could not be extracted from an erratic study of complexes with different metal atoms and ligands.

In particular one can (1) explain the stability of the [Fe(II)Pc] complex *vs.* the iron porphyrins, in which the stable iron complex is the trivalent one; (2) find an interesting magnetic transition between low and high spin upon oxidizing the [Fe(II)TPP] complex; (3) find hf interaction of a ligand unpaired π electron with the nuclear spin of the cobalt; (4) identify a Ni(III) square-planar complex in solution; and (5) find triplet states with one unpaired electron on the metal atom (Fe and Cu) and the other on the ligand. Most of the experimental results agree with Gouterman's theoretical calculations; however, they point out that upon removal of one ligand electron the whole energy level scheme is affected.

The assignments of the electrochemical trends were proven by spectroscopic (optical and magnetic) measurements; therefore, it would be gratifying if future studies could prove the existence of the predicted Cu(III)–dithiolate complex, and thus generalize our observations beyond the metalloporphyrins.

Acknowledgment. The authors are grateful to Drs. J. M. Fritsch and J. P. Petrovich for the use of their electrochemical equipment and their many helpful suggestions.

Ligand Binding by Metalloporphyrins. I. Thermodynamic Functions of Porphyriniron(II)–Pyridine Complexes

S. J. Cole,[1a] G. C. Curthoys,[1a] and E. A. Magnusson[1b]

University of Newcastle, N.S.W., Australia, and Avondale College,
Cooranbong, N.S.W., Australia. Received July 29, 1969

Abstract: Enthalpy, entropy, and free energy values for the addition of a series of substituted pyridine ligands to a number of porphyriniron(II) dimethyl esters in benzene solution were measured spectrophotometrically. Variations in free energy of reaction with the changes in ligand and metalloporphyrin were not great but changes in enthalpy and entropy of reaction were large. The results obtained best fit a model in which the ligand is bound by both σ bonding from the pyridine nitrogen to the metal atom and π bonding by back-donation from the iron atom to the pyridine. While π bonding becomes more important in the order 4-methyl < 4-vinyl < pyridine < 4-carboxybutyl ester < 4-cyanopyridine, σ bonding is most important for 4-methylpyridine and decreases in importance in the same order. π bonding was not as important for 2,4-diacetyldeuteroporphyriniron(II) dimethyl ester as it was for the meso-, deutero-, and protoporphyrin complexes. Entropies of reaction were large and variable and were well correlated with the enthalpy changes observed. This and the compensatory nature of the entropy change in relation to the enthalpy change gave an isoequilibrium temperature of 340°K. The close proximity of this to the temperature at which the reactions were studied led to the small changes observed in the free energy of reaction.

Recent work[2] on the addition of ligands to metalloporphyrins highlights the need for an explanation of the physiochemical properties in terms of the electronic structures involved. This is of both chemical and biochemical importance. In the latter case there is a need to be able to explain in electronic and structural terms the changes in properties of hemoproteins when the same heme (usually protoheme) is attached to various proteins and various ligands.

It has been suggested that the affinity of iron porphyrins for ligands is partly explained in terms of π bonding occurring by back-donation of electrons from the metal atom to the π system of the ligand.[3] The suggestion was prompted by the changes observed in the stability constants of heme–pyridine complexes as the ligand changes from 4-aminopyridine, through pyridine, to 4-cyanopyridine—a potent π acceptor. However, there are strong objections to using stability constant data alone to derive conclusions about the nature of chemical bonding.

The effect of the entropy term is usually unknown, but comparison of a series of stability constants to obtain information about bonding presupposes that the effect of entropy is negligible. This is not so in zinc and nickel tetraphenylporphyrin complexes where the entropy terms are large and variable.[4] Complete enthalpy and entropy data as well as free-energy changes (stability constants) are needed before reactions of metalloporphyrins with basic ligands can be properly understood.

In this study the equilibrium constant for the reaction between metalloporphyrin and ligand symbolized by the equation

$$MP + nL \longrightarrow MPL_n$$

was determined spectrophotometrically. The equilibrium constant $K = [MPL_n]/[MP][L]^n$ can be evaluated readily provided the ratio of the two metalloporphyrin species can be determined and the equilibrium concentration of ligand is known. In the systems studied here the concentration of ligand is so far in excess of the metalloporphyrin concentration that the amount of ligand added may be taken as the equilibrium concentration.

---

(1) (a) University of Newcastle, N.S.W., Australia; (b) Avondale College, Cooranbong, N.S.W., Australia.

(2) (a) J. E. Falk in "Porphyrins and Metalloporphyrins," Elsevier Publishing Co., New York, N. Y., 1964, p 41; (b) W. S. Caughey, W. Y. Fujimoto, and B. P. Johnson, *Biochemistry*, 5, 3830 (1966).

(3) J. E. Falk in ref 2a, p 51.

(4) S. J. Cole, unpublished results, 1967.

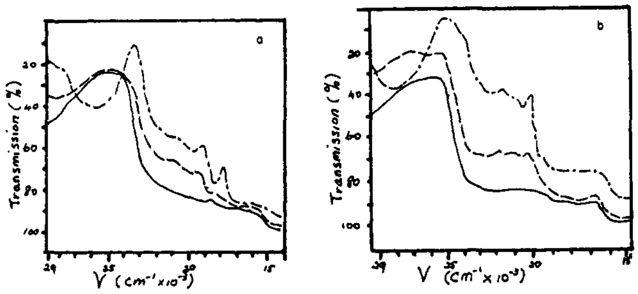

Figure 1. Ultraviolet and visible absorption spectra of Fe porphyrin species in benzene solution: (1a) solid line, Fe(III) protoporphyrin dimethyl ester; — —, Fe(II) protoporphyrin dimethyl ester; —-—, Fe(II) protoporphyrin dimethyl ester dipyridinate. (1b) solid line, Fe(III) deuteroporphyrin dimethyl ester; — —, Fe(II) deuteroporphyrin dimethyl ester; —-—, Fe(II) deuteroporphyrin dimethyl ester dipyridinate.

The total metalloporphyrin concentration was kept constant as the ligand concentration varied. It can be shown that

$$\frac{[\mathrm{MPL}_n]}{[\mathrm{MP}]} = \frac{A_\mathrm{m} - A_\mathrm{p}}{A_\mathrm{l} - A_\mathrm{m}}$$

where A_p = the absorbance of a solution of unliganded metalloporphyrin, A_l = the absorbance of a solution of liganded metalloporphyrin, and A_m = the absorbance of a solution of mixed species. If it is not possible to measure the absorbance of a solution in which all the metalloporphyrin is liganded it is still possible to calculate the ratio of species by estimating the value of A_l itself obtained by solving simultaneous equations as outlined by Rossotti.[5]

Experimental Section

Equilibrium measurements were made spectrophotometrically using a Unicam SP700 spectrophotometer fitted with a constant-temperature accessory and with cells designed so that water from the constant-temperature bath circulated through a jacket surrounding the cells. The temperature of the cells was controlled to ±0.1° and measured by means of a thermistor inserted in the cell just above the light path.

The total concentration of porphyrin used was approximately 10^{-5} *M*. Solutions containing a range of ligand concentrations were prepared by mixing different volumes of solutions containing metalloporphyrin and known concentrations of ligand.

The absorption spectrum was measured in the region of the α band of the pyridine hemochrome (around 550 nm) and the equilibrium constant determined at a number of wavelengths (usually about 20) on either side of the peak absorption. Individual calculations were screened by comparing the variance in results obtained from different wavelengths and different ligand concentrations with the residual error variance, and the average equilibrium constant was calculated.

In order to produce and retain Fe(II) hemochromes in benzene solution it was necessary to find suitable reducing agents which do not affect the porphyrin ring or the ligands being used. After many trials three satisfactory reducing systems were found—hydrazine hydrate, hydroquinone, and phenidone (1-phenyl-3-pyrazolidone). The optimum quantity of reducing agent was determined experimentally by titrating it with a solution of heme and pyridine in benzene and observing the intensity of the α and β hemochrome bands in the visible spectrum.

Changes in the visible spectrum indicate that at very high concentrations hydrazine is capable of acting as a ligand to Fe(II) porphyrins. There is no danger when used at the concentrations adopted in this study (1 drop of benzene saturated with hydrazine monohydrate satisfactorily reduces a 4-ml portion of hemin solution), nor when phenidone and hydroquinone are used as reducing agents. Both compounds are relatively insoluble in benzene and there is no evidence of the formation of a hemochrome-type spectrum even in saturated solutions (see Figure 1). Furthermore, the equilibrium constant for the ligand-addition reaction is quite insensitive to the amount of reducing agent present.

It is possible to follow the reduction of porphyriniron(III) esters to the Fe(II) state spectrophotometrically. Full spectra for protoporphyriniron(II) and deuteroporphyriniron(II) are shown in Figure 1. Fairly large changes in the Soret region and the low-wavelength visible spectrum can be seen when the reducing agent is added; the resulting spectrum is that of the Fe(II) compound. Much greater changes can be seen as ligands are brought into the 5th and 6th coordination positions. Evidence for the reduction is also seen in the effect of the porphyriniron on the position of resonance of the internal reference substance (TMS) in the high resolution nmr spectra of these compounds. Spectra were recorded on a Perkin-Elmer Model R20 nmr spectrometer at room temperature and revealed a downfield shift of the order of 100 cps in the position of the TMS resonance in chloroform when the solution was saturated with protoporphyriniron(III) dimethyl ester. The shift is reversed by the addition of hydrazine or phenidone, indicating the spin-state change to be expected on reduction to the diamagnetic Fe(II) state.

It was found that slow breakdown of the pyridine hemochrome occurred over a period of some hours after the addition of the hydrazine. This was negligible if measurements were taken within 5 min of addition of hydrazine. Accordingly the time between addition of the reducing agent and measurement of spectra was kept constant.

Heats of reaction between iron(II) porphyrins and ligands were determined from a plot of log *K vs.* reciprocal temperature in the temperature range 18–40°. At least four temperatures were used for each study. The plots of log *K vs.* $1/T$ displayed high linearity over the temperature range studied, and in each case ΔS was constant within experimental error.

The compounds were prepared as follows and checked for purity by thin layer chromatography on alumina and the positions and intensities of the spectral bands.

(a) Protohemin was obtained from citrated horse blood by the glacial acetic acid method.[6]

(b) Deuterohemin was prepared by heating protohemin with resorcinol at 150–160° for 45 min;[7] the crude product was purified by washing with ether and recrystallizing from glacial acetic acid.

(c) Insertion of iron into mesoporphyrin by the method of Lemberg and Morrell[8] yielded mesohemin.

(d) Mesoporphyrin was obtained by column chromatography on alumina of commercial mesoporphyrin dimethyl ester (K & K Laboratories) and subsequent hydrolysis with aqueous HCl.[9]

(e) 2,4-Diacetyldeuteroporphyrin dimethyl ester was made from deuterohemin by acetylation with acetic anhydride using $SnCl_4$ as catalyst,[10] followed by removal of iron and esterification by the Grinstein method.[11] The crude product was chromatographed on alumina using $CHCl_3$-ether solutions of increasing $CHCl_3$ concentration as eluent.

(f) 2,4-Diacetyldeuterohemin was obtained by insertion of iron into the porphyrin by the method of Lemberg and Morrell.[8]

(g) The dimethyl esters of protohemin, deuterohemin, mesohemin, and 2,4-diacetyldeuterohemin were prepared from the corresponding hemins by esterification with 5% H_2SO_4 in methanol at 0° in the dark.[12] All hemin esters were recrystallized from $CHCl_3$-MeOH.

The pyridines were prepared as follows. (h) Pyridine and 4-methyl pyridine were distilled over KOH pellets and the fractions boiling between 114.5 and 115.5° and 142 and 144°, respectively, were collected.

(i) 4-Vinylpyridine and 4-carboxy-*n*-butylpyridine were purified by vacuum distillation of the commercially available products

---

(5) F. J. C. Rossotti and B. Rossotti in "The Determination of Stability Constants and Other Equilibrium Constants in Solution," McGraw-Hill Book Co., New York, N. Y., 1961, p 276.

(6) H. Fischer, *Org. Syn.*, **3**, 442 (1955).

(7) J. E. Falk in ref 2a, p 179; O. Schumm, *Z. Physiol. Chem.*, **178** 1 (1928).

(8) R. Lemberg, B. Bloomfield, S. Caizer, and W. Lockwood, *Aust. J. Exptl. Biol.*, **33**, 435 (1955); D. B. Morrell, J. Barrett, and P. S. Clezy, *Biochem. J.*, **78**, 793 (1961); D. B. Morrell and M. Stewart, *Aust. J. Exptl. Biol.*, **34**, 211 (1956).

(9) J. E. Falk in ref 2a, p 126.

(10) D. B. Morrell, private communication.

(11) M. J. Grinstein, *J. Biol. Chem.*, **167**, 515 (1947).

(12) J. E. Falk in ref 2a, p 125.

(Aldrich Chemical Co. and Koch-Light Laboratories, respectively) and the middle fractions collected.

(j) 4-Cyanopyridine was recrystallized from water and dried in a vacuum desiccator over silica gel. Crystals of mp 80° (lit. mp 78.5-80°[13]) were obtained.

Results and Discussion

The determination of heats of reaction by the indirect method used in this study has commonly been employed in studies of metal-ligand systems.[14] Results obtained by the method are usually in reasonable agreement with results from direct calorimetric measurements although serious anomalies sometimes arise.[15] It is an assumption of the method that ΔH remains constant over the temperature range studied. Any great change in ΔH would be reflected in a departure of the plot of log K *vs.* $1/T$ from linearity. In no case was there any significant departure from linearity in the equilibria studied here.

It was not the purpose of this study to determine ΔH values to high accuracy, measurements on a greater range of compounds being preferred to this. Standard errors of ΔH values are between 0.5 and 1.0 kcal mol^{-1}. A typical Van't Hoff plot is shown in Figure 2. In the metalloporphyrin systems it is not possible to employ direct calorimetry for the determination of ΔH because of solubility problems.

Due consideration must be given to the possibilities of aggregation and dimerization of the porphyriniron (II) esters in benzene. There is ample evidence for association of one type or another in metal porphyrin compounds,[16] especially those of magnesium in organic solvents,[17] but we conclude that these phenomena are unimportant for Fe(II) porphyrins at the concentrations at which we have worked, for the following reasons.

(a) We have determined the molecular weights of a series of porphyriniron(III) complexes (hemins) in carbon tetrachloride, chloroform, and benzene by means of vapor-pressure osmometry. The results will be published soon. The sensitivity limit of the method is 10^{-3} M, at which concentration deutero- and mesohemins aggregate only slightly. Difficulties in isolating the Fe(II) species free from reducing agent have prevented our measuring the molecular weights of the Fe(II) porphyrins themselves in these solvents.

(b) The degree of aggregation found by Doughty and Dwiggins[18] for mesoporphyrinnickel(II) in chloroform (equilibrium constant for dimerization = 61.6) is insufficient to produce any significent quantity of dimer at the 10^{-5} M concentrations employed in this study.

(c) The equilibrium constants of the ligand addition reactions are independent of the concentration of the porphyriniron(II) compound. Equilibrium constants were determined over a range of concentrations so that the percentage of complex formation varied from 15 to 85% of the total porphyriniron(II) present, but there was never any evidence of a trend in the equilibrium constant with concentration. The extreme values of the equilibrium constants obtained correspond to free energy changes within 0.4 kcal mol^{-1} of the average value.

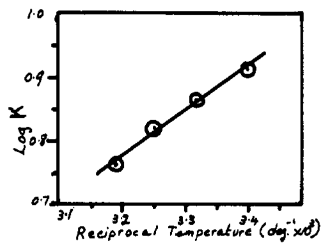

Figure 2. Typical Van't Hoff plot for Fe(II) porphyrin dimethyl ester-pyridine complexes.

The equilibria were studied in benzene. It is not clear how to apply corrections for activity coefficients; however the low concentration of metalloporphyrin (10^{-5} M) and of the ligand in most cases (10^{-2} M) would seem to make them unnecessary. Over the range of concentrations varying by a factor of 10 there was no evidence of a change in equilibrium constant with changing ligand concentration. Hence there was no need to extrapolate answers to pure solvent conditions.

The stoichiometry of the reaction was measured by plotting the logarithm of the ratio of the complexed and uncomplexed species against the logarithm of ligand concentration.[19] The slope of this line gives the number of ligands adding to form the complex. In all cases studied here the slope of the line was not significantly different from two. Further, it was not possible to fit the experimental data to any other stoichiometry. The spectra at low ligand concentration were examined for evidence of stepwise addition of ligand but no changes in spectra were noted until the concentration of ligand was sufficiently high for the dipyridine complex to be formed. Our unpublished studies on pyridine ligand addition to nickel(II) porphyrins[4] have demonstrated that stepwise addition is readily detected by investigating the stoichiometry of the reaction. As expected from previous studies,[20] the results reported here definitely exclude it.

Direct estimation of the absorbance at high ligand concentration was possible in most instances. In such cases the porphyriniron is completely complexed and the equilibrium constants are obtained immediately from the data.

When ligand solubility problems prevented this approach it was found that the previously cited method of solving simultaneous equations for the absorbance, A_l, gave reproducible results whenever a spectrum in which more than 85% of the metalloporphyrin is complexed could be obtained. Test cases in which the calculated absorbance has been compared with measured absorbance have shown that the errors introduced by this method of estimation are small.

---

(13) D. G. Leis and B. C. Cunan, *J. Amer. Chem. Soc.*, **75**, 67, 79 (1953).

(14) F. J. C. Rossotti in "Modern Coordination Chemistry," J. Lewis and R. C. Wilkins, Ed., Interscience Publishers, New York, N. Y., 1960, p 17.

(15) W. R. May and M. M. Jones, *J. Inorg. Nucl. Chem.*, **25**, 507 (1963); L. G. Sillen, *ibid.*, **8**, 176 (1958); F. Basolo and R. K. Murmann, *J. Amer. Chem. Soc.*, **76**, 211 (1954).

(16) J. Shack and W. M. Clark, *J. Biol. Chem.*, **171**, 143 (1947); W. Scheler, P. Mohr, K. Hecht, and K. Gustarv, *Z. Chem.*, 303 (1967).

(17) G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Amer. Chem. Soc.*, **85**, 3809 (1963).

(18) D. A. Doughty and C. W. Dwiggins, Jr., *J. Phys. Chem.*, **73**, 423 (1969).

(19) H. E. Dent and C. L. French, *J. Amer. Chem. Soc.*, **63**, 568 (1941); J. R. Miller and G. D. Dorough, *ibid.*, **74**, 3977 (1952).

(20) J. E. Falk in ref 2a, p 50.

Table I. Thermodynamic Constants for Binding of Pyridines in Benzene by Various Iron(II) Porphyrins[a]

Porphyrin type	Ligand	$\Delta G(298)$, kcal mol^{-1}	ΔH, kcal mol^{-1}	ΔS, cal mol^{-1} deg^{-1}
Mesoporphyriniron(II) dimethyl ester	4-Methylpyridine	−5.1 ± 0.8[b]	−8.7 ± 1.2[b]	−12 ± 6[b]
	4-Vinylpyridine	−5.0 ± 0.8	−10.2 ± 1.0	−16 ± 5
	Pyridine	−3.3 ± 0.8	−5.2 ± 1.4	−6 ± 7
	4-Carboxy-*n*-butylpyridine	−4.3 ± 0.8	−8.9 ± 1.0	−15 ± 5
	4-Cyanopyridine	−3.8 ± 0.8	−11.7 ± 1.1	−24 ± 5
Deuteroporphyriniron(II) dimethyl ester	4-Methylpyridine	−3.3 ± 0.6	+0.8 ± 0.6	+13 ± 4
	4-Vinylpyridine	−3.9 ± 0.6	−1.6 ± 0.9	+8 ± 4
	Pyridine	−3.5 ± 0.6	−2.4 ± 0.2	+4 ± 3
	4-Carboxy-*n*-butylpyridine	−4.8 ± 0.6	−6.7 ± 1.0	−7 ± 5
	4-Cyanopyridine	−4.8 ± 0.6	−6.9 ± 1.1	−7 ± 5
Protoporphyriniron(II) dimethyl ester	4-Aminopyridine	−7.7 ± 0.8	−16.1 ± 2.2	−28 ± 9
	4-Methylpyridine	−9.0 ± 0.6	−14.0 ± 1.0	−16 ± 5
	4-Vinylpyridine	−5.1 ± 0.6	−7.4 ± 1.1	−8 ± 5
	Pyridine	−1.8 ± 0.6	−3.3 ± 0.6	−5 ± 4
	4-Carboxy-*n*-butylpyridine	−4.0 ± 0.6	−10.1 ± 0.4	−20 ± 3
	4-Cyanopyridine	−4.1 ± 0.6	−9.0 ± 1.0	−16 ± 5
Diacetyldeuteroporphyriniron(II) dimethyl ester	4-Methylpyridine	−1.5 ± 0.9	−2.6 ± 0.7	−4 ± 5
	4-Vinylpyridine	−3.6 ± 0.9	−3.6 ± 0.2	0 ± 3
	Pyridine	−1.8 ± 0.9	−7.5 ± 0.9	+32 ± 5
	4-Carboxy-*n*-butylpyridine	−5.0 ± 1.0	+7.0 ± 0.4	+42 ± 4
	4-Cyanopyridine	−1.7 ± 1.0	+7.5 ± 0.4	+32 ± 4

[a] Values refer to the reaction porphyriniron(II) + 2(ligand) = Fe-porph–(ligand)$_2$. [b] Standard error of estimate.

Calculated entropy changes rather than enthalpy changes are most affected by errors in the estimation of A_l provided that the same method is used for all temperatures studied. This occurs because a similar error is introduced into all equilibrium constants and the resultant plot of log K *vs.* reciprocal temperature is parallel to the plot of true values. The value of ΔH is not affected but an error in ΔS occurs. Results obtained in this way were compared with those obtained by the method of Ramette,[21] in which a plot of $(A_m - A_p)/L^n$ *vs.* A_m gives a straight line with slope equal to the reciprocal of the equilibrium constant. Results obtained by both methods were comparable. The former method was used here.

The thermodynamic constants in Table I emphasize the problems associated with interpretations of stability constants in terms of electronic effects on bond strengths. Because of the difficulties involved there have been very few determinations of enthalpy data in the metalloporphyrin systems. Examination of the results obtained shows that there is very poor correlation between the free-energy and the enthalpy values and any attempt to use the free-energy values as a measure of the metal–nitrogen bond strengths would be unsafe. Changes in bond energy are best deduced from changes in the enthalpy of formation of the complexes, and this method of comparison has been used throughout the following discussion.

Since the changes in entropy were neither small nor constant it is not surprising that correlation between free energy and enthalpies of ligand addition is so poor. A wide range of values (−30 to +40 cal deg^{-1} mol^{-1}) has been obtained in the series of porphyrin-iron(II)–pyridine complexes studied. In addition to this the magnitude of the $T\Delta S$ term is of the same order of magnitude as the ΔH term. This means that variation in the entropy values will greatly affect the free energy of reaction and thus the stability constant.

The extent to which the nitrogen lone-pair electrons are available for σ bonding with the metal is likely to be sensitive to the presence of substituents in pyridine. Presumably, metal–ligand σ bonding is affected by pyridine substituents in much the same way as proton binding, and acid dissociation constants may be used as a guide to σ-bonding strength provided that there are no significant entropy changes associated with proton binding by pyridines. If this is true here, it is obvious from pK_a data that 4-methylpyridine should form the strongest and 4-cyanopyridine the weakest σ bonds. As Table I shows, the bond strengths of the heme compounds do not follow this pattern; not only do bond strength variations caused by varying the ligand depend on the particular porphyriniron(II) being used but there is a factor involved which tends to strengthen the bond as pK_a is decreased.

Overlap between the pyridine nitrogen pπ orbital and one of the dπ orbitals of the iron atom permits the π systems of the heme and the ligand to interact. There is good evidence that this occurs in this series of compounds and that the overall electron distribution of the compound is affected by the withdrawal or release of π electrons by substituents on both heme and ligands. The fact that iron(II) has a d^6 configuration leads one to expect that iron–nitrogen π-bond strength will be increased by the presence of electron-attracting substituents in the 4 position and that ΔH changes will become more negative from 4-methylpyridine to 4-cyanopyridine.

Examination of Figure 3 shows that the direction of the departure from a linear dependence of bond strength on pK_a implies that the additional factor affecting the bonding is of this type. Clearly, the effect of π bonding on the enthalpy of ligand addition is most likely to be dominant at the low end of the pK_a scale, with σ-bonding effects dominant at the high end. V-shaped plots of ΔH *vs.* pK_a are, therefore, not surprising.

In no case is a strictly linear relation between pK_a and bond strength expected, nor is the relationship between pyridine substituent, heme substituent, and metal–nitrogen bond strength likely to be a simple one. That the orientation of the V is different in each case probably is a consequence of the effects of the different heme substituents.

---

(21) R. W. Ramette, *J. Chem. Educ.*, **44**, 647 (1964).

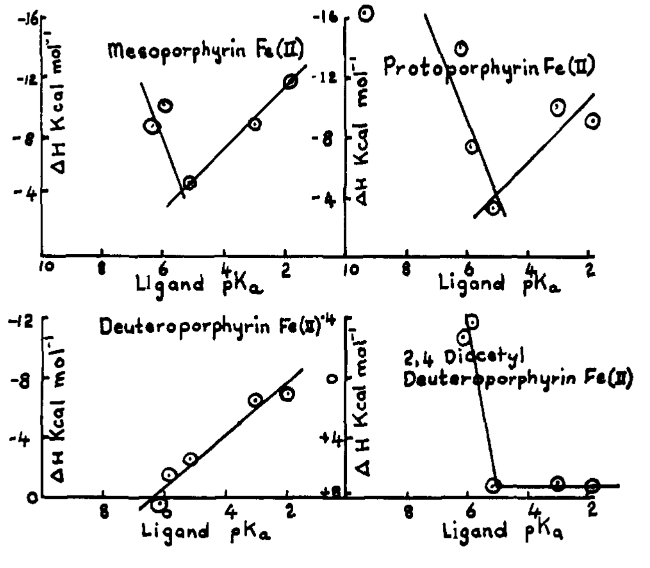

Figure 3. Relationship of ligand pK_a to enthalpy of complex formation at 25° for pyridine–iron(II) porphyrin complexes.

It is not easy to eliminate other contributions (*e.g.*, solvation enthalpy changes) to the enthalpy changes observed here, nor is there an immediately obvious explanation for the different behavior of the same series of ligands with the different hemes. One way of rationalizing the data is to suppose that, of all the heme substituents used, there is one which imposes optimum electron density on the iron dπ orbitals involved in overlap with pπ orbitals of pyridine nitrogen atoms. This particular heme provides conditions most favorable for π bonding with the widest range of ligands. The data suggest that deuteroporphyrin fills this role, π bonding being dominant for the whole range of ligands available. π bonding becomes relatively less important for both protoporphyrin, where electron attraction to the periphery of the heme is stronger, and mesoporphyrin, where it is weaker. In the case of diacetyldeuteroporphyrin, π bonding is sufficiently unimportant for the differences between the pyridines with the most strongly electron-attracting substituents that it disappears. The right-hand arm of the V-shaped curve is horizontal.

The effect on the bond strength as the porphyrin is changed for any given ligand is bound to be dependent on the relative importance of the σ and π bonding for that ligand. It is shown in Figure 3 that 4-aminopyridine, 4-methylpyridine, and 4-vinylpyridine can be viewed as σ-bonding-type ligands and 4-carboxybutyl- and 4-cyanopyridine as π-bonding types. Pyridine itself is somewhat intermediate. The above statements are not meant to imply that only one type of bonding is operative, but only that σ bonding predominates in one group and π bonding in the other.

For the ligands 4-methyl- and 4-vinylpyridine the order of bond strength is proto > meso, 2,4-diacetyl > deutero, whereas for the cyano- and carboxybutyl-substituted pyridines the order is meso > proto > deutero, 2,4-diacetyl. These results reflect the relatively unimportant contribution of π bonding in iron–2,4-diacetyldeuteroporphyrin complexes and what appears to be a very significant enhancement of bond strength by π bonding in the other iron porphyrins.

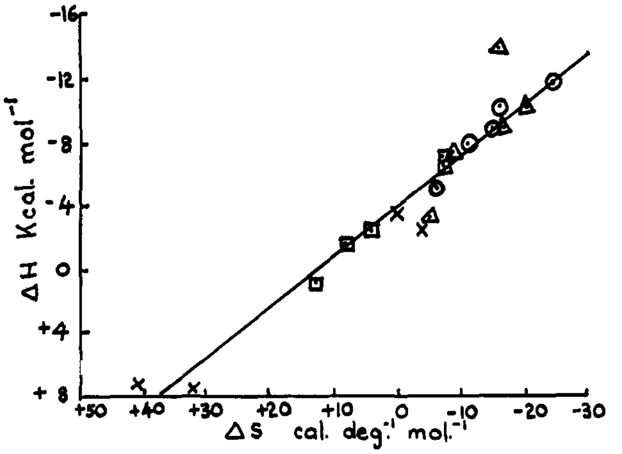

Figure 4. Enthalpy–entropy relationships for pyridine–iron(II) porphyrin complexes in benzene solution: ⊙, mesoporphyrin Fe(II); ⊡, deuteroporphyrin Fe(II); ◬, protoporphyrin Fe(II); ×, 2,4-diacetyldeuteroporphyrin Fe(II).

The free energy changes for the reaction porphyrin-iron(II) + ligand = complex do not conform to the values that would be predicted from the enthalpy values. An examination of the relationship between enthalpy and entropy changes in this system shows why this is so. If, for a given system in which the solvent and/or ligand are changed, ΔH is plotted against ΔS, the slope of the resulting line is called the "isoequilibrium" temperature.[22] At this temperature enthalpy and entropy effects cancel. No shift in free-energy change will occur as the ligand and/or solvent are varied at this temperature in a system in which there is a perfectly linear enthalpy–entropy relationship. Such relationships are seldom encountered and even at the isoequilibrium temperature small variations in free energy are usually observed as the solvent and ligand are varied. Nevertheless, the closer the temperature is to the isoequilibrium temperature, the poorer will be the relationship between the free-energy change and the enthalpy change and the less satisfactory will be any attempt to relate the equilibrium constant to the bond strength.

In the pyridine (substituted pyridine)–porphyrin-iron(II) systems which we have investigated, the enthalpy–entropy relationship has a correlation coefficient of 0.98 (Figure 4) and an isoequilibrium temperature at 340°K. It is not surprising, therefore, that at 298°K, which is only about 40°K below the isoequilibrium temperature, there is only a poor relationship between ΔG and ΔH as the heme and/or ligand are changed. It is interesting to note that the ΔH and ΔS data for all the complexes of the iron(II) porphyrins studied are described by the same straight line and the same isoequilibrium temperature is obtained for all of them. The slope is positive as is usual in systems of this type. Thus the changes induced by the various ligands and solvents are of the same nature for the range of iron(II) porphyrins and ligands studied. A similar relationship has also been found for the addition of piperidine to various nickel porphyrins.[2b]

For reactions in which two ligand molecules become complexed to a central atom, an entropy loss of 40–50

---

(22) J. E. Leffler and E. Grunwald in "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1963, p 324.

Table II. Average Entropy Changes on Ligand Addition for Metalloporphyrins and Pyridine Ligands

Metalloporphyrin	ΔS(298),[a] cal deg^{-1} mol^{-1}	Ligand	ΔS(298),[b] cal deg^{-1} mol^{-1}
Mesoporphyriniron(II)	−14.6	4-Methylpyridine	−4.8
Deuteroporphyriniron(II)	+2.2	4-Vinylpyridine	−4.0
Protoporphyriniron(II)	−13.0	Pyridine	+6.2
2,4-Diacetyldeuteroporphyriniron(II)	+20.4	4-Carboxybutylpyridine	0.0
		4-Cyanopyridine	−3.7

[a] Averaged over all ligands. [b] Averaged over all iron(II) porphyrins.

cal deg^{-1} mol^{-1} is to be expected.[23] π bonding would also cause an additional entropy loss due to restricted rotation around the iron–nitrogen bond and a general increase in the rigidity of the molecule. The solvent also plays a role in determining the value of the entropy change, in that the latter will depend upon the relative solvation of the iron(II) porphyrin, with and without ligands attached, and also on the degree of solvation of the ligand itself. If the unliganded porphyriniron(II) binds more solvent molecules than it does when ligand is attached, which is highly probable, there will be a gain in entropy from a complex formation due to release of solvent molecules. Likewise, a favorable entropy change will be associated with the release of any solvent from the ligand.

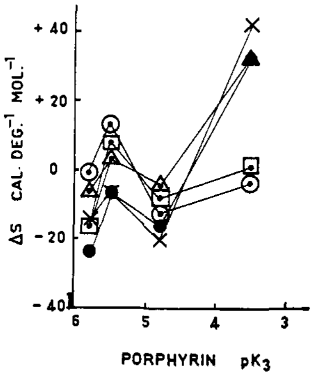

Figure 5. Changes in entropy of complex formation (25°) for porphyrin Fe(II) complexes as a function of porphyrin pK_3: ⊙, 4-methylpyridine; ⊡, 4-vinylpyridine; ◬, pyridine; ×, 4 carboxy-*n*-butylpyridine; ⊕, 4-cyanopyridine.

The average entropy changes resulting from the addition of pyridines and substituted pyridines to various iron(II) porphyrins are compared in Table II.

An analysis of variance of the data from which Table II is derived shows that there is a significant difference, at the $P < 0.001$ level, between the average entropies of the different Fe(II) porphyrins. This may reflect a difference in the change in solvation of the hemes, with the change in solvation being the greatest for diacetyldeuteroporphyriniron(II) and least for meso- and protoporphyriniron(II). The analysis of variance also shows that there is no significant difference at the $P < 0.05$ level between the average entropy of the different ligands. It may be seen from Figure 5 that the entropy changes which occur when pyridine, 4-carboxybutylpyridine, and 4-cyanopyridine are complexed with diacetyldeuteroporphyriniron(II) are much more positive than are to be expected. The possibility that π bonding is relatively weak in diacetyldeuteroporphyriniron(II) may be the reason for this.

Whatever the contribution from π bonding may be, the enthalpy changes attending ligand binding are much less favorable for this heme than for the others and the overall decrease in the rigidity of the complex may be sufficient explanation for the more favorable entropy changes.

Conclusions

Determination of the pyridine ligand equilibria of a series of iron(II) porphyrins over a range of temperatures has yielded data from which we have been able to assess the contributions of enthalpy and entropy changes to the free energies of ligand addition. There is an unexpectedly close relationship between ΔH and ΔS values for the ligand-binding reactions of these compounds (correlation coefficient 0.98) and this has two important consequences. Firstly, there is the compensatory effect of ΔS for the effect of ΔH on ΔG which makes free-energy data an unsatisfactory guide to the magnitude of the bond energies of these complexes. Secondly, the fact that the isoequilibrium temperature (340°K) is so little different from the temperatures at which these compounds are usually studied implies that a linear free-energy relationship is not likely to be found. Free-energy data thus give no reliable indication of the direction of the bond-energy changes from one compound to another. To the extent that pK_a data of pyridine ligands may be taken to indicate donor power, the pattern of enthalpy changes associated with attachment of substituted pyridines to these hemes can be explained only if π bonding, as well as σ bonding, contributes to the energy of the iron–nitrogen bond. This has been suggested before but in the earlier studies the free-energy changes alone were reported, a procedure which now appears unreliable because of the magnitude and range of the entropy changes which occur. Nevertheless, the conclusions about the dominance of σ bonding with ligands of high pK_a and of π bonding when the pK_a is low are confirmed by the enthalpy data. The effect of substituents on the heme is felt in the way in which they modify the relative importance of σ and π bonding of the various pyridine ligands complexed to a particular heme. This is most easily seen in the plots of ΔH against ligand pK_a for each heme compound.

---

(23) F. J. C. Rossotti in "Modern Coordination Chemistry," J. Lewis and R. C. Wilkins, Ed., Interscience Publishers, New York, N. Y., 1960, p 20.