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The Equilibria Between Various Ligands and a Ferric Metalloporphyrin in Aqueous Solutions

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The equilibrium constants between the ligands imidazole, pyridine, and histidine and the monomer Fe(III)–TPPS and the dimeric O–(Fe(III)–TPPS)$_2$ have been measured by spectrophotometric methods.

$$Fe(III)\text{–}TPPS + 2L \rightleftharpoons Fe(III)\text{–}TPPS[L]_2 \quad K_1$$

$$O\text{–}(Fe(III)\text{–}TPPS)_2 + 4L \rightleftharpoons 2Fe(III)\text{–}TPPS[L]_2 + 2OH^- \quad K_2$$

At 25 °C the values for K_1 are 1.7×10^6, 1.8×10^5, and 1.5×10^3 for imidazole, histidine, and pyridine, while the values of K_2 are 2.8×10^{-7} and 4.6×10^{-10} for imidazole and histidine.

Introduction

There have only been a few quantitative studies of the equilibria between various ligands and ferric metalloporphyrins [1–7] and very limited data have been obtained on well-behaved water-soluble porphyrins. This paper reports the measurement of the equilibria involved between the ligands pyridine, imidazole, and histidine, and the water-soluble porphyrin, tetra(*p*-sulfonato)phenylporphinatoiron(III) [Fe(III)–TPPS].

Experimental

Synthesis of the $Li_3Fe(III)$–TPPS

A slight modification of the method of Fleischer was employed in the synthesis of the water-soluble ironporphyrin complex [1]. Several grams of the sodium salt of TPPS was weighed out, dissolved in water and filtered, and then passed through a column of Dowex 50W × 8 cation exchange, lithium form to change the sodium salt to the lithium salt. A small excess (less than twofold) of ferrous chloride was added to the solution, and the resulting solution heated on a steam bath for one hour. The pH of the solution was kept above 6 during the heating. When the spectrum of the solution showed that no unconverted TPPS free base remained, the solution was passed through the cation exchange column in the lithium form to remove any excess ferrous iron. The solution was then concentrated by evaporating most of the solvent on a rotavapor, and the metalloporphyrin precipitated out with acetone. The precipitate was collected and oven dried and then Soxhletted with methanol. Another acetone precipitation followed by oven drying yielded the pure $Li_3Fe(III)$–TPPS used in this study. The purity was checked by comparison with the known extinction coefficients.

In studying the equilibria of various bases with the iron-porphyrin system, care was taken to be in the pH range where the iron complex is either in its monomeric form and the ligands are in their unprotonated forms. The literature values for the equilibrium constants for the Fe(III)–TPPS, pyridine, imidazole, and histidine were used to calculate appropriate pH regions to study the reactions.

$$2Fe(III)\text{–}TPPS \rightleftharpoons (Fe(III)\text{–}TPPS)_2\text{–}O + 2H^+ \quad K_D$$

$$LH^+ \rightleftharpoons L + H^+ \quad K_a$$

The value of $K_D = 0.79 \times 10^{-8}$ and the pK's of 5.25 (pyridine), 7.05 (imidazole), and 6.04 (histidine) were employed in our studies. It should be noted that the fraction of monomer to dimer in the Fe(III)–TPPS is concentration dependent. Figures 1 and 2 demonstrate the % of protonated ligand and % monomer calculated from the known equilibrium constants. At pH < 4 the ligands are all protonated and do not complex with the Fe(III)–TPPS. In the pH range of 5–8, where the unprotonated ligand species are dominant, the Fe(III)–TPPS monomer–dimer equilibrium takes place. One would like to study the simpler reactions of the ligands with either monomer, or ligands with dimer, but not with a mixture of the species. If we assume that a reaction can be studied in a pH region where at least 5% of the ligands are unprotonated, and at least 98% of the iron is in the form of one species (monomer or dimer), then one can calculate the ranges available for study.

Fe(III)–TPPS conc. *M*	pH range where study of monomer–base feasible: Pyridine	Histidine	Imidazole	pH range where study of dimer–base reaction feasible (all ligands)
1×10^{-4}	4.0–4.5	–	–	>7.7
1×10^{-5}	4.0–5.0	4.8–5.5	–	>8.2
1×10^{-6}	4.0–5.8	4.8–6.0	5.7–6.0	>8.7

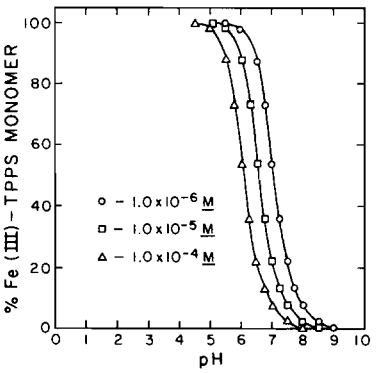

Figure 1. The percent of Fe(III)-TPPS monomer as a function of pH for (○) 1×10^{-6} *M*, (□) 1×10^{-5} *M*, (△) 1×10^{-4} *M* total iron porphyrin concentration.

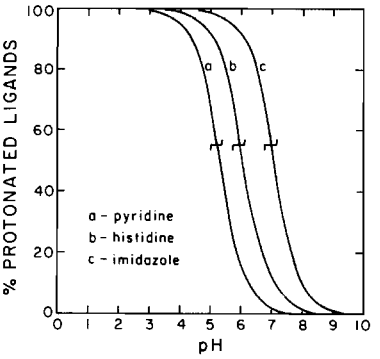

Figure 2. Calculated percent of ligand protonated as a function of pH for (a) pyridine, (b) histidine, and (c) imidazole.

In each of the three organic bases studied, addition of base to an aqueous solution of Fe(III)–TPPS of suitable pH resulted in formation of a complex; the same complex was formed upon addition of ligand to either monomer or dimer. This indicates that the complexes formed are an adduct of the monomeric Fe(III)–TPPS with the particular organic base, as complexation can take place at pH's in which only the monomer exists.

Formation of the complexes was detected by spectrophotometric measurement. Complexation caused a color change from yellow–brown of the monomer or yellow–green of the dimer to a distinct orange color. Examination of the spectra showed that for iron concentrations in the range of 10^{-5}–10^{-4} *M*, the visible region between 450 and 650 nm was suitable for study, while in the concentration range 10^{-6}–10^{-5} *M* the Soret region (375–450 nm) may be used.

Experimental Procedure

Buffer solutions of the desired pH were prepared according to the recipes given in the Handbook of Chemistry and Physics (50th Ed., D102). Stock solutions of Fe(III)–TPPS, imidazole, and histidine were prepared by weighing out the pure compounds and dissolving them in the buffer solutions. Imidazole was Aldrich Co. 99%, L-histidine was Aldrich Co. 98%. Stock solutions of pyridine were prepared by pipetting appropriate volumes of reagent grade pyridine and diluting with buffer solution. The pH of the stock solutions was checked on a Beckman Century SS pH meter. A series of solutions having a constant iron concentration and variable base concentrations were prepared, and the appropriate spectral region scanned. All spectral measurements were made on a Beckman Acta III spectrophotometer at 25 °C.

Analysis of the Data

In all cases, addition of organic base eventually produced a spectrum which showed no alteration upon further addition of base. The "limiting" spectrum was taken to be the spectrum of the pure ligated complex. Good isosbestic points were obtained in most of the spectral titrations, indicating a two species equilibrium. Absorbance data at suitable wavelengths were treated in the following manner. If A is the observed absorbance of a solution of some ligand concentration, A_o is the absorbance of the pure unligated iron–TPPS species, and A_∞ is the absorbance of the pure complex, then the fraction of the total iron (Fe_{TOT}) in the form of the complex is given by

$$fc = \frac{A - A_o}{A_\infty - A_o}$$

Subsequent treatment depends on whether complexation of the monomer or the dimer is being studied. In the case of the monomer, the reaction is Fe(III)–TPPS + nL $\underset{}{\overset{K_1}{\rightleftharpoons}}$ Fe(III)–TPPS$(L)_n$. If fc = fraction of iron in form of complex, then [Fe(III)–TPPS$(L)_n$] = fc Fe_{TOT} and [Fe(III)–TPPS] = (1 – fc)Fe_{TOT} where Fe_{TOT} is the total iron porphyrin

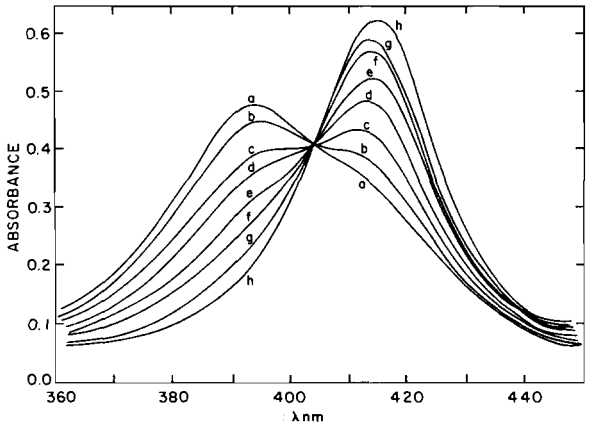

Figure 3. Absorption Spectra of Fe(III)-TPPS plus Imidazole at pH = 6.0 and Fe(TOT) = 7.5 × 10^{-6} *M*. Imidazole concentrations (a) 0, (b) 2.4 × 10^{-3}, (c) 4.8 × 10^{-3}, (d) 7.2 × 10^{-3}, (e) 9.6 × 10^{-3}, (f) 14.4 × 10^{-3}, (g) 24.0 × 10^{-3}, (h) 96.0 × 10^{-3} *M*.

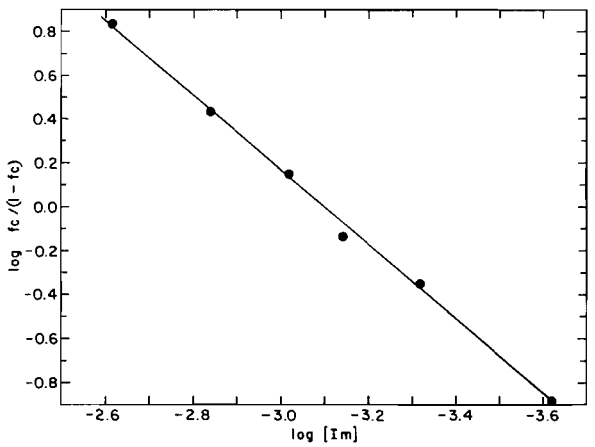

Figure 4. A plot of log [fc/(1 – fc)] *versus* log [Im] for the spectral titration of Fe(III)-TPPS by imidazole.

concentration in the solution. Substituting into the equilibrium expression leads to

$$\frac{fc}{1-fc} = K_{eq}[L]^n$$

Hence a plot of log[fc/(1 – fc)] *versus* log [L] should yield a straight line of slope n and intercept $-\log K_{eq}/n$. One can also plot fc/(1 – fc) *versus* $[L]^n$; if a straight line is obtained, this verifies the value of n, and the slope of the line equals the equilibrium constant.

The treatment differs slightly for the case of the dimer. Since the experiments indicate the same complex is formed as in the case of the monomer the reaction can be written

$$O\text{–(Fe(III)–TPPS)}_2 + 2nL + H_2O \overset{K_2}{\rightleftharpoons} 2Fe(III)\text{–TPPS(L)}_n + 2\,OH^-$$

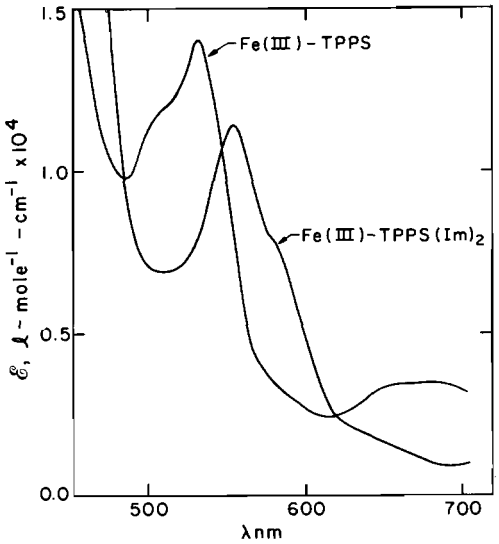

Figure 5. Visible absorption spectra for Fe(III)-TPPS and Fe(III)-TPPS$(Im)_2$.

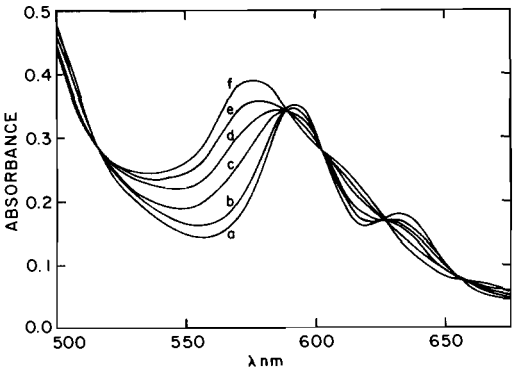

Figure 6. Spectral titration of the O-(Fe(III)-TPPS)$_2$ with imidazole at pH 9.8. Imidazole concentrations are (a) 0, (b) 1.0 × 10^{-2}, (c) 2.0 × 10^{-2}, (d) 3.0 × 10^{-2}, (e) 4.0 × 10^{-2}, and (f) 10.0 × 10^{-2} *M*.

It can be shown that

$$\frac{fc^2}{1-fc} = \frac{K_{eq}}{2Fe_{TOT}[OH^-]^2}[L]^{2n}$$

A plot of $\log[fc^2/(1-fc)]$ *versus* log[L] should yield a straight line with slope 2n and an intercept equal to $K_{eq}/2Fe_{TOT}[OH^-]^2$. Since the Fe_{TOT} and OH^- are known, the equilibrium constant can be derived from the plot as well as the number of ligands bound to the iron complex.

Results

Results for Imidazole

The complexation of imidazole with the Fe(III)–TPPS was carried out at pH = 6.0 and metalloporphy-

rin concentration of 7.5×10^{-6} *M*. The results of the spectral titration are shown in Figure 3. The concentration of unprotonated imidazole at pH 6.0 was calculated assuming the pK_b for imidazole of 9.1×10^{-8}. A plot of log[f/(1 − f)] *versus* log [Im] is a straight line with slope of 1.8 and 1.9 for plots of data at λ of 393 and 415 nm, respectively, and equilibrium constants of 1.6×10^6 and 1.8×10^6 respectively. See Figure 4 for such a plot. Figure 5 shows the visible spectra of the Fe(III)–TPPS monomer and the imidazole adduct Fe(III)–TPPS$(Im)_2$.

Addition of imidazole to solutions of O–(Fe(III)–TPPS$)_2$ dimer yields the same complex produced by addition to the monomer; if this complex is the bis adduct, as indicated by the results for the monomer, then the net reaction for the dimer would be

$$\text{O–(Fe(III)–TPPS)}_2 + 4\text{Im} + \text{H}_2\text{O} \overset{K_2}{\rightleftharpoons} 2\text{Fe(III)–TPPS(Im)}_2 + 2\text{OH}^-$$

If this equation is correct, then the reaction should be repressed at high pH. This was verified experimentally. Solutions 4×10^{-5} *M* in Fe and 0.03 *M* in imidazole were examined at various pH's. At pH 11.5 the iron existed mainly as the O–(Fe(III)–TPPS$)_2$ dimer, whereas at pH 9–10 over 50% of the iron was in the form of the imidazole complex. There should also be a dependence on the iron concentration, and this too was observed. No such dependence was found for the monomer–imidazole equilibrium.

The dimer-imidazole equilibrium was studied at several pH's; the best results were obtained at pH 9.5 - 10.0. The equilibrium could be studied in the visible region; Figure 6 gives such a spectral titration. A plot of log $[fc^2/(1 - fc)]$ yields a straight line with slope 3.8 and intercept −1.52 at pH 9.5. This yields n = 2 and an equilibrium constant of 4.2×10^{-7}. The titration at pH 9.8 gave a slope of 4.2 and an equilibrium constant of 1.4×10^{-7}. This is reasonable agreement considering the uncertainty in pH and that the calculation depends on $[OH^-]^2$.

Results for Pyridine

Since pyridine has a pK of 5.25 it is possible to study the reaction at lower pH and over a wider range of iron concentrations than is possible with imidazole. The pH selected for the studies reported here is 5.0, a pH at which pyridine is 36% unprotonated and at which the Fe(III)–TPPS is entirely in the form of the monomer. The equilibrium was examined in both the Soret and visible regions and the absorbance data at 395 and 415 nm were treated as previously described. Plots of log [fc/(1 − fc)] *versus* log[py] yielded straight lines with slope 2.0 and an equilibrium constant of 1.5×10^3 for the reaction.

Pyridine does not react with the dimer to any appreciable extent. A solution in neat pyridine is very similar to the dimer. This implies a very small K for the dimer–imidazole equilibrium.

TABLE I. Reactions of Fe(III)–TPPS with Various Ligands at 25 °C.

Ligand	K_1	K_2
Imidazole	1.7×10^6	2.8×10^{-7}
Histidine	1.8×10^5	4.6×10^{-10}
Pyridine	1.5×10^3	[a]

[a]Too small to be observed.

Results for Histidine

The behavior of histidine is analogous to that of imidazole; it forms a complex with both the monomer and the dimer. The data were treated as before and the results of this and the other equilibrium studies are given in Table I.

Discussion

The equilibrium constants that were measured are listed in Table I. There is an internal check of these equilibria constants since K_D has been independently measured. The following relationship holds

$$K_D = \frac{K_1^2 K_w^2}{K_2}$$

where K_1 is the monomer–imidazole equilibrium constant, K_2 is the dimer–imidazole equilibrium constant, while K_w is the hydrolysis constant of water which is taken as 1.0×10^{-14}. For the imidazole case, substitution into this equation leads to a calculated $K_D = 1.0 \times 10^{-9}$ while the measured value of K_D is 7.9×10^{-9}. Considering the sensitivity of these measurements to pH the agreement is reasonable. The calculated value of K_D for the histidine case is 7.0×10^{-9} which is in better agreement than the imidazole case. We can also calculate the value of K_2 for the pyridine case and obtain a value of 2.8×10^{-14} which is too small to be observed under the conditions of our experiment.

The nonexistence of any substantial amount of monoimidazole complex has been previously established in the Fe(III)–TPP study in nonaqueous solvents such as methylene chloride [2, 3]. Thus in solution the predominant species in the iron(III) case are the Fe(III)–TPPS(H_2O) and the Fe(III)–TPPS-$(Lig)_2$ with little Fe(III)–TPPS(H_2O)(L) or Fe(III)–TPPS(L) present. The low affinity of one ligand is probably related to the cross-over from high-to-low spin and the relative affinity of low-spin Fe(III) for the ligands compared to high-spin Fe(III).

There is the expected correlation of the equilibrium constants with basicity; the strongest base imidazole has the largest K and the weakest base pyridine has the smallest equilibrium constant. The equilibrium constants of these bases with hemin C in aqueous solutions were very similar to our values [8]. It is both interesting and rather unusual that the equilibrium constant with hemin C and our synthetic porphyrin are similar (1.1×10^5 compared to 1.7×10^5) despite the rather large difference in the substituents on the porphyrin skeleton.

Acknowledgment

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