on the weight given to $4Me_2N$-py (uppermost point). It is, however, known that this derivative shows a slightly anomalous behaviour in, and has therefore been excluded from, other linear free-energy relationships.[26] Excluding this last point gives the linear relationship log K = 0.36 pK + 0.8 which is shown as the solid line in Fig. 1; within experimental error, this relationship also includes the 1,3- and 1,4-diazines pyrimidine (pym) and pyz. Whatever values might be adopted for a and b, the value of log K for pydz will appear anomalously high.

We have previously found[1] that the 'baseline' for sp^3 amines with MP-8 corresponds to the relationship $\log K = 0.43\ pK - 0.5$, *i.e.* the slope (*a*) is the same within experimental error, but the intercept (*b*) is somewhat different. Possible interpretations of the slopes and intercepts will be discussed later, together with results for the five-membered heterocycles and for cobalt(III) corrinoids, but the close parallel between the two series suggests that π-back bonding from the Fe to the ligand is negligible in the pyridine series.

The spectra of the products reveal an interesting difference between the sp^3 aliphatic amines and the sp^2 heterocycles. In the case of the amines the Soret band remains virtually unchanged at 405 ± 0.5 nm even though the p*K* of the base varies over the range 5.3–10.6. In the case of the heterocycles (pyridines and diazines combined) the Soret band shows a relatively small but fairly systematic shift from 403 to 407 nm as the p*K* increases from 0.4 to 9.8. The fact that this regular shift includes the two diazines provides further justification for including pym and pyz in the 'baseline' of Fig. 1; the anomalously high value of log *K* shown by pydz is paralleled by an anomalously long wavelength of the Soret band. The fact that the position of the Soret band is far more sensitive to the effect of substituents in the heterocycles than in the amines presumably reflects the greater polarisability of the π electrons in the latter.

Steric Effects.—The value of $\log K \leqslant 1$ (but probably 0) calculated for 2Me-py indicates that steric hindrance has suppressed log *K* by $\geqslant 2$ (most probably by 3) below the 'baseline'. This is comparable to the suppression by $\geqslant 3$ below the 'baseline' of the sp^3 amines by NH_2Bu^t where steric repulsion should be similar.[1]

The α Effect.—Inspection of Fig. 1 shows that the value of log *K* for pydz is *ca.* 0.7 above that expected from the baseline of the pyridines and other diazines. We have also found (details to be published elsewhere) that pydz exhibits a similarly enhanced binding constant compared to substituted pyridines and other diazines with cobalt(III) corrinoids. As in the case of the enhanced nucleophilic reactivity[6,7] and hydrogen-bonding capacity,[8] we ascribe the enhanced log *K* of pydz to operation of the so-called α effect. This effect has, therefore, been established in the co-ordination of NH_2NH_2, NH_2OH[1] and pydz with the Fe^{III} in MP-8 at the thermodynamic, and not merely the kinetic, level; these represent the first three examples of the α effect demonstrated for any metal complexes. Studies of the co-ordination of the diazines by Ag^I and various divalent metal ions yielded much more complex patterns which were discussed in terms of π-back bonding;[12,13] such back bonding can, however, probably be ignored with the higher-valent iron(III) ion.

The origin of the α effect is still controversial and most or all theories can be applied only to the kinetic aspects. The inclusion of pydz allows the origin to be related to the analysis of proton-binding equilibria by Taft *et al.*[10] They successfully explained the different gas- and aqueous-phase basicities of pydz and pym (also imidazole and pyrazole) on the basis of two main effects, *viz.* (*a*) electrostatic repulsion, which destabilises those forms where adjacent N atoms both possess unshared pairs of electrons by *ca.* 6.5 kcal mol^{-1} in the gas phase (attenuated to *ca.* 2.5 kcal mol^{-1} in aqueous solution), and (*b*) the inductive effect of an aza substituent, which destabilises the NH^+ form by *ca.* 12.5, 8.5 and 0 kcal mol^{-1} in the gas phase (7.8, 5.3 and 0 kcal mol^{-1} in solution) when the substituent is in the 2, 3 or 4 position respectively. Comparing pydz and pym shows that the difference in basicity exhibited towards different acids (H^+ in the gas phase or solution, metal cations, hydrogen bond) will reflect the difference between two significant but opposing trends [*viz* (*a*) and (*b*)] which will probably show a different dependence on the degree of electron density removed from the base under the polarising influence of the Lewis acid. It is reasonable to assume that (*a*) will be relatively more important, and (*b*) less important, for lower degrees of charge removal and that pydz will be relatively better than pym as a base towards those acids that remove less charge (*viz.* carbonyl carbon, hydrogen bond, metal cation) than the proton itself. We therefore suggest that the relatively enhanced donor power of pydz and other 'α effective' nucleophiles (whether acting as hydrogen-bond acceptor, ligand or other nucleophile) over that expected from their p*K* towards the proton, which is considered anomalous and termed the 'α effect', is the natural consequence of the existence of opposing factors with a different dependence on the extent of negative charge removed by the Lewis acid; similar arguments would apply to NH_2NH_2, NH_2OH, HO_2^-, *etc.* It would, of course, be equally valid to use values of log *K* as the 'standard' and to plot values of p*K* against log *K*; pydz, NH_2NH_2 and NH_2OH would then exhibit anomalously low values of p*K* and the term 'α effect' could then be applied to their anomalously low basicity towards the proton compared to their Lewis basicity towards a metal or acylium ion. The magnitude of any observed α effect may be sensitive to other neighbouring effects (including adjacent ligand atoms in metal complexes) and hence susceptible to manipulation by the protein in metalloenzymes (*e.g.* through a critically positioned hydrogen bond or net charge). We have already emphasised[1] that the α effect is of particular interest to inorganic biochemists because of the important role played by ligands such as O_2, O_2^-, H_2O_2 and HO_2^- in the enzymatic reactions of haemoproteins.

Acknowledgements

We thank the University of Surrey for a research studentship and the Arab Student Aid International for financial support (to M. S. A. H.).

References

1 Part 8, M. P. Byfield, M. S. A. Hamza and J. M. Pratt, preceding paper.
2 H. M. Marques, M. P. Byfield and J. M. Pratt, *J. Chem. Soc., Dalton Trans.*, 1993, 1633.
3 J. Aron, D. A. Baldwin, H. M. Marques, J. M. Pratt and P. A. Adams, *J. Inorg. Biochem.*, 1986, **27**, 227.
4 D. A. Baldwin, H. M. Marques and J. M. Pratt, *J. Inorg. Biochem.*, 1986, **27**, 245.
5 J. O. Edwards and R. G. Pearson, *J. Am. Chem. Soc.*, 1962, **84**, 16.
6 J. A. Zoltewicz and L. W. Deady, *J. Am. Chem. Soc.*, 1972, **94**, 2765.
7 J. A. Zoltewicz and H. L. Jacobson, *Tetrahedron Lett.*, 1972, 189.
8 M. H. Abraham, P. P. Duce, D. V. Prior, D. G. Barratt, J. J. Morris and P. J. Taylor, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1355.
9 L. Joris and P. v. R. Schleyer, *Tetrahedron*, 1968, **24**, 5991.
10 R. W. Taft, F. Anvia, M. Taagepera, J. Catalan and J. Elguero, *J. Am. Chem. Soc.*, 1986, **108**, 3237.
11 M. Tišler and B. Stanovnik, *Adv. Heterocycl. Chem.*, 1979, **24**, 363.
12 J. Kulig, B. Lenarcik and M. Rzepka, *Pol. J. Chem.*, 1986, **60**, 715.
13 J. Kulig and B. Lenarcik, *Pol. J. Chem.*, 1988, **62**, 351.
14 G. D. Fasman, *Handbook of Biochemistry and Molecular Biology, Physical and Chemical Data*, 3rd edn., CRC Press, Cleveland, OH, 1976, vol. 1.
15 K. Sakata, M. Hashimoto and H. Yoshino, *Inorg. Chim. Acta*, 1985, **99**, 231.
16 K. L. Brown, D. Chernoff, D. J. Keljo and R. G. Kallen, *J. Am. Chem. Soc.*, 1972, **94**, 6697.
17 F. A. Walker, *J. Am. Chem. Soc.*, 1973, **95**, 1150.
18 Y. Murakami, Y. Aoyama and K. Tokunaga, *J. Am. Chem. Soc.*, 1980, **102**, 6736.
19 J. E. Falk, J. N. Phillips and E. A. Magnusson, *Nature (London)*, 1966, **212**, 1531.
20 S. J. Cole, G. C. Curthoys and E. A. Magnusson, *J. Am. Chem. Soc.*, 1970, **92**, 2991; 1971, **93**, 2153.
21 P. Mohr and W. Scheler, *Eur. J. Biochem.*, 1969, **8**, 444.
22 P. A. Adams, M. P. Byfield, R. C. de L. Milton and J. M. Pratt, *J. Inorg. Biochem.*, 1988, **34**, 167; P. A. Adams, M. P. Byfield, R. D. Goold and A. F. Thumser, *J. Inorg. Biochem.*, 1989, **37**, 55.

23 D. D. Perrin, W. L. F. Armarego and D. R. Perrin, *Purification of Laboratory Chemicals*, 2nd edn., Pergamon, Oxford, 1980, p. 182.
24 D. W. Urry and J. W. Pettegrew, *J. Am. Chem. Soc.*, 1967, **89**, 5276.
25 G. S. Collier, J. M. Pratt, C. R. de Wet and C. F. Tshabalala, *Biochem. J.*, 1979, **179**, 281.
26 M. J. Kamlet, J. Gal, P. C. Maria and R. W. Taft, *J. Chem. Soc., Perkin Trans. 2*, 1985, 1583.

Received 5th January 1993; *Paper* 3/00059A