Complex Formation between Porphyrins and Metal Ions[1]

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Abstract: Certain metal salts, *e.g.*, $FeCl_3$, $SnCl_2 \cdot 2H_2O$, $CoCl_2$, $CuBr_2$, etc., when added to chloroform solutions of porphyrin esters, cause characteristic changes in the visible absorption spectra of the porphyrins. The resulting spectra are generally similar to, but can be distinguished from, the spectrum of the same porphyrin in the HCl-dication form. The species so formed have never been adequately characterized. The problem has been studied by Mössbauer spectroscopy, which concerns the central metal atom, and by electronic absorption measurements on the porphyrin ligands as well as by direct chemical analysis. The results can be interpreted in terms of salt formation between the porphyrin dication and the anion of the solvated metal. The particular anion paired with a porphyrin dication has a pronounced effect on the visible spectrum of the dication in anhydrous solvents, while in aqueous solvents the anion has little effect.

Three groups of metal tetrapyrroles are of importance in biological systems, the hemes, the chlorophylls, and the cobalamins, which contain as central metal ions iron, magnesium, and cobalt, respectively.

We are interested in the biosynthesis of functional metal tetrapyrroles, and these interests have been focused upon the metal incorporation reaction.

The generalized reaction for metal incorporation into a porphyrin can be written

$$M^{2+} + H_2P \longrightarrow PM + 2H^+$$

where H_2P represents the neutral porphyrin molecule with two protons attached to the pyrrole nitrogen atoms.

Discussions of the mechanism of metal incorporation usually present two alternatives.[3] These are: (i) dissociation, which requires that the porphyrin be transformed to the monoanion or dianion before reacting with the metal

$$H_2P \longrightarrow HP^- + H^+ \longrightarrow P^{2-} + H^+$$
$$HP^- + M^{2+} \longrightarrow PM + H^+$$

or

$$P^{2-} + M^{2+} \longrightarrow PM$$

and (ii) displacement, requiring that a metal–porphyrin complex forms prior to the loss of the two protons

$$M^{2+} + H_2P \longrightarrow H_2PM^{2+} \longrightarrow PM + 2H^+$$

Fleischer and Wang[4] reported a type of metal–porphyrin complex which seemed to shed additional light on the incorporation reaction. This complex had the unique property, for metalloporphyrins, of being unstable in the presence of water. The authors designated the species as a "sitting-atop" complex, and proposed that the metal in the complex would appear to meet the requirements for the metal–porphyrin intermediate in the displacement mechanism for incorporation.

The sitting-atop proposal of Fleischer and Wang has received criticism, and experimental evidence which would clearly define the situation is lacking. This paper describes an investigation undertaken to establish definitely the nature of these complexes.

Results

The physical properties of the sitting-atop complex closely resemble those of the dicationic porphyrin esters, H_4P^{2+}. In order to establish whether the product of the action of metal ions is in fact the dication, or a metal–porphyrin complex, H_2PM^{2+}, the porphyrin esters were titrated with both acids and metal salts in organic solvents.

In anhydrous chloroform the neutral porphyrin was converted directly to the diprotonated species by both HCl and CF_3COOH. The single set of isosbestic points clearly rules out the formation of the monocationic porphyrin as an intermediate (see Figure 1). In absolute ethanol, however, protonation of the porphyrin took place stepwise and two sets of isosbestic points were obtained. The spectrum of the intermediate titration product is that reported for the porphyrin monocation.[5,6]

Formation of the proposed metal–porphyrin complexes was studied by titrating the neutral porphyrin esters in anhydrous chloroform with $MgClO_4$, $CuBr_2$, $FeCl_3$, and $SnCl_2 \cdot 2H_2O$ all in organic solvents. No reaction was observed when $MgClO_4$ was the titrant. Each of the other metals caused the four-banded spectrum of the neutral porphyrin to shift to a two-banded spectrum. This two-banded spectrum, that of the so-called sitting-atop complex, closely resembles the HCl-dication spectrum. However, the wavelength of maximum absorption differs for each metal, and none correspond to the wavelength of maximum absorption of the HCl-dication. We found further, that the porphyrin HCl-dication could be directly converted to the apparent metal complex (see Figure 2). The conversion is not reversible. The fact that each metal causes the appearance of a species with a characteristic absorption spectrum is presumptive evidence that each metal forms a unique complex with the porphyrin.

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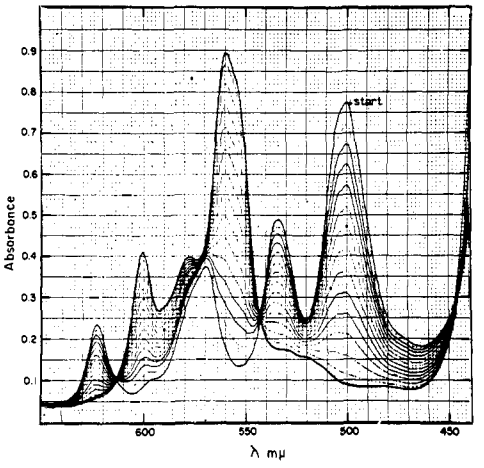

Figure 1. Reproduction of the spectrophotometric titration of 150 nmol of haematoporphyrin dimethyl ester in 2.65 ml of anhydrous chloroform. Each curve was measured following the addition of 2-μl. aliquots of 20 m*M* HCl in chloroform prepared as described in the text.

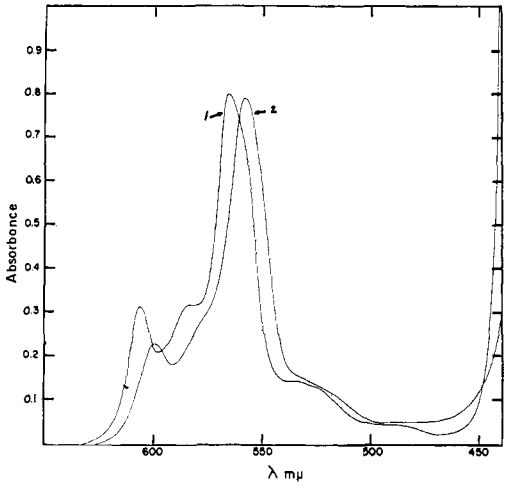

Figure 2. Curve 1, 150 nmol of uroporphyrin I octamethyl ester in 2.65 ml of anhydrous chloroform converted to the HCl-dication by the addition of HCl in chloroform prepared as described in the text. Curve 2, spectrum of the same sample following the addition of 600 nmol of $FeCl_3$ in chloroform.

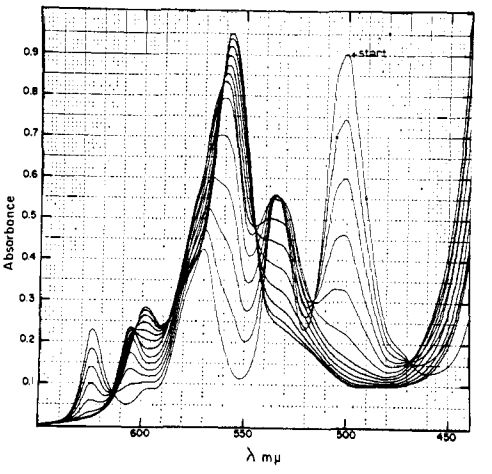

Figure 3. Reproduction of the spectrophotometric titration of 150 nmol of uroporphyrin I octamethyl ester in 2.65 ml of anhydrous chloroform. Each curve was measured following the addition of 2-μl. aliquots of 27.5 m*M* $FeCl_3$ (0.37 equiv) in chloroform.

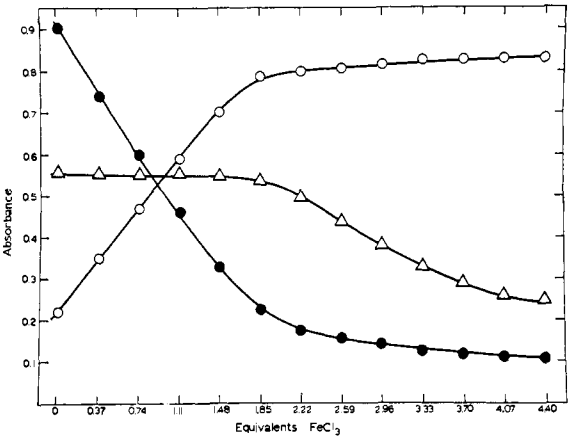

Figure 4. Spectrophotometric titration of uroporphyrin I octamethyl ester with $FeCl_3$ in chloroform. Absorbance at 562, ○, 534, △, and 503 mμ, ●, is plotted as a function of equivalents of $FeCl_3$ added to the reaction system.

The details of the interaction between porphyrin esters and metal salts in organic solvents were studied most extensively with $FeCl_3$ and $SnCl_2 \cdot 2H_2O$. Titrations of porphyrin esters with $FeCl_3$ reveal that complex formation takes place in two steps in contrast to titrations with HCl. Two distinct sets of isosbestic points form as the neutral porphyrin is first converted to a species with spectral characteristics of the monocation, and then as this intermediate is converted finally into a complex with spectral characteristics similar to the dication. (See Figure 3.)

The stoichiometry of the Fe^{3+}–porphyrin interaction was determined graphically from the change in absorption and the total added $FeCl_3$. Figure 4 shows absorbancy changes at three different wavelengths as a function of added $FeCl_3$. The neutral porphyrin was half-titrated to the intermediate complex at the point where the 562- and 503-mμ absorbancy lines cross (see Figure 4). The iron:porphyrin ratio at that stage of the titration was 0.96:1 (±5%). The intermediate complex was half-titrated to the final complex at the inflection point of the 534-mμ absorbancy line. The iron:porphyrin ratio at that stage of the titration was 2.65:1 (±12%).

Apparently the intermediate complex consists of one porphyrin and two iron atoms and the final complex consists of one porphyrin and four iron atoms. It is known that $FeCl_3$ exists as a dimer in poorly coordinating solvents like chloroform, and it appears that the iron participates in its interaction with the porphyrin as a dimer. These results are at variance with those reported by Fleischer and Wang[4] who found an iron:porphyrin ratio of 1. Differences in solvent composition in the two studies might account for this descrepancy. Since Fleischer and Wang did not indicate the solvent composition in their study, a comparison is not possible.

During the course of some of the titrations it became apparent that the solubility of some of the metal–porphyrin complexes differed markedly from both the neutral porphyrin and the porphyrin dication prepared with HCl. This was manifested by the appearance of turbidity toward the end of the titration, with the order of solubility: uroporphyrin > coproporphyrin > deu-

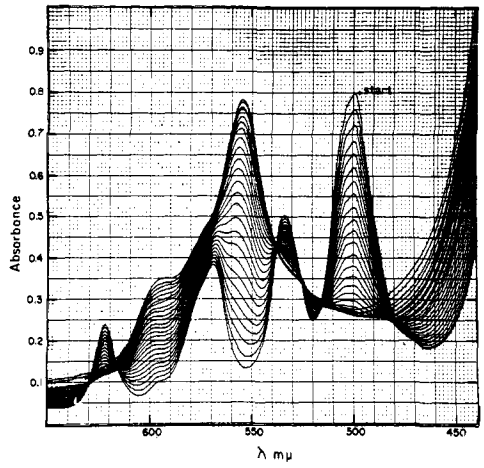

Figure 5. Reproduction of spectrophotometric titration of 150 nmol of haematoporphyrin dimethyl ester in 2.65 ml of anhydrous chloroform. Each curve was measured following the addition of 2-μl. aliquots of 30 m*M* $SnCl_2$ (0.40 equiv) prepared as described in the text.

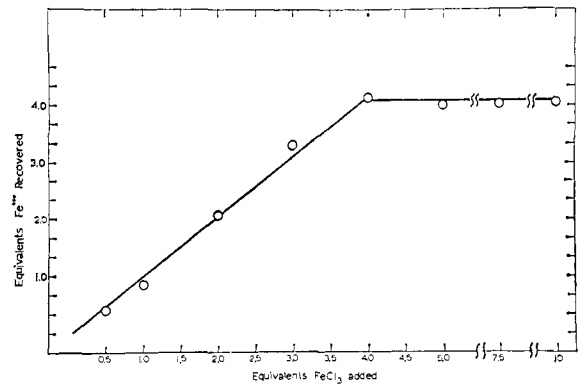

Figure 6. Graded amounts of $FeCl_3$ in chloroform were added to a series of 30-nmol samples of deuteroporphyrin dimethyl ester in anhydrous chloroform. After precipitation and washing as described in the text, the complex was analyzed for iron and porphyrin content. The equivalents of iron recovered in the complex is plotted against equivalents of iron added to each sample.

teroporphyrin > hematoporphyrin. Large differences in solubility were found at the two extremes.

The tin complexes of the porphyrin esters are considerably less soluble in chloroform than the corresponding iron complexes. A typical titration is shown in Figure 5. A single isosbestic point was formed during the early stages of the titration but after the tin:porphyrin ratio exceeded 2, turbidity became a problem. The shape of the spectrum, however, did not change on addition of more tin halide. Allowing for the turbidity-induced changes observed in Figure 5, it is apparent that the titration does not proceed through the formation of an intermediate as was the case during the iron titrations. The mean tin:porphyrin stoichiometry of the final product was $7 \pm 1:1$.

The metal titrated porphyrin esters were analyzed as follows: $FeCl_3$ was added to a series of anhydrous chloroform solutions containing a constant amount of deuteroporphyrin dimethyl ester. The iron–porphyrin complex was precipitated with benzene, centrifuged, and reprecipitated from benzene–chloroform. Analysis of the precipitate yielded the iron to porphyrin ratio (see Figure 6), which increased linearly with iron added below four where the deuteroporphyrin ester became limiting. The iron:porphyrin ratio then remained constant at $4 \pm 0.5:1$. The mirror experiment, where increments of deuteroporphyrin ester were added to a constant quantity of iron, yielded the identical ratio at saturation. A number of attempts were made to observe metal incorporation by prolonged heating of several preparations of the iron–porphyrin complex. No metalloporphyrin formation was observed.

At least two interpretations are possible. (1) The observed spectral difference between the porphyrin HCl-dication and the metal-containing complex could be due to a concentration effect. However, addition of a large excess of HCl to the preformed HCl-dication does not cause any significant change in the spectrum, and, likewise, addition of a large excess of metal ion does not cause any significant change in the spectrum of the metal–porphyrin complex. (2) A metal–porphyrin complex could be formed. The fact that an iron–porphyrin complex could actually be isolated supports this likelihood as do the titration data. The question, of course, remains as to the nature of the complex. How might two dimeric $FeCl_3$ molecules interact with a single porphyrin molecule?

The key lies in the observation that small amounts of ethanol are necessary to dissolve anhydrous $FeCl_3$ in pure chloroform. The ethanol apparently serves to solvolyze the metal, producing an anion

$$M_2X_6 + (1 + m)HOEt \longrightarrow [M_2X_{6-m}(1 + m)OEt]^- + (1 + m)H^+ + mX^-$$

In the presence of a porphyrin ester, the labilized or released hydrogen could protonate one of the pyrrolinine ring nitrogen atoms, with the solvolyzed metal anion acting to neutralize the charge. Two metal dimer anions would then associate with a dicationic porphyrin.

The particular gegenion associated with a porphyrin dication in chloroform has a pronounced effect on the visible spectrum of the dication. When small quantities of the strong acids CF_3COOH, HCl, and H_2SO_4 are added to uroporphyrin octamethyl ester in chloroform, the resultant absorption maxima of band II differ by as much as 9 mμ (554, 563, and 556, respectively). In aqueous solution where the ions are presumably independent, the maxima differ by less than 2 mμ (551, 552, and 551, respectively). In chloroform the absorption maximum of the iron–uroporphyrin complex is different from the HCl-dication by 8 mμ, of the same order of magnitude as the differences produced by the simple acid anions in this solvent.

Sn^{119m} Mössbauer spectra were studied in order to help elucidate the nature of the metal-containing gegenion and its interaction with the porphyrin. Because of the relative opacity of dilute chloroform solution to γ rays, the more γ-transparent solvent acetone was used. The spectrum of tin(II) chloride, examined in a solid matrix of acetone by rapidly freezing a dilute solution in liquid nitrogen, gave a doublet with isomer shift (IS) 3.38 ± 0.06 and quadrupole splitting (QS) of 1.46 ± 0.12 mm/sec compared with anhydrous solid $SnCl_2$ variously reported as a singlet with IS 3.8–4.8 mm/sec.[7] [*Cf.* tin(II) chloride dihydrate with a doublet sepctrum (IS in the range 3.52–4.8 and QS 0.90–1.30 mm/sec).[7]]

(7) J. J. Zuckerman, *J. Inorg. Nucl. Chem.*, **29**, 2191 (1967).

The spectrum of frozen dilute solutions of anhydrous tin(II) chloride in acetone changes (IS = 2.78 ± 0.06 and QS = 1.81 ± 0.12 mm/sec) on addition of excess solid porphyrin ester to the original samples when thawed and refrozen. The spectrum of the solid tin–porphyrin complex precipitated from chloroform was similar to that obtained from the preparation in acetone (IS = 2.94 ± 0.06 and QS = 1.46 + 0.12 mm/sec).

Sn^{119m} Mössbauer spectra of samples where tin had been incorporated from acetic acid into a variety of porphyrins were recorded for comparison. The visible absorption spectra are those of a typical metalloporphyrin: two bands of approximately equal intensity. The Mössbauer spectrum, recorded using a narrow-line $BaSn^{119m}O_3$ source, is an unresolved doublet with IS = 0.47 ± 0.06 and QS = 0.84 ± 0.06 mm/sec. We conclude from the IS value that tin has undergone oxidation to tin(IV) on incorporation. These data rule out the possibility that the species under study here is the "tin-in" incorporated product.

Discussion

Several lines of evidence lead to the conclusion that a specific complex is formed between metal ions and porphyrin esters when they are mixed in certain organic solvents. These complexes have several of the properties that Fleischer and Wang[4] have observed for the species they designate sitting-atop complexes.

Two possibilities exist for the structure of the metal–porphyrin complexes: (i) direct coordination between the metal ion and the two pyrrolinine nitrogen atoms of the porphyrin to give H_2PM^{2+} (or $H_2PM_2X_4^{2+}$, taking into account the dimeric nature of iron halides in chloroform) as proposed by Fleischer and Wang,[4] or (ii) an ion pair between the porphyrin dication and solvated metal anion formed in solvents incapable of supporting separate charged species.

Alcohol can solvolyze the metal ion, the labilized or released protons effecting the conversion of the neutral porphyrin to the dication. All the reactions described in this report as carried out in anhydrous chloroform required the presence of some alcohol to proceed. The overall reaction can be formulated in a general way as

$$H_2P + 2M_2X_6 + (2 + m)EtOH \longrightarrow H_4P^{2+}\cdot 2[M_2X_{6-m}(1 + m)OEt]^- + mHX$$

to satisfy the 4:1 iron to porphyrin ratio found. The visible spectrum of the product is thus that of the porphyrin dication perturbed by the gegenion; the close association of the two species in the ion pair gives rise to characteristic spectral features for each metal or acid anion used, while in aqueous solution where ion pairing is reduced the nature of the anion has only slight effect. Only metal ions capable of undergoing alcoholysis are able to take part; thus no metal complex is formed with $MgClO_4$. The reduction of IS in the Mössbauer spectrum of the tin complex is consistent with the conversion to higher coordination, and the spectrum is very different from that of the final incorporated tin product.

Our results do not support the sitting-atop model as defined by Fleischer and Wang, and we propose the ion-paired species as an alternate explanation. This metal–porphyrin ion pair is not the proposed intermediate in the displacement mechanism for metalloporphyrin formation.

In a recent report on the separation of dicarboxylic porphyrin esters by thin layer chromatography where ferric chloride impregnated silica gel was used as an absorbant, it was noted that the spectrum of the ester on the plate was that of the dication. Plates impregnated with H_2SO_4, while giving spots with similar color, did not provide separation of the porphyrin esters.[8] These observations correlate well with the spectral analysis and solubility properties described in the present work.

A recent attempt to prepare the iron sitting-atop species led to $H_4P^{2+}\cdot FeCl_4^-,Cl^-$ as determined by X-ray crystallography, presumably due to water contamination of the chloroform–$FeCl_3$ system used.[9] Other evidence for the occurrence of sitting-atop species has been presented by Fleischer, *et al.*,[10] from studies carried out in an aqueous system involving α,β,γ,δ-tetra(4-pyridyl)porphine, where no comparisons with the present investigation are possible.

Experimental Section

Hematoporphyrin was purchased from Calbiochem, Los Angeles, Calif.; deuteroporphyrin was prepared from hemin according to the method of Fischer, Triebs, and Zeile;[11] coproporphyrin I and uroporphyrin I were generous gifts of Dr. S. Schwartz, University of Minnesota. The porphyrins were esterified with anhydrous methanol·HCl and purified chromatographically. Following preparative chromatography the porphyrin esters were crystallized. Samples of the crystalline porphyrin esters were examined by paper chromatography, and each ran as a single spot. Reagent grade metal salts were used throughout. Quantitative iron determinations were performed with *o*-phenanthroline. Visible spectra were obtained using a Bausch and Lomb Spectronic 505, or a Cary Model 14.

Two acid solutions (HCl and CF_3COOH) were prepared for the titrations at 2×10^{-2} *M*. The HCl solution was prepared by adding 12.6 *N* HCl to chloroform containing 20% v/v acetone. Nonquantitative HCl–chloroform solutions were prepared by saturating anhydrous chloroform with HCl gas. Titrations with this reagent were qualitatively similar to those using HCl in the chloroform–acetone mixture. Trifluoroacetic acid solutions were prepared by diluting the acid with anhydrous chloroform. Titrations were carried out on samples of porphyrin both in anhydrous chloroform and in absolute ethanol.

Ferric chloride solutions were prepared by dissolving anhydrous $FeCl_3$ in reagent grade chloroform containing 0.75% ethanol. Stannous chloride was put into solution by dissolving a weighed amount of $SnCl_2\cdot 2H_2O$ in a volume of absolute ethanol equal to 2% of the final volume. This ethanol solution of $SnCl_2$ was then diluted to full volume with anhydrous chloroform.

Spectrophotometric titrations were carried out on the porphyrin esters in 2.65 ml of anhydrous chloroform by adding successive 2–10-μl. portions of the metal salt or acid solutions. The maximum total volume of titrant was 50 μl.

Sn^{119m} Mössbauer spectra were recorded at liquid nitrogen temperatures using $Sn^{119m}O_2$ and $BaSn^{119m}O_3$ (New England Nulcear Corp., Nuclear Science and Engineering Corp.) sources with positive velocities indicating relative motion of source toward absorber. The constant-acceleration, cam-drive spectrometer and method of data refinement have been previously described.[12] The velocity constant and standard zero of motion were determined using SnO_2 and white tin absorbers, both at ambient temperatures. Resonances observed were of strengths ranging between 6 and 10%. A statistically reliable number of counts was used, yielding an error of no more than 1% in the number of counts per channel.

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