Aqueous Complexation Equilibria of *meso*-Tetrakis(4-carboxyphenyl)porphyrin with Viologens: Evidence for 1:1 and 1:2 Complexes and Induced Porphyrin Dimerization

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UV−vis spectra of 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (TCPP) have been studied in dilute (1.0 μM) aqueous solution at pH 7.0 as a function of added electrolytes, including phosphate buffer, methyl viologen dichloride (MV), and propyl viologen sulfonate (PVS). At concentrations $\leq$1.0 μM and pH $\geq$ 7.0, TCPP is considered to be tetraanionic and unaggregated. It shows a strong B (Soret) band at 414.2 nm that can be decomposed into three Gaussians, and follows Beer's law with $\epsilon = 4.8 \times 10^5$ M^{-1} cm^{-1} at pH 7.0 in 5.0 mM phosphate buffer. Deviations from Beer's law appear above 2 μM under the above conditions; linear regions for Beer's law plots depend on the pH, temperature, and ionic strength. Even around 1 μM, the Soret absorption strength is a very sensitive function of electrolyte concentration; for example, $\epsilon = 3.6 \times 10^5$ M^{-1} cm^{-1} at pH 7.0 in 62 mM phosphate buffer, and $\epsilon = 5.1 \times 10^5$ M^{-1} cm^{-1} in aqueous solution of minimal ionic strength (0.1 mM NaOH, pH 10). In the titration of TCPP with MV, two types of experiments were performed, at low ionic strength (which necessarily increased in the later stages of the titration) or at high (effectively constant) ionic strength. In 5.0 mM phosphate buffer at pH 7.0 two isosbestic points are observed, at 418.4 nm (in the range of 40−300 μM MV) and at 421.5 nm (15−90 mM MV), considered to represent the formation of 1:1 and 1:2 complexes (porphyrin−viologen). By nonlinear regression analysis, the complexation constants are calculated to be $K_1 = 3350$ M^{-1} and $K_2 = 68$ M^{-1}. Application of principal component analysis identifies the spectra of both complexes and gives $K_1 = 2200$ M^{-1} and $K_2 = 100$ M^{-1}. The complexation constants, the spectra of the complexes, and the positions of the isosbestic points vary with the buffer concentration. At 62 mM phosphate buffer, $K_1 = 600$ M^{-1} and $K_2 = 40$ M^{-1} by nonlinear regression analysis ($K_1 = 1200$ M^{-1} and $K_2 = 140$ M^{-1} by principal component analysis). ^{1}H NMR spectra indicate that both TCPP and MV undergo concentration-dependent resonance shifts that can be correlated with the calculated complexation constants. Similar titration of TCPP with PVS also shows evidence for 1:1 and 1:2 complexes, but with lower equilibrium constants compared to those from titration with MV. At high PVS concentrations (>25 mM) a blue-shifted Soret band attributed to TCPP dimer is observed. The same spectrum is observed under a variety of other conditions, including the presence of a cationic surfactant (4 μM CTAB), very high ionic strength (3 M NaCl), or a polycationic complexing agent. These effects illustrate the variety of influences that affect the porphyrin absorption spectrum in aqueous solution and offer cautions about the conditions of experimental control and data analysis that are necessary to extract meaningful titration data.

Introduction

Porphyrins are frequently prepared with ionic substituents to confer water solubility; the special properties of such porphyrins have been thoroughly reviewed recently.[1] In particular, ionic porphyrins are commonly used as water-soluble electron-transfer photosensitizers,[2] and are often used in conjunction with viologens (bipyridinium dications) as electron acceptors.[3] Porphyrin−viologen pairs have been used for photoinduced electron transfer in a variety of solution-phase and supramolecular assemblies.[4,5] Numerous complications can arise from even this simple pairing of functional units. For example, it has been well-established that porphyrins have a tendency to form homoaggregates in aqueous solution.[1,6−13] Porphyrins of opposite charge type show a strong tendency to form heterodimers or aggregates.[1,14−18] In addition, porphyrins undergo complexation with charged species ranging from DNA to metal ions.[1] Complexation with viologens is most pronounced with anionic porphyrins,[19−33] but neutral porphyrins can also complex viologens under appropriate circumstances.[34,35] Furthermore, solvent and other environmental influences exert significant effects on porphyrin spectroscopy.[36]

In this paper, we characterize in detail the behavior of a dilute aqueous solution of an anionic free base porphyrin (5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin, TCPP, at pH 7.0) upon the addition of methyl viologen dication (MV), as monitored over a wide MV concentration range by absorption and NMR spectra. We also characterize the effect of porphyrin concentration (Beer's law plots) and the effect of ionic strength (phosphate buffer concentration) to sort out the factors that affect the porphyrin spectra during a typical titration experiment. Large numbers of spectral data points were taken, with each titration consisting of 30−100 individual spectra of 3200 points each. These data were analyzed both by nonlinear least squares (NLLS) at three different wavelengths and at 200 regular samplings of the full spectral data by principal component analysis (PCA) to obtain complexation constants and spectra of the complexes.

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Experimental Section

Materials. TCPP was purchased from Porphyrin Products (now Frontier Scientific, Inc., Logan, UT). Traces of solvents (DMF and acetic acid) were detectable by NMR and were satisfactorily removed by vacuum-drying a finely ground sample for 4 days in the dark at a pressure of 10^{-5} Torr with the temperature gradually raised from ambient to 100 °C. The absence of any detectable metals was verified by atomic emission spectroscopy, and the sample showed a single spot on TLC elutions. *meso*-Tetrakis(4-sulfonatophenyl)porphyrin, tetrasodium salt (TSPP), was purchased from Porphyrin Products and dried by the same procedure described for TCPP, except the maximum temperature was 40 °C. Methyl viologen dichloride (MV), 1,1′-dimethyl-4,4′-bipyridinium dichloride, was purchased from Pfaltz and Bauer (Waterbury, CT), treated with activated charcoal and recrystallized three times from 95% ethanol under an inert atmosphere and red light, and dried in a vacuum oven. Propyl viologen sulfonate (PVS), 1,1′-bis(3-sulfonatopropyl)-4,4′-bipyridinium, was prepared by the addition of 4,4′-bipyridine, which had been recrystallized from water six times, to an excess of 1,3-propanesultone (Aldrich Chemical, Milwaukee, WI); the reaction mixture was held at 50−70 °C for 2 h, dissolved in water, precipitated with acetone, and then recrystallized and dried as described for MV.[37] Cetyltrimethylammonium bromide (CTAB) was from Southwest Analytical Chemical (Austin, TX) and was recrystallized twice from ethanol. Phosphate buffer solutions were prepared from monobasic potassium phosphate, 99.999% (Aldrich), and ultrapure sodium hydroxide (Alfa Chemicals, Ward Hill, MA). Sodium chloride used to adjust ionic strength was reagent grade. Water either was distilled in a Corning apparatus or was Millipore deionized water, passed through a Millipore 0.45 μm filter just prior to use in solution preparations.

UV−Vis Spectrophotometry. Spectra in the ultraviolet−visible range were taken with a Shimadzu model UV260 spectrophotometer. The instrument was interfaced to a PC with UV-265 software or custom FORTRAN programs implementing GPIB bus commands. The minimum slit width (0.5 mm) and slowest scan speed were routinely used for optimum resolution. Quartz cuvettes of path lengths 0.5 mm, 1.0 mm, 1.0 cm, 2.0 cm, and 5.0 cm were used to obtain optimum spectra for the range of porphyrin concentrations studied. All spectra are reported normalized to the absorbance of a 1.0 cm path length. The temperature of the sample during the spectroscopic measurements was controlled by a Lauda K-2/RD constant-temperature bath that circulated water through an aluminum heat exchanger and sample holder that was custom designed to fit within the spectrophotometer sample compartment. The temperature was measured with a thermistor calibrated in the range 19−50 °C.

NMR Spectrometry. Routine ^{1}H NMR spectra were obtained on a Varian EM390 90 MHz spectrometer using internal TMS as reference. Studies of the complexation equilibria between TCPP and MV were performed on a 300 MHz General Electric QE-300 spectrometer in D_2O solution. Stock solutions in D_2O were prepared by evaporating all solvent from the corresponding aqueous stock solution, redissolving in D_2O, and repeating the process shortly before the experiment (less than 4 h before all spectra were completed). The D_2O solutions for NMR spectrometry were prepared from the stock solutions a few minutes before their use.

Solution Preparations. Glassware and spectroscopic cuvettes were thoroughly washed with detergent, KOH in ethanol, and aqueous nitric acid, with intermediate water rinsings. Without the acid washings, traces of TCPP remained adsorbed on the glass surfaces, a general problem noted before with porphyrins.[9] The glassware was next rinsed with an EDTA solution to remove trace metals, then with distilled water, then with dilute ultrapure NaOH solution to leave all acidic surface sites with sodium counterions, and finally with distilled water. Aqueous TCPP solutions were prepared from a stock 100 μM solution, which was prepared by first dissolving the requisite TCPP in a small volume of 0.10 M NaOH solution, diluting with buffer, and adjusting the pH with 0.10 M NaOH; the final pH of the stock solution was 10.2. The stock solution was stable in the dark for several months, indicated by a constant absorption spectrum of an aliquot diluted to 1.0 μM. Viologen stock solutions were prepared in distilled water and used the same day; the viologen concentration was confirmed by the ultraviolet absorption spectrum.[38] Phosphate buffer solutions were prepared from ultrapure KH_2PO_4, adjusted to pH 7.0 with ultrapure NaOH, filtered through a 0.45 μm Millipore filter, and stored in the dark. Solution pH values were determined with an Orion model 811 pH meter. Solutions for analyses involving various concentrations of porphyrin, viologen, and buffer were either prepared individually and spectra taken immediately (always within 3 h after preparation) or monitored during a titration experiment, as described in the following section.

Titration Procedures. A Gilmont model GS-3200-B ultraprecision microburet was fitted to the spectrophotometer sample compartment to allow microliter titrations with sample stirring and no intrusion in the light path. At each dispensation of titrant, stirring was halted, the buret tip was lowered just below the sample surface, titrant was dispensed, the tip was raised above the sample solution, and stirring was resumed for 1 min before a reading was taken. Typically 30−100 spectra (385−700 nm at 0.1 nm intervals) were taken during a titration, with the data transferred to a PC for analysis. Four types of titration experiments were performed: (a) variable porphyrin concentration (Beer's law experiments) with constant buffer, (b) constant porphyrin concentration with variable salt, (c) constant porphyrin concentration with increasing viologen and constant buffer (but necessarily variable total ionic strength), (d) constant porphyrin concentration with increasing viologen but constant total ionic strength (via variable salt or buffer concentration). In the experiments with constant porphyrin and/or constant buffer, the solution in the microburet was exactly matched in concentration to the solution in the sample cuvette to avoid any dilution corrections. For example, a typical experiment of type c used a solution in the sample cuvette containing 1.0 μM TCPP in 5.0 mM phosphate buffer at pH 7.0, and the titrant solution was also 1.0 μM TCPP in 5.0 mM phosphate buffer at pH 7.0, but also contained high viologen concentration (up to 0.1 M, depending on the concentration range under study). Note that, in such experiments, the total ionic strength of the sample solution increases significantly as the addition of methyl viologen titrant increases past about 1 mM. In experiments of type d, the sample cuvette contained 1.0 μM TCPP in 62 mM phosphate buffer at pH 7.0 (ionic strength $\mu = 0.15$ M), and the microburet solution contained 1.0 μM TCPP in a mixture of MV and phosphate buffer that was also at an ionic strength of $\mu = 0.15$ M and pH 7.0.

Data Analysis. The Soret regions of the TCPP spectra were decomposed into three Gaussian functions and a small linear function using the Marquardt−Levenburg algorithm.[39] The linear function was used as a baseline for the entire spectrum, and absorbances were corrected by subtracting this linear component. The complexation constants and extinction coef-

ficients of the complexes were evaluated from corrected absorbance data consisting of at least 30 points taken during a viologen titration experiment. Two independent methods were applied: nonlinear regression analysis for absorbances at three selected wavelengths, and principal component analysis using 200 regular samplings of the full wavelength range. The nonlinear regression analysis fit the data to a cubic equation derived from two equilibrium constants, mass balance, and total absorbance equations. PCA[40−43] identified the spectrum of the 1:1 complex from the spectra observed in the two disjoint viologen concentration ranges that showed clear isosbestic points with TCPP. The two disjoint sets of two-component spectra were independently treated in the Lawton−Sylvestre technique[43] whereby two allowable sectors in eigenvalue coefficient space are calculated. Since the 1:1 complex is present in both of the two-component sets, its spectral line shape was determined by locating the ray in the corresponding allowable sectors that resulted in congruent line shapes for this component. The spectral line shape for the 1:2 complex was determined by the ray on the edge of the allowable sector of eigenvalue coefficient space nearest to the highest concentration data point. PCA also verified that there were only two significant components in the isosbestic ranges and only three components in the entire range. From these data, specific concentrations of all of the components of the equilibria could be calculated throughout the concentration range and equilibrium constants evaluated.

Results

One of the most commonly used water-soluble anionic porphyrins is TSPP. TSPP is expected to be a tetraanion in the basic and neutral pH ranges; below pH about 4.8, protonation of TSPP is at both porphyrin nitrogens,[1] rather than the sulfonates, and the resulting dianion has been studied as an interesting case of J-aggregate formation, with a unique spectroscopic signature of a sharp band at 490 nm.[10,12,44,45] The related carboxy derivative TCPP is a weaker acid; no specific measurements of the pK_a values for TCPP have appeared in the literature, although estimates based on the analogy to benzoic acid (pK_a = 4.2) have been occasionally assumed.[6,46] Aqueous pH titrations are complicated by the very low solubility of TCPP in its un-ionized form; however, back-titration with acid of a basic aqueous solution of TCPP shows that the highest pK_a value is about 6.6, with the others lying in the range 5−6. All of the studies reported here were performed in aqueous solutions of pH 7 or higher. Although we will consistently refer to the porphyrin as TCPP, without designation of the state of ionization, it is expected to be multiply ionized (primarily tetraanionic) under all the conditions used in these studies. Formation of J-aggregates from TCPP has also been suggested,[45] but in these studies we never observed the characteristic spectral features that are so prominent with TSPP.

Electronic Absorption Spectrum of TCPP. The UV−vis spectra of porphyrins include two major features: a series of strong visible bands (the Q bands) and an extremely intense near-ultraviolet band (the B, or Soret, band). For most free base tetraphenylporphyrins, there are four well-defined Q bands, assigned to the 0 → 0 and 0 → 1 vibronic transitions of the distinct *x* and *y* components of the lowest $\pi \rightarrow \pi^*$ transition. The Soret band, assigned to the second $\pi \rightarrow \pi^*$ transition, generally appears as a single intense band; however, magnetic circular dichroism studies of tetraphenylporphyrin (TPP) have shown that the Soret band also has *x* and *y* components, appearing at nearly identical positions.[36] In addition, the Soret region of tetraphenylporphyrins shows a weaker shoulder at about 400 nm, which has been considered to be the corresponding 0 → 1 transition[47] or a completely separate n → π^* transition.[36]

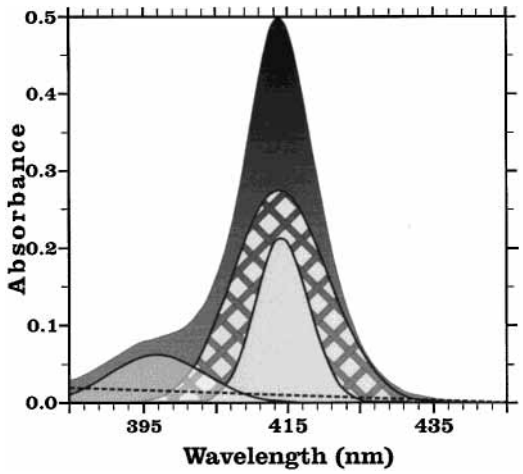

Figure 1. Soret band of 1.0 μM TCPP in 5.0 mM aqueous phosphate buffer at pH 7.0. The band is decomposed into three Gaussians and a small linear baseline.

For the purposes of the studies reported here, which used porphyrin concentrations of 1.0 μM, the Q bands are effectively invisible, and only the Soret regions were analyzed in detail. The Soret region of the TCPP spectrum under our standard experimental conditions (1.0 μM TCPP in 5.0 mM phosphate buffer at pH 7.0) was subjected to line shape analysis. Three Gaussians and a small linear baseline were necessary for a satisfactory fit, as illustrated in Figure 1. The presence of two peaks under the main Soret band is consistent with *x* and *y* components, as assigned in the MCD spectrum of TCPP.[36]

Solvent and Solute Effects on the TCPP Soret Band. The overall spectrum of TCPP in its ionized form in aqueous solution at pH 7.0 is very similar to that of neutral TCPP in various organic solvents. The Q bands appear in the same relative positions and intensities (decreasing intensities at increasing wavelengths, called the etio pattern[36]), and the Soret region has its characteristic shape. Thus, to a first approximation, ionized TCPP in aqueous solution is considered to be a well-behaved porphyrin, whereby water solubility is simply conferred by peripheral ionic substituents that do not significantly affect the characteristic porphyrin spectroscopy. It is important to note that the class called hyperporphyrins can show relatively drastic effects on porphyrin spectra, especially with changes of ionization state.[36] Hyperporphyrins involve an extension of the π conjugation beyond the porphyrin ring via quinone-like interactions with the phenyl rings, and new bands appear in the longer wavelengths, typically >700 nm. Although there are some cases of para-substituted tetraphenylporphyrins that show hyperporphyrin spectra, in particular those with strongly electron-donating substituents,[48,49] it is clear that the carboxy substituent in both its ionized and un-ionized forms does not create such effects.

Nevertheless, we have found that the exact location and intensity of the Soret band of TCPP varies significantly depending upon environmental conditions, in particular the solvent and the presence of other solutes (Table 1). It is important to note that all of the data in Table 1 are for highly dilute solutions where Beer's law is obeyed, although the range in which Beer's law holds is often rather low (Figure 2). The deviations from Beer's law at increased concentrations have been well-documented and are generally considered to represent the formation of dimers or higher aggregates.[6,7] Thus, the range

TABLE 1: Solvent and Solute Effects on the Soret Absorption of TCPP

solvent	solute	λ_{max} (nm)	linear region[a]	ϵ ($\times 10^5$ M^{-1} cm^{-1})
DMSO		419	70 μM	4.4
DMF		419		3.9
THF		418		4.0
90:10 EG/H_2O[b]		418		4.9
50:50 EG/H_2O[b]		417		4.8
10:90 EG/H_2O[b]		415		4.7
H_2O (pH 10)	0.1 mM NaOH	414	8 μM	5.1
H_2O (pH 7)	5 mM buffer[c]	414.2	2 μM	4.8
H_2O (pH 11)	33 mM buffer[d]	413	3 μM	4.1
H_2O (pH 7)	0.5 M NaCl	410	none	<4
H_2O (pH 7)	2.0 M NaCl	408	none	<4
H_2O (pH 7)	TMAP[e]	416		4.2

[a] From Beer's law plots. [b] Ethylene glycol/water (v/v). [c] Phosphate buffer. [d] Carbonate buffer. [e] Tetramethylammonium perchlorate at 80% of its saturated concentration.

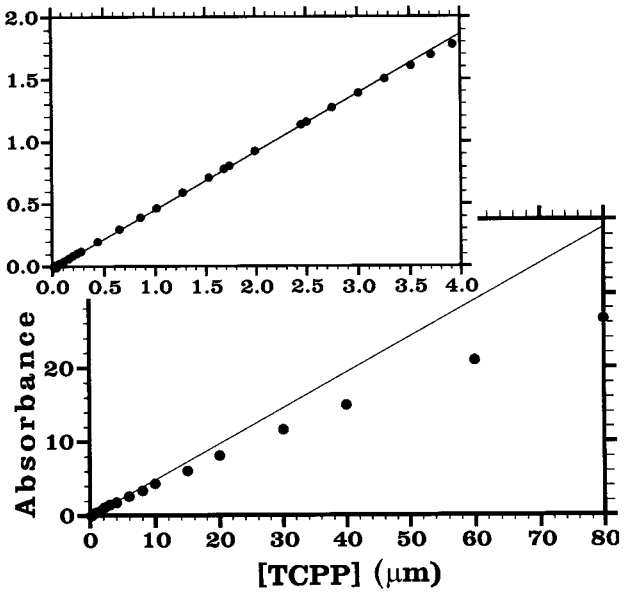

Figure 2. Beer's law plots for TCPP in 5.0 mM aqueous phosphate buffer at pH 7.0. Least-squares lines are shown for the data ranges 0.08−2.0 μM (top) and 0.08−70 μM (bottom). The slope in the lower concentration range is 4.84×10^5 M^{-1} cm^{-1}.

of observations displayed in Table 1 do not represent formation of porphyrin dimers, but rather the effects of the environment upon the absorption spectrum of the monomeric form of TCPP. In general, more polar conditions lead to a blue shift, including more polar solvents and greater ionic strength in aqueous solution. Note that the addition of tetramethylammonium perchlorate leads to a red shift compared to other ionic solutes, indicating that it effectively provides a more "organic" environment around the porphyrin, probably by ion-pairing with the TCPP anionic sites.

The specific effect of increasing the buffer concentration is a slight blue shift as the ionic strength increases, but the dominant change is the decrease in absorptivity. Figure 3 shows that the decrease in absorbance is not a simple linear function of buffer concentration. At extremely low ionic strength (micromolar range), there is little buffering capacity and the initial rapid change in this region could be due to pH as well as ionic strength effects. However, beyond about 0.5 mM buffer concentration, the pH is constant and a significant absorbance decrease continues. If the spectra are analyzed by decomposition into their three Gaussian components (see Figure 1), it is found that all three components decrease in area comparably. The

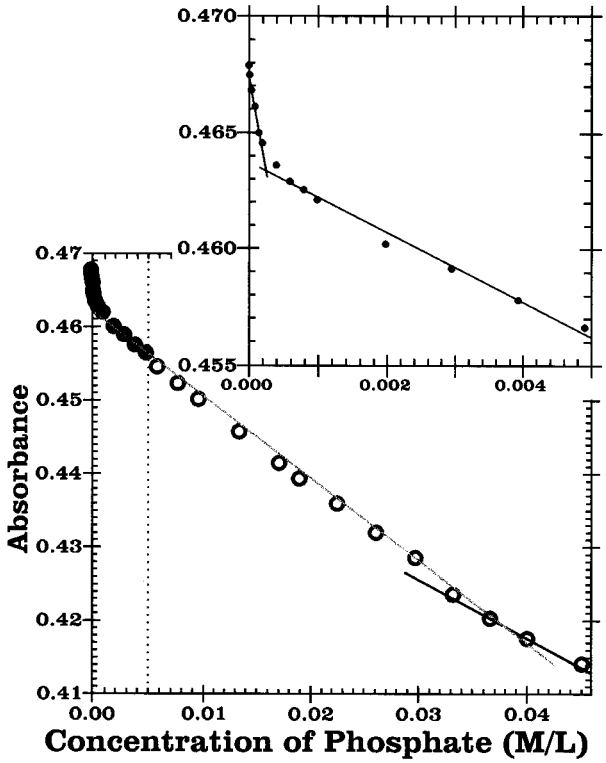

Figure 3. Effect of phosphate buffer concentration on the absorbance of 1.0 μM TCPP at 414.2 nm. Inset: low concentration range of the same plot.

decrease in area is not as great as the decrease in peak absorptivity, however, indicating that the bands are widening as well as weakening. Again, the absorption decrease is not considered to be due to dimer formation under these conditions, since these experiments are performed at a TCPP concentration (1 μM) and ionic strengths (<50 mM) well below those that correlate with dimerization. Typical dimerization conditions reported for TCPP are >10 μM in 10 mM buffer or >1 μM in 100 mM KNO_3.[6]

Titration of TCPP with MV. Given the strong effect of buffer concentration (ionic strength) on the absorptivity of the porphyrin, any titration with an ionic titrant may be expected to lead to nonspecific spectral changes. To identify the specific effects of complexation of TCPP with MV, we adopted two different strategies for carrying out titration experiments: with minimal buffer concentration (5 mM), in which case the ionic strength will necessarily increase during the titration, and with high, effectively constant buffer concentration (62 mM). The two types of titration experiment gave different results, since the complexation constants vary with ionic strength.

Low Buffer Titrations. Addition of MV to TCPP leads to a gradual red shift and decrease in the Soret band. Careful analysis reveals that distinct isosbestic points exist during two stages of the titration. As illustrated in Figure 4, the spectral changes can be categorized into five different stages during the titration: (a) The initial porphyrin spectrum and spectra at very low MV concentrations typically do not exhibit an isosbestic point. (b) In the range 43−340 μM MV, the spectra go through an isosbestic point at 418.4 nm. (c) An extended range without an isosbestic point appears, followed by (d) another range of much higher MV concentration (14−90 mM), in which all spectra pass through an isosbestic point at 421.5 nm. (e) At even higher MV concentrations, no further changes in the spectra are observed except a slight red shift. We interpret these results to indicate the formation of an initial 1:1 TCPP−MV complex

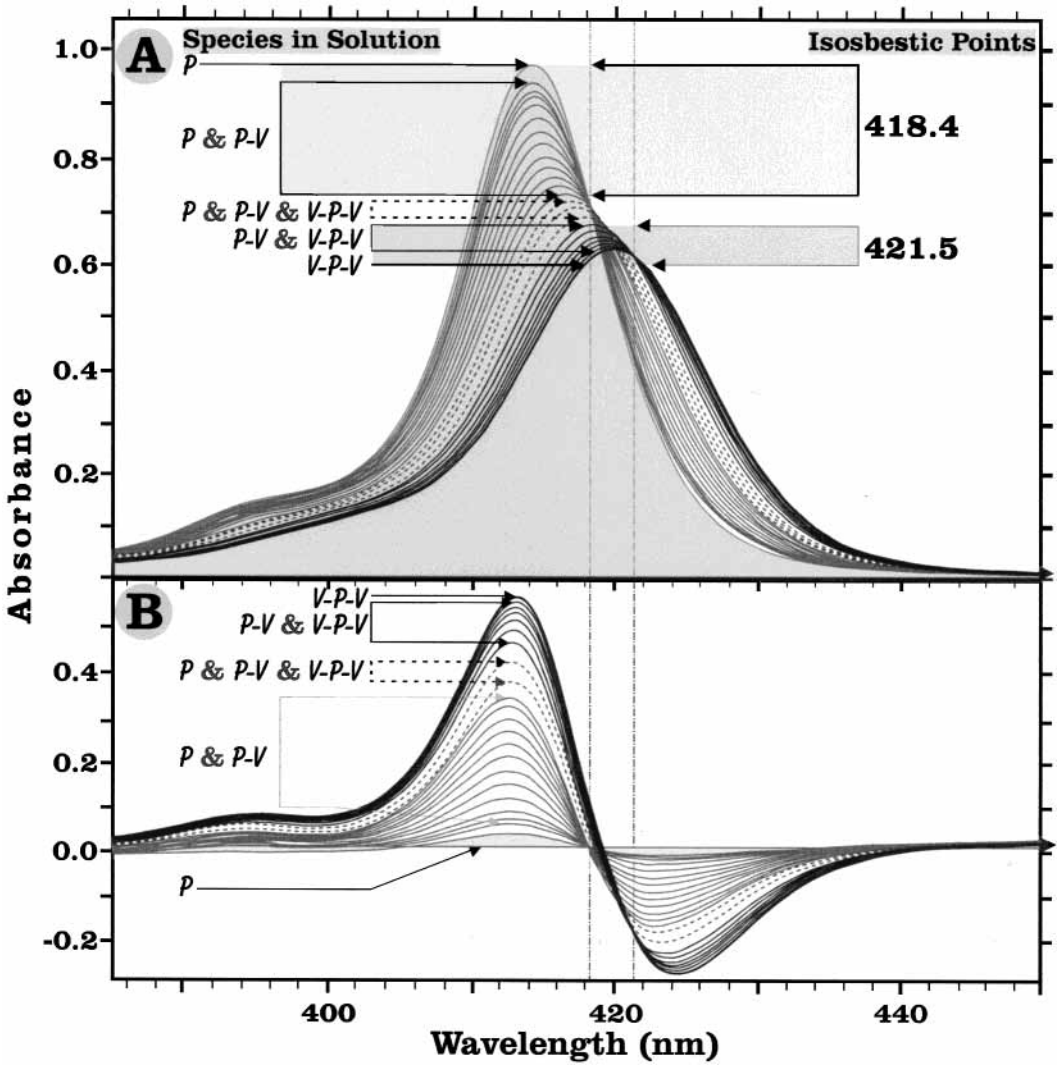

Figure 4. Representative spectra (A) from the titration of 1.0 μM TCPP in 5.0 mM phosphate buffer with MV. Difference spectra (B) were obtained by subtracting each new spectrum from the original spectrum. The predominant species present in each of the five distinct stages of the titration are noted.

(abbreviated PV in the figures) followed by a 1:2 TCPP−MV complex (abbreviated VPV, without implying any specific structural arrangement).

Data from three different wavelengths (413.7, 418.5, and 420.1 nm) were independently used for NLLS analysis of the complexation constants, using mass balance equations and the following two equilibrium constants:

$$\mathrm{TCPP} + \mathrm{MV} \Leftrightarrow \mathrm{TCPP}\cdots\mathrm{MV}\ (K_1) \qquad (1)$$

$$\mathrm{TCPP}\cdots\mathrm{MV} + \mathrm{MV} \Leftrightarrow \mathrm{TCPP}\cdots(\mathrm{MV})_2\ (K_2) \qquad (2)$$

For the MV titration in 5.0 mM phosphate buffer the NLLS method calculated equilibrium constants of $K_1 = 3350\ \mathrm{M}^{-1}$ and $K_2 = 68\ \mathrm{M}^{-1}$.

The existence of two distinct equilibria was confirmed by the method of PCA. Using the full spectral data for the titration (at 200 selected wavelengths from 25 spectra), PCA clearly indicated the existence of three (and only three) components. When selected spectra from the isosbestic ranges of the titration were similarly analyzed, two components each were identified.

Using the PCA analyses, it was possible to determine the spectra of all three components. The TCPP spectrum is available from the beginning of the titration, the 1:2 complex spectrum is a constant spectrum at the end of the titration, and the 1:1 complex spectrum could be calculated by PCA analysis of each of the two isosbestic regions (Figure 5). Although the spectra are very similar, it is clear that MV complexation leads to a distinct red shift and weakening of the TCPP Soret absorption.

Compared to the NLLS method, PCA calculates a slightly lower K_1 value ($2200\ \mathrm{M}^{-1}$) and a slightly higher K_2 value ($100\ \mathrm{M}^{-1}$). An overlay of the speciation plot comparing the two methods shows a good degree of agreement (Figure 6).

High Buffer Titrations. Comparable titration data were taken with the phosphate buffer concentration at 62 mM, a concentration comparable to the final stages of the previous titration experiments. Thus, the ionic strength was effectively constant throughout the titration. Similar results were obtained, although the equilibrium constants were significantly smaller ($K_1 = 600\ \mathrm{M}^{-1}$ and $K_2 = 40\ \mathrm{M}^{-1}$). The same stages of the titration were observed, with isosbestic points at 417.3 and 419.5 nm. A summary of all titration data is presented in Table 2.

NMR of TCPP/MV Solutions. Because of solubility and sensitivity issues, NMR studies were undertaken at substantially increased concentrations of both TCPP and MV. These experiments used a constant concentration of MV and variable TCPP, as described and illustrated in Figure 7 and Table 3. There is clear evidence for interactions between MV and TCPP, with the aromatic protons of MV showing a clear upfield shift as

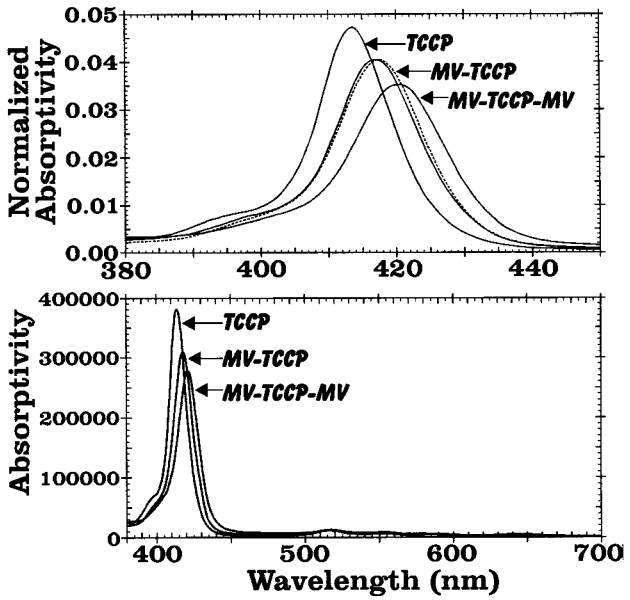

Figure 5. Spectra of TCPP and its 1:1 and 1:2 complexes with MV. The solid and dashed lines for the TCPP···MV complex are obtained by independent principal component analyses of the two isosbestic regions of the titration of 1.0 μM TCPP with MV in 5.0 mM phosphate buffer.

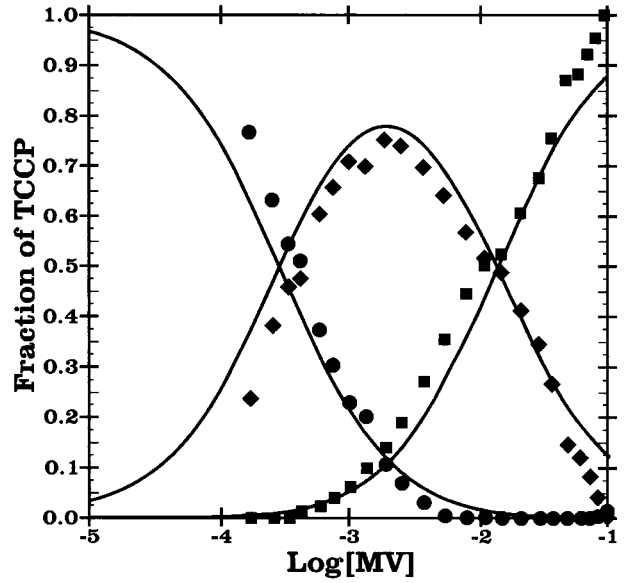

Figure 6. Speciation plot for the titration of 1.0 μM TCPP with MV in 5.0 mM phosphate buffer: (circles) TCPP, (tilted squares) 1:1 complex, (squares) 1:2 complex. The solid lines are calculated from the two equilibrium constants derived from NLLS analysis, and the individual points are from PCA analysis of the spectra.

the concentration of TCPP increases. The absence of any change in splitting patterns suggests that there is a mobile equilibrium of the components of the complex which is rapid on the NMR time scale; i.e., MV molecules all experience the same average environment, but the average environment is strongly shifted upfield because MV spends a significant amount of time in the shielding region of the TCPP ring current. Figure 7 also shows that the chemical shifts can be correlated with the extent of complexation, as calculated from the equilibrium constants from the NLLS analysis. The positions of the TCPP protons are affected by MV much less than those of MV are affected by TCPP, as would be expected from the much larger magnitude of the porphyrin ring current effect. Furthermore, essentially the same fraction of TCPP (65−70%) is in the complexed form in each of the intermediate spectra, since the total MV concentration is constant.

Titration of TCPP with PVS. Comparable titration experiments were carried out with PVS, a neutral (zwitterionic) viologen. The appearance of two isosbestic regions and the conclusions regarding both 1:1 and 1:2 complexes were similar to those from the MV experiments, although the complexation was distinctly weaker. Figure 8 shows an unexpected feature, however—the appearance of a new blue-shifted spectrum under conditions of high viologen concentration, where the MV titration had simply leveled off at a final 1:2 complex spectrum. This weak, blue-shifted absorption has been observed under a variety of conditions and is normally attributed to a face-to-face porphyrin dimer.[11,13,50] We observed a comparable spectrum from the titration of TSPP with CTAB, as noted in other studies.[21,51]

Discussion

Ionic Strength and Solvent Effects on the TCPP Soret Absorbance. Ionic porphyrins in aqueous solution often deviate from Beer's law behavior, most prominently noted by a weakened Soret band at increasing concentration. This effect is nearly always attributed to a dimerization or higher aggregation, and dimerization equilibrium constants have been reported for TSPP,[10,50,52−55] TCPP,[6,52] and other substituted TPPs.[9,11] In general, aggregation is strongly dependent on the state of ionization of the porphyrin. TSPP is reportedly monomeric over extended concentration ranges as long as the pH is above 5 (i.e., while TSPP is a tetraanion).[44,54] Earlier studies of TCPP were done at pH 7.5 in 10 mM Tris buffer; under these conditions, TCPP was considered monomeric.[6] Aggregation effects in aqueous solution also depend on ionic strength, with addition of salts inducing dimerization, e.g., 0.1 M KNO_3 added to the TCPP solution just mentioned.[6]

We began our studies in 1.0 μM TCPP at pH 7.0 in very weak buffer (5.0 mM phosphate), under which conditions we expect TCPP to be a monomeric tetraanion. Even using the reported dimerization equilibrium constant for high ionic strength[6] of 4.55×10^4 M^{-1}, less than 1% of the TCPP would be in dimer form. We see the beginnings of deviations from Beer's law only above 2 μM under our experimental conditions (Figure 2). Furthermore, our value of the extinction coefficient (4.84×10^5 M^{-1} cm^{-1} at the Soret peak, 414.2 nm) is significantly higher than previously reported for monomeric TCPP at pH 7.5 (3.86×10^5 M^{-1} cm^{-1} at 415 nm),[6] suggesting that our initial conditions involve minimal perturbation of the porphyrin absorbance. The maximum extinction coefficient we observe for TCPP is 5.08×10^5 M^{-1} cm^{-1}, under conditions of minimal ionic strength, i.e., at pH about 10 in 0.1 mM NaOH.

In 1 μM TCPP solutions at very low buffer concentrations, the observation of a significant decrease in absorbance of the Soret band with increasing buffer concentration (Figure 3) marks an effect other than simple dimerization. Note that a significant decrease in absorbance occurs at buffer concentrations well below the 5 mM range, where the Beer's law experiments (Figure 2) indicate monomer. The decrease in absorbance from 0 to 5 mM buffer is about 2%, and from 0 to 62 mM it is about 25%, in a nearly linear correlation, except for the initial sharp decrease below 1 mM, which may be related to pH changes in the region below effective buffer capacity. Over this range there is only a very small blue shift in the position of the Soret peak. This effect of buffer concentration on the intensity of the Soret absorbance seems to be a specific electrolyte−porphyrin interaction. Such effects have been suggested before, for

TABLE 2: Summary of Spectral Data for TCPP−MV Complexes

	TCPP···MV	TCPP···$(MV)_2$		TCPP···MV	TCPP···$(MV)_2$
From the Titration of 1.0 μM TCPP with MV in 5.0 mM Phosphate Buffer at pH 7.0					
K (by NLLS) (M^{-1})	3350	68	λ_{max} (nm)	419	422
K (by PCA) (M^{-1})	2200	100	ϵ_{max} (M^{-1} cm^{-1})	3.1×10^5	2.8×10^5
$\lambda_{isosbestic}$ (nm)	418.4	421.5			
From the Titration of 1.0 μM TCPP with MV in 62 mM Phosphate Buffer at pH 7.0					
K (by NLLS) (M^{-1})	600	40	λ_{max} (nm)	418	420
K (by PCA) (M^{-1})	1200	140	ϵ_{max} (M^{-1} cm^{-1})	2.8×10^5	2.4×10^5
$\lambda_{isosbestic}$ (nm)	417.3	419.5			

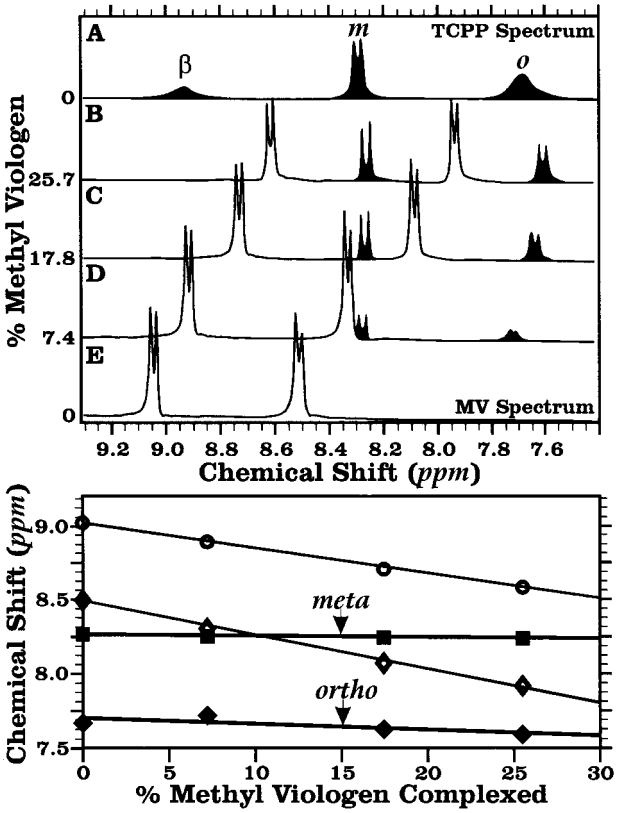

Figure 7. ^{1}H NMR (300 MHz) spectra of 500 μM MV with variable concentrations of TCPP in D_2O solution with 250 mM phosphate buffer at pD 7. Data are summarized in Table 3. Chemical shifts of the MV protons (open points) and TCPP phenyl protons (filled points) as a function of the fraction of MV in complexed form are calculated using the equilibrium constants derived from NLLS analysis.

TABLE 3: Calculated Concentrations of TCPP−MV Complexes (μM)[a]

	TCPP	TCPP···MV	TCPP···$(MV)_2$	MV
A	625	0	0	0
B	65	118	5.1	371
C	40	81	3.9	411
D	15	34	1.8	462
E	0	0	0	500

[a]Solutions corresponding to the five NMR spectra shown in Figure 7.

example, in the specific effects observed on TSPP spectra when cations are complexed with crown ethers.[53,55] In these studies, we have not addressed the specific effects of counterions further except to carry out our studies of viologen complexation under conditions designed to minimize those effects.

TCPP Complexation with Viologens. Careful titration studies of TCPP with MV indicate that there are two distinct isosbestic points occurring at different stages of the titration (Figure 4). We take this as evidence for formation of a 1:1 complex followed by a 1:2 complex. There have been many

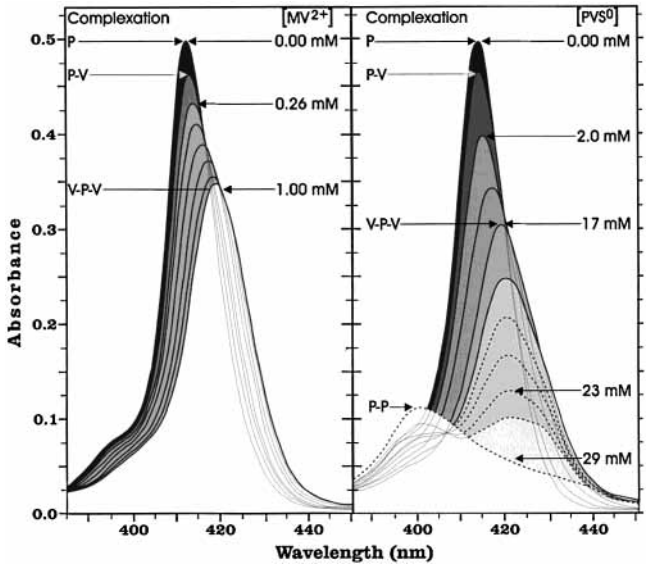

Figure 8. Representative spectra taken from the titration of 1.0 μM TCPP with PVS in 5.0 mM phosphate buffer at pH 7.0. PVS concentrations are noted, as are the spectra most closely corresponding to the 1:1 and 1:2 complexes. The dotted lines indicate spectra at the highest concentrations of PVS.

determinations of complexation equilibrium constants between TSPP (or ZnTSPP) and viologens, with values typically around 10^4 M^{-1} at low ionic strength and significantly lower at high ionic strength.[19,21−27,29−32] Most of the studies, however, used fluorescence quenching as the indicator of complex formation, and that method will not detect whether complexes of more than one stoichiometry exist. Spectrophotometric studies would require extraordinary care to detect the shift in isosbestic point, and we believe these may have been missed in the case of many of the previous complexation studies.

There have been no reported complexation constants between TCPP and MV, although viologen complexes with other carboxyporphyrins have been noted, including the uroporphyrins,[28] a membrane-bound dicarboxyporphyrin,[56] and a myoglobin analogue.[57] Complexation of TCPP or ZnTCPP with an antibody has been reported to facilitate photoinduced charge transfer to methyl viologen.[58] Particularly strong complexation occurs with the metallouroporphyrins, which bear a −8 charge, with equilibrium constants ranging from 10^4 to 10^6 M^{-1}, with the free base at 10^5 M^{-1}. In the case of zinc uroporphyrin, clear evidence for 1:1 and 1:2 complexes is observed because the K_1 and K_2 values are distinct ($10^{5.9}$ and $10^{2.3}$ M^{-1}, respectively), while for the other uroporphyrins K_1 and K_2 are not distinguished but suggested to be very similar.[28] Porphyrin−viologen complexes have also been the basis for supramolecular assemblies of catenanes.[34,35]

The spectra of the two different complexes formed between TCPP and MV are increasingly red-shifted and weakened, as typically observed for porphyrin−viologen complexes. Lower

equilibrium complexes at higher ionic strength are also consistent with observations from other porphyrin−viologen complexes. NMR chemical shifts for porphyrin−viologen complexes have been used to determine structures, especially for linked porphyrin−viologen molecules.[59−61] The observed chemical shifts are consistent with a viologen overlaying the porphyrin ring system, allowing for interactions between the oppositely charged sites.

The complexation of TCPP with PVS proceeds very similarly to that with MV, with two distinctions. The complexation is distinctly weaker with PVS, as observed with complexes of TSPP−PVS compared to TSPP−MV.[30] Furthermore, at high PVS concentration a new feature is observed—a very weak and blue-shifted band (Figure 8). This spectral feature has been assigned to a face-to-face porphyrin dimer and has been observed under a variety of conditions, including complexation of TSPP and TCPP with a +8 macrocycle.[50]

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