

The Incorporation of Zinc Ion into a Synthetic Water-Soluble Porphyrin¹

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Abstract: The synthesis of a water-soluble porphyrin, $C_{38}H_{54}N_{12}O_2 \cdot 8H_2O$, is described. Kinetics of the zinc incorporation of the porphyrin are reported for aqueous solutions. The rate law at fixed pH (6.23) and 30° is $d[ZnP]/dt = (14.2 M^{-1} \text{ min}^{-1})[Zn^{2+}][H_2P]$. The reaction is reversible, and the first-order rate constant for zinc replacement is $1.7 \times 10^{-4} \text{ min}^{-1}$. The forward reaction is shown to be catalyzed by pyridine, 2-, 3-, and 4-methylpyridines, imidazole, and methylimidazole. Of these, imidazole shows the greatest catalysis, and it also catalyzes the reverse reaction. There is no catalysis by the sterically hindered nitrogen base collidine which serves as buffer for the rate experiments. The activation energy measured for the uncatalyzed zinc incorporation is 11.7 kcal/mole, and 12.3 kcal/mole for the pyridine-catalyzed path indicating a preequilibrium having favorable entropy. Formation and absorption spectra of metalloporphyrins of Ag(II), Sn(IV), Cu(II), In(III), and Mn(II) are also described.

The study of metal ion incorporation into porphyrins represents an interesting extension of complex ion substitution reactions to biologically important reactions. A principal obstacle to the direct extension is the fact that most porphyrins are not sufficiently soluble in aqueous solution. Attempts to remedy the situation involve work in methanol solution,³ in aqueous detergent mixtures,⁴ and in aqueous pyridine⁵ and use of the synthetic porphyrin tetrapyridylporphine which is water soluble at low pH.⁶ In order to remove the restrictions imposed by each of these approaches, a new water-soluble porphyrin was synthesized. The present study reports the synthesis and the incorporation of several metal ions, as well as a kinetic study of the incorporation of Zn(II) in aqueous solution.

The starting point for the preparation of the water-soluble porphyrin was the reaction of ethylenediamine with protoporphyrin IX dimethyl ester described by Fleischer and Wang.⁷ The reaction was found to yield variable results and gave mixed products, a portion of which was water soluble. A homogeneous product was obtained by raising the temperature and refluxing protoporphyrin IX dimethyl ester for 24 hr in a large excess of redistilled ethylenediamine with added ethylenediammonium chloride. After chromatographic purification the product remained water soluble at pH as high as 8.

Experimental Section

Synthesis. Protoporphyrin IX dimethyl ester (1 g) was refluxed for 24 hr with redistilled ethylenediamine (500 ml) and ethylenediammonium chloride (2 g). The solution was then evaporated to dryness on a rotary evaporator under vacuum. The product was completely soluble in water. Chloride was removed from the

product by column chromatography on Dowex 50-WX2 cation-exchange resin. The column was washed with a water-ethylenediamine gradient. The porphyrin moved as a band at 75 vol % ethylenediamine. Ethylenediamine was then removed by vacuum distillation. Drying under vacuum over phosphoric oxide gave a product of formula $C_{38}H_{54}N_{12}O_2 \cdot 8H_2O$. *Anal.* Calcd: C, 53.39; H, 8.20; N, 19.67; O, 18.74. Found: C, 53.24; H, 8.27; N, 19.93; O, 18.56.

Characterization. Chemical argument suggests that the methyl propionate side chains should react with ethylenediamine to give the corresponding amide. The infrared spectrum (potassium bromide disk) shows the presence of an amide carbonyl at 1636 cm^{-1} and the absence of an ester carbonyl band.

Further comparison of the infrared spectrum with that of protoporphyrin IX dimethyl ester shows that the product had lost a peak at 985 cm^{-1} . This peak is commonly ascribed to a vinyl group.⁸

The nmr spectrum of the porphyrin is very complex and shows much broadening of peaks due to nitrogen-hydrogen interactions. A computer-averaged transient nmr spectrum showed the complete absence of vinyl protons in the region τ 3-5, where they would be expected to occur.^{9,10}

That the reaction was not the simple removal of vinyl groups as in the resorcinol melt reaction of Schumm¹¹ was shown by an experiment in which deuteroporphyrin IX dimethyl ester (identical with protoporphyrin IX dimethyl ester except that the vinyl groups in the 2 and 4 positions have been replaced by hydrogens) was refluxed with ethylenediamine plus ethylenediammonium chloride. Although reactions occurred, the product was not that obtained from the protoporphyrin IX dimethyl ester reaction as shown by the fact that it was not water soluble.

Apparently, with protoporphyrin IX dimethyl ester reaction occurred at the vinyl groups so as to cause the product to be water soluble. Replacement of the vinyl groups by $-NH-CH_2-CH_2-NH_2$ gives the observed empirical formula.

The visible absorption spectrum of the product is qualitatively similar to that of other porphyrins. The spectrum is summarized in Table I and shown later in Figure 3. It should be noted that the extinction coefficients are lower than those of protoporphyrin and most other porphyrins.¹²

However, it should be noted that 2,4-diacetyldeuteroporphyrin IX dimethyl ester also has extinction coefficients well below those of other porphyrins. Apparently ring substitution can greatly lower the extinction coefficients, possibly due to removal of electron density from the ring system by electron-withdrawing substituents.

Kinetic Measurements. Free porphyrin has the maximum of the

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Table I. Porphyrin Extinction Coefficients

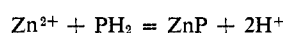
| Peak | λ_{\max} | ϵ_{\max} |
|------|------------------|-------------------|
| I | 621 | 980 |
| II | 569 | 2,020 |
| III | 535 | 2,400 |
| IV | 504 | 3,310 |
| V | 394 | 35,000 |

Soret band at 394 $m\mu$ and the difference spectrum has a maximum at 406 $m\mu$. Buffer (2 ml) was placed in a cuvette, and porphyrin powder added. The optical density was read at 394 $m\mu$, and the system allowed to equilibrate thermally for 5 min. A zinc solution in collidine perchlorate (1 ml) which had been equilibrated at 30° was pipetted in, and the increase in optical density at 406 $m\mu$ with time was monitored using a Gilford 2000 spectrophotometer with thermostated cell compartments.

Results

In order to show that the porphyrin does indeed form true solution in water, Beer's law experiments were run for concentrations of 4×10^{-3} to 7×10^{-6} *M*. For the entire range the optical density was found to be directly proportional to concentrations. Figure 1 shows results for both a peak maximum (504 $m\mu$) and a minimum (475 $m\mu$).

For the over-all reaction



a variety of kinetic measurements were made at 30° and a pH of 6.23 (0.20 *M* collidine perchlorate-2,4,6-trimethylpyridine).¹³ The initial porphyrin species at this pH are designated PH_2 and the product metalloporphyrin, MP.

In all experiments porphyrin was in limiting concentration and initial (first-order) rates were observed. Experiments were run to determine the dependence of rate on porphyrin concentration. For a series of constant zinc concentrations 5.13×10^{-3} to 3.42×10^{-4} *M*, strict linearity of rate with porphyrin concentration was observed in the range of porphyrin concentrations 4.4×10^{-6} to 4.4×10^{-5} *M*.

The dependence of rate on Zn^{2+} (Figure 2) concentration was found from these experiments to be linear with an intercept at the origin showing that the reaction is first order in zinc as well as porphyrin and therefore second order over-all. Figure 2 is a plot of $(\Delta/\Delta P)(d[\text{MP}]/dt)$ vs. $[\text{Zn}^{2+}]$, and each point required at least eight separate initial rate determinations with porphyrin concentrations ranging between 4.4×10^{-6} and 4.4×10^{-5} *M* at constant zinc. The data fit the second-order rate law

$$\frac{d[\text{MP}]}{dt} = (14.2 \text{ M}^{-1} \text{ min}^{-1})[\text{Zn}^{2+}][\text{H}_2\text{P}]$$

Direct second-order plots were not obtained because, as discussed below, there is significant back-reaction.

To see whether a visibly detectable concentration of an intermediate is formed during the reaction, a series of spectra was taken during the course of the reaction. These are displayed in Figure 3. The spectra show two isosbestic points, and there is a third one at 394 $m\mu$ indicating that a detectable concentration of intermediate is not present in the reaction mixture. To show that

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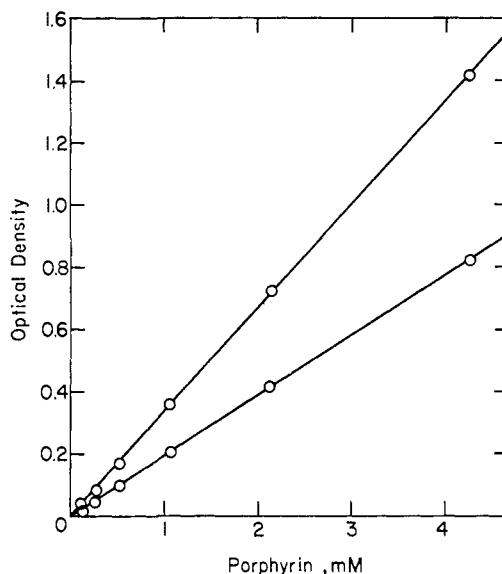


Figure 1. Beer's law plot for water-soluble porphyrin: upper curve for 503- $m\mu$ maximum; lower curve for 475- $m\mu$ minimum.

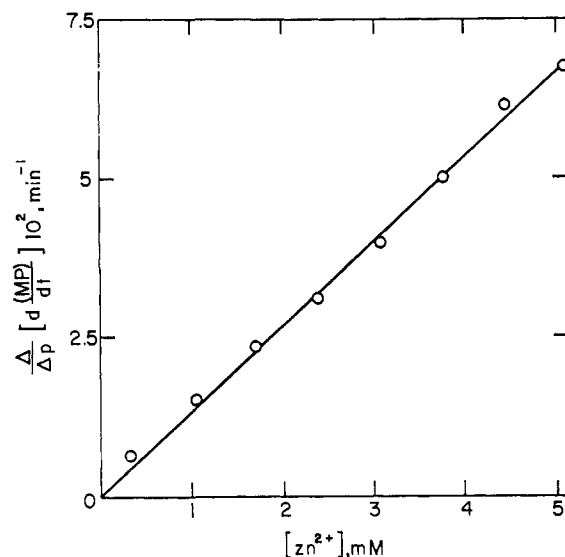


Figure 2. Zinc dependence of rate of metal incorporation. Each point summarizes eight separate rate determinations for porphyrin concentrations varying from 4.4×10^{-6} to 4.4×10^{-5} *M*.

there is not an initial spectral change, a spectrum was run immediately after mixing at 5°. A difference spectrum run vs. the free porphyrin failed to show any significant features.

In order to determine whether the reverse reaction (zinc removal) is significant, zinc porphyrin was dissolved in buffer and the rate of decrease of OD at 406 $m\mu$ followed. The initial rate found corresponds to a first-order specific rate of $1.7 \times 10^{-4} \text{ min}^{-1}$. The equilibrium constant for the zinc incorporation at fixed pH (6.23) can be approximated by dividing the second-order forward rate ($14.2 \text{ M}^{-1} \text{ min}^{-1}$) by the reverse rate ($1.7 \times 10^{-4} \text{ min}^{-1}$) which gives $8.4 \times 10^4 \text{ M}^{-1}$.

Imidazole, along with other aromatic nitrogen bases, was found to catalyze the zinc incorporation reaction. The catalysis in each case was found to be first order in nitrogen base so that an extended rate law can be written

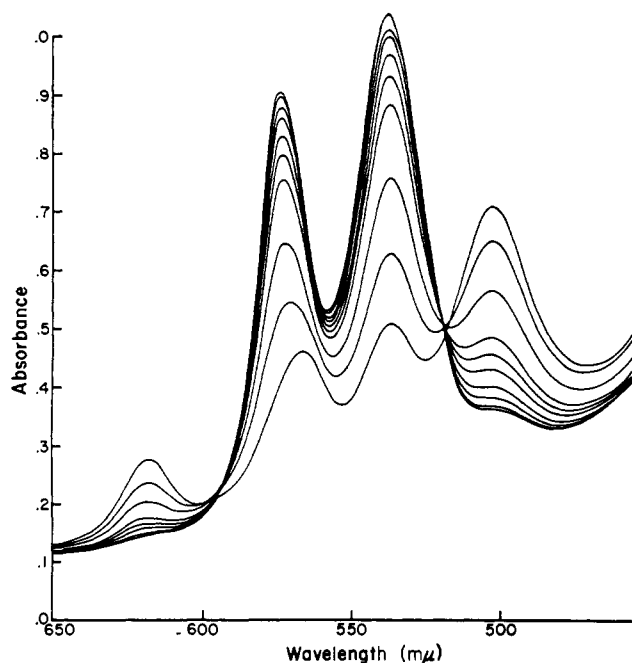


Figure 3. Spectral changes of porphyrin during zinc incorporation

$$\frac{d[\text{ZnP}]}{dt} = k[\text{Zn}][\text{PH}_2] + k'[\text{Zn}][\text{PH}_2][\text{B}]$$

Values of the third-order rate constant k' for a variety of bases are given in Table II. The experiments with nitrogen base catalysis were run at a fixed pH (6.23)

Table II. Catalytic Rate Constants

| Base | Concn range, M | k' , $M^{-2} \text{min}^{-1}$ |
|------------------|----------------------|---------------------------------|
| Pyridine | 0.4×10^{-2} | 1,350 |
| 2-Methylpyridine | 0.4×10^{-2} | 6,980 |
| 3-Methylpyridine | 0.4×10^{-2} | 8,280 |
| 4-Methylpyridine | 0.4×10^{-2} | 10,200 |
| Methylimidazole | 0.9×10^{-5} | 32,200 |
| Imidazole | 0.9×10^{-5} | 32,100 |

controlled by the buffer collidine perchlorate which does not catalyze the reaction. For the catalyzed rate, experiments showed that the dependence on zinc remains first order as for the uncatalyzed rate. It was also found that imidazole catalyzed the rate of the reverse reaction.

Because it was not possible to determine the various dissociation constants of the porphyrin and obtain equilibrium concentrations of the various possible species at various pH values, a systematic dependence of rate on pH was not attempted. Measurements were, however, made in the pH range 5.7–7.1 by changing the buffer ratio. These experiments showed a general increase of rate with increasing pH. The increase is moderate up to 6.5 (40% between 5.7 and 6.5), but above this it increases greatly (greater than a factor of 2 between 6.5 and 7.1).

In order to assess the activation parameters, kinetic experiments were performed in the temperature range 23–48° for both the uncatalyzed and the pyridine-catalyzed reaction. The results are plotted in Figure 4. It should be noted that the buffer ratio was fixed for all

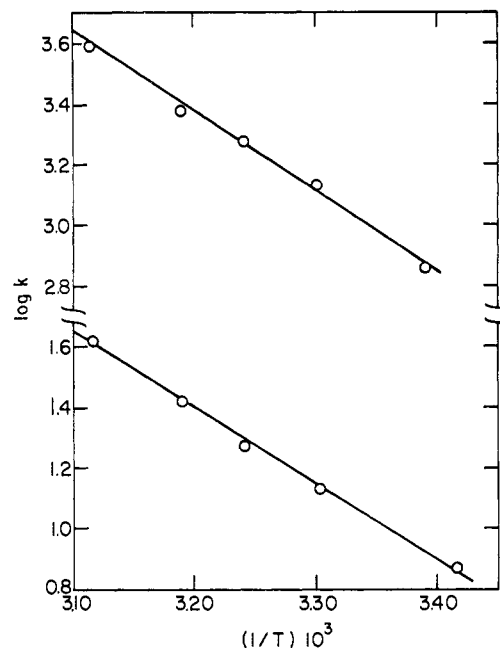


Figure 4. Change of rate constants with temperature: upper curve for pyridine catalysis; lower curve for uncatalyzed rate.

experiments so as to give pH 6.23 at 30°. The empirical activation energies obtained for the two paths are 11.7 kcal/mole for the uncatalyzed path and 12.3 kcal/mole for the catalyzed path.

In addition to zinc, other metal cations were treated with aqueous solutions of the porphyrin. With silver nitrate in acetate buffer at pH 4.9, an interesting reaction occurred even in the absence of light. Within a few hours of mixing, the reaction vessel became coated with a silver mirror and the solution turned cherry red. ESR spectra of the resulting metalloporphyrin solution were consistent with the product being Ag(II). A similar reaction was carried out with stannous chloride solution. Again reaction occurred within hours to produce a reddish solution plus some precipitate. The solution was chromatographed on Dowex 50-WX2, using ethylenediamine as eluent. Removal of the solvent by vacuum distillation produced a purple solid whose Mössbauer spectrum showed it to contain Sn(IV). Similar isolation procedures were successfully used for metalloporphyrins which formed with solutions containing Cu(II) (in seconds), Co(II) (in days), In(III) (in days when catalyzed by imidazole), and Mn(II) (in weeks when catalyzed by imidazole). The absorption spectra of these products are summarized in Table III. The me-

Table III. Metalloporphyrin Spectral Maxima (mμ)

| Metal | Soret | Other maxima | |
|-------|-------|--------------|-----|
| Zn | 406 | 539 | 576 |
| Ag | 390 | 544 | 565 |
| Sn | 402 | 537 | 573 |
| Cu | 382 | 530 | 565 |
| Co | 418 | 528 | 560 |
| In | 401 | 528 | 575 |
| Mn | 365 | 538 | 575 |

talloporphyrins and their formation are being further characterized and will be the subject of future publications.

Discussion

In the kinetics of the zinc incorporation reaction for the water-soluble porphyrin, the nitrogen-base-catalyzed term is of considerable interest. The catalysis is similar to that observed for magnesium ion in methanol and acetone solutions,³ indicating that the catalysis is a general phenomenon which could be of biological importance. In understanding the mechanism of the catalysis, of significance is the fact that collidine does not catalyze the reaction whereas the closely related cyclic nitrogen compounds of comparable base strength do catalyze the reaction. If the catalysis is of the general base type there should be a Brønsted correlation between catalytic rate and base strength. There is a reasonable correlation except for collidine. The obvious difference between collidine and the other bases is the fact that it contains two methyl groups *ortho* to the ring nitrogen. Whereas these groups apparently offer but slight steric hindrance to addition of a proton so that collidine has a base strength comparable to that of pyridine, the steric hindrance for a cation such as Zn^{2+} must be quite significant. Thus the failure of collidine to catalyze the reaction implies that the nitrogen base coordinates to Zn^{2+} during the course of the reaction. Confirmatory

evidence comes from studies which show that, under the conditions of the kinetic experiments, Zn^{2+} and the nitrogen bases do indeed associate.^{14,15}

Finally, the fact that the empirical activation energy of the pyridine catalyzed path exceeds that for the uncatalyzed reaction is noteworthy. The situation is similar to that observed for the magnesium replacement from deuteroporphyrin ester.¹⁶ In both cases, the observation must indicate the existence of preequilibria having favorable entropies. Use of the water-soluble porphyrin makes possible relaxation studies, now underway, which should prove useful in understanding the preequilibria.

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Thermodynamic and Infrared Studies of Tertiary Amine Oxides with Bis(2,4-pentanedionato)oxovanadium(IV)

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Abstract: The donor properties of a series of substituted pyridine N-oxide bases toward the reference acid bis(2,4-pentanedionato)oxovanadium(IV) have been studied. Heats of reaction have been determined calorimetrically and, in the case of the 4-substituted pyridine N-oxides, correlate well with σ_{PyNO} . The change of the vanadium-vanadyl oxygen stretching frequency ($\Delta\bar{\nu}_{V=O}$) and the vanadium-2,4-pentanedionate stretching frequency ($\Delta\bar{\nu}_{V-O}$) upon coordination were studied and were also found to correlate well with enthalpies of reaction. Substitution of alkyl groups in other than the 4 position of the pyridine N-oxide ring causes a decrease in the effective basicity toward the reference acid, $VO(acac)_2$. The usefulness of $VO(acac)_2$ as a reference acid is discussed. The system is extended to the tertiary amine oxides *p*-bromo-N,N-dimethylaniline N-oxide and trimethylamine N-oxide.

The use of the coordination compound bis(2,4-pentanedionato)oxovanadium(IV), $VO(acac)_2$, as a reference acid has been examined.^{2,3} The heats of reaction of a variety of nitrogen and oxygen donors interacting with $VO(acac)_2$ were determined, and it was found that enthalpy values did not correlate well with the pK_a 's of the bases.² Selbin, *et al.*,³ studied the decrease of the vanadium-vanadyl oxygen stretching frequency ($\Delta\bar{\nu}_{V=O}$) in the infrared upon coordination to a large variety of bases. It was concluded that there was no

general correlation between $\Delta\bar{\nu}_{V=O}$ and the pK_a values of the bases. Shifts in the visible absorption spectrum of $VO(acac)_2$ have been used as parameters for ordering solvent basicity.⁴

The structure of $VO(acac)_2$ has been established as being a slightly rectangular pyramid⁵ with the vanadium near the center of gravity of the pyramid rather than at the base. The sixth position in the coordination sphere of $VO(acac)_2$ has been shown to be solvated in solution.^{6,7} As a result of the structure of $VO(acac)_2$, the steric effects of the base can be quite important to

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