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Association of Small Molecule Cosolutes with Water-Soluble Polymers. Effects of Polymer Binding on the Reactivity of Water-Soluble Porphyrins

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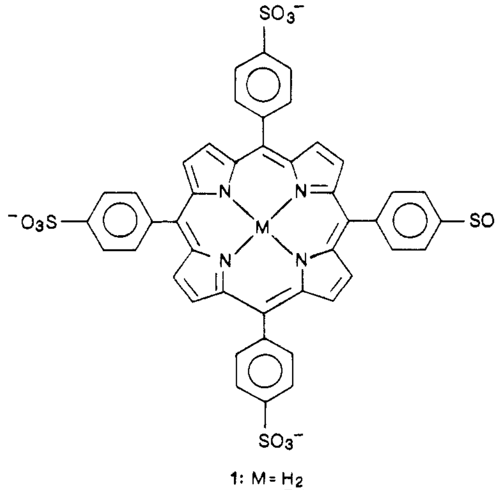
The tetraanionic porphyrin, **1**, associates strongly with poly(vinylpyrrolidone) (PVP) in dilute buffered aqueous solutions. The equilibrium constant for association of **1** with PVP in pH 3 acetate at 298 K is 1.4×10^7 M^{-1} for $M = 48\,000$ PVP. Chelation of Cu^{2+} by the porphyrin is slowed dramatically in the presence of PVP. The rate expression is $K[\mathbf{1}]_b[Cu^{2+}]/(k'[PVP] + k''[Cu^{2+}])$, which fits a mechanism in which only free porphyrin, $[\mathbf{1}]_f$, coordinates to Cu^{2+} ($[\mathbf{1}]_b$ is the concentration of polymer-bound porphyrin). The palladium(II) porphyrin, **2**, also associates with PVP. The efficient quenching of the phosphorescence of **2** by methylviologen, MV^{2+}, in aqueous solution is completely inhibited upon association of **2** with PVP. The lack of reactivity of **1** and **2** bound to PVP indicates that the porphyrin microenvironment involves little contact with the bulk aqueous solution.

Introduction

Research on the binding of small solute molecules by synthetic polymers and biopolymers in solution has focused on analyzing the factors governing the association equilibrium.[1-8] For such a complex process there are numerous interdependent variables including the polymer conformation,[2] ionic strength,[2,4b] charges of the polymer[2,3] and the solute,[5] and polymer molecular weight which influence the associative process. An aspect of equal importance in the associative interaction is the nature of the microenvironment of the adsorbed solute, since the reactivity of the polymer bound solute should be strongly influenced by the degree of interaction with the bulk medium. However, there are only a few reports of reactions of polymer-bound cosolutes in aqueous solutions.[9,10,25] Assessments of local environments based upon spectral shifts of organic dyes bound to polymers and salt effects on association equilibria are frequently complicated by subtle effects such as changes in polymer conformation.[11] For example, for poly(vinylpyrrolidone) (PVP) studies of association with the organic dyes methyl orange[4a,b] and 2-*p*-toluidinonaphthalene-6-sulfonic acid[5] (TNS) have resulted in contradictory interpretations regarding the presence of "protected" hydrophobic pockets for dye encapsulation. If such pockets do exist and serve as binding sites for dye molecules, the reactivity of the incorporated dye with solutes restricted to the aqueous phase should be retarded.

This work illustrates association of the tetraanionic porphyrins, **1** and **2**, with PVP in aqueous solution and reactivity of the bound porphyrins with ions in the aqueous phase. The PVP sample used is characterized by both static and dynamic scattering methods to provide an accurate measure of the weight average molecular weight and hydrodynamic radius.



1: M = H_2
2: M = Pd

The insertion of Cu^{2+} into **1** (eq 1a) was examined as a function of reactant concentrations and bulk pH. The phosphorescence quenching of **2** (eq 1b) by methylviologen, MV^{2+}, was also examined both in the presence and in the absence of PVP. Results obtained support the existence of hydrophobic binding sites for incorporation of **1** and **2** in PVP.

$$H_2P^{4-} + Cu^{2+} \rightarrow CuP^{4-} + 2H^+ \qquad (1a)$$

$$PdP^{4-} \xrightarrow{h\nu} PdP^{4-*} + MV^{2+} \rightarrow PdP^{3-} + MV^+ \qquad (1b)$$

Experimental Section

Materials. Poly(vinylpyrrolidone), PVP, having an average molecular weight of 40 000 was obtained from Polysciences, Inc., and was used without further purification. Tetrasodium tetrakis(*p*-sulfonatophenyl)porphyrin (Strem Chemical), methylviologen (Sigma), copper acetate (Baker), sodium acetate (MCB), and glacial acetic acid (Mallinckrodt) were all used as received. The Pd(II) complex, **2**, was prepared as described elsewhere.[12]

Apparatus. Absorption spectra were recorded on a Hewlett-Packard 8451 single beam diode array spectrophotometer equipped with a thermostated cell holder. Emission studies were performed using a Spex Model 111C spectrofluorimeter modified to include a 450-W Xe arc lamp, thermostated cell holder, and a cooled

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(13) The complex tris(2,2′-bipyridyl)ruthenium(II) exhibits no change in any physical or chemical property in solutions having PVP. The complex also absorbs the 488-nm emission of the Ar ion laser and has an emission quantum yield similar to **1**. Thus the complex can be used to test the effects of local heating on the hydrodynamic diameter observed for PVP under dynamic scattering conditions.

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photomultiplier tube housing (Hammamatsu R928 PMT). Static and dynamic light scattering measurements were made using the 488-nm line from a 5-W vertically polarized Ar ion laser (Spectra Physics) and detecting scattered light from a cylindrical cell at automatically stepped angles between 20° and 90° relative to the excitation source with a Thorn EMI 9863 PMT. A Brookhaven Instruments Model BI 2030 Autocorrelator was employed in dynamic scattering measurements, where the on-board microprocessor was also used for data storage and analysis. The custom-built compartment was thermostated to 0.1 °C accuracy. A more complete description of the scattering apparatus will be presented elsewhere. Luminescence lifetimes were measured by flash photolysis using facilities of the Center for Fast Kinetics Research, University of Texas, Austin.[27]

Results and Discussion

Characterization of the PVP. Both static and dynamic light scattering were employed to obtain a measure of the molecular weight and polydispersity of the commercial PVP used.

Dynamic scattering measurements were made in the homodyne mode, at angular increments of 5° from 20° to 90° in the horizontal scattering plane. The sample time at each angle was determined according to eq 2, where $(\mathrm{st})_{90}$ represents the sample

$$\mathrm{st}(\theta) = 0.5(\mathrm{st})90°/\sin^2(\theta/2) \qquad (2)$$

time at $\theta = 90°$ and was set to 3 μs to allow an intensity autocorrelation function decay of approximately two e-folds over the entire 64-channel curve.

The intensity autocorrelation curves were analyzed according to the standard cumulant procedure,[14] in which the electric field autocorrelation function $g^{(1)}(t)$ is expanded to yield the z average decay rate $\bar{\Gamma}$ and the second moment, μ_2, whence the polydispersity index is calculated, $Q = \mu_2/2\bar{\Gamma}^2$. The $\bar{\Gamma}$ for each angle was plotted against $\sin^2 \theta/2$ and the translational diffusion coefficient, D, determined by the slope.

Dynamic scattering measurements were carried out at 20.0 °C on 0.25%, 0.5%, and 1.0% PVP solutions, and an 0.28% PVP solutions in the presence of 1.7×10^{-5} M porphyrin. D for the pure PVP solution, which showed no measurable dependance on concentration over the range studied, was determined to be 3.47 $(\pm 0.1) \times 10^{-7}$ cm^2 s^{-1}. The z-average hydrodynamic diameter, d_{H}, for the spherical approximation of the particle is 123 ± 5 Å, based upon the Stokes–Einstein equation.

When scattering measurements were made on the PVP-incorporated porphyrin, the optimum amounts of PVP and porphyrin were 0.28% and 1.7×10^{-5} M, respectively, which allowed an average of one porphyrin per four PVP molecules. The diffusion coefficient for this solution was determined to be 4.4 $(\pm 0.4) \times 10^{-7}$ cm^2 s^{-1}. The results indicate a higher D for the bound porphyrin case, leading to the notion that the PVP has hydrodynamically contracted. In the bound porphyrin case, however, absorption of the laser light at 488 nm causes local heating of the solution across the approximately 0.2 mm Gaussian laser profile. This local heating was confirmed by measuring the D of PVP when a nonbinding, absorbing chromophore, a ruthenium complex,[13] was added. Using identical laser power and equal absorbance as in the porphyrin case, the measured D for PVP was 4.4 $(\pm 0.4) \times 10^{-7}$ cm^2 s^{-1}. This strongly suggests that the difference in D for the PVP above and that with bound porphyrin is purely a thermal effect; the local viscosity has decreased by 28%, giving a local temperature in the beam profile of about 31 °C. Hence, the association of the porphyrin with PVP does not measurably affect the translational diffusion coefficient and hence does not appreciably change the size or overall conformation of the PVP. Nor does the presence of the porphyrin lead to aggregation of the PVP, as any aggregation, even of two PVP molecules would lead to a measurable change in D, since for PVP, $D = 10^{-4}(\mathrm{MW})^{-1/2}$.[21] A decrease of D by a factor of 1.4, due to dimerization, would easily fall within the resolution of the scattering measurements.

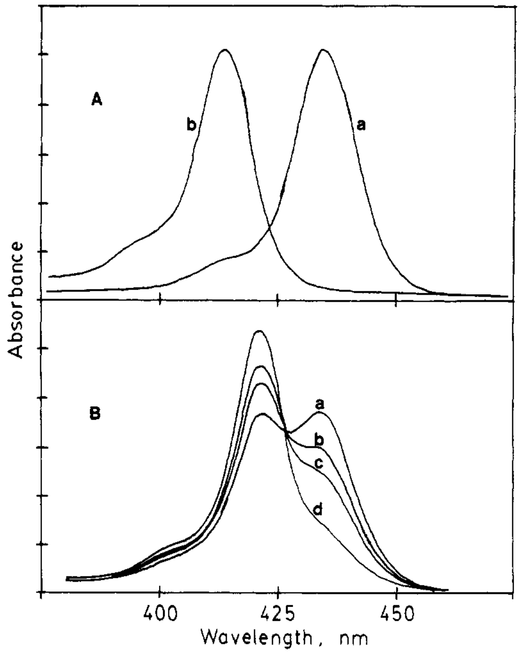


Figure 1. (A) Absorption spectra of the Soret band of **1** at pH 3 (a) and pH 6.5 (b). (B) Absorption spectra of **1** (10^{-6} M) in pH 3 solutions containing 4.0 μM (a), 6.0 μM (b), 11.0 μM (c), and 20.0 μM (d) PVP.

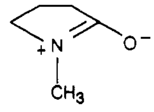
The polydispersity index, Q, of the pure PVP solution, was between 0.12 and 0.18, indicating a fairly narrow molecular weight spread. For both the PVP/porphyrin and PVP/ruthenium complex solutions, Q was in the range of 0.28–0.36. Rather than indicating a true increase in polydispersity it is presumed that this increase in the z-average width of the translational diffusion coefficient distribution is due to a nonhomogeneous temperature and hence viscosity distribution over the laser beam profile, all of which is sampled by the photomultipler.

Weight average molecular weight determinations were carried out in the total intensity mode. The value of dn/dc was found to be 0.169 ± 0.002 cm^3 g^{-1} measured on a Brice-Phoenix differential refractometer. The calculated weight-average molecular weight for pure PVP solution was 47 600 ± 4000 where the error reflects uncertainties in the Kc/R extrapolation, dn/dc determination, and error margin published for the toluene[14] calibration factor.

PVP–Porphyrin Association. The tetraanionic porphyrin, **1**, exhibits spectrophotometric changes of the intense Soret band with changes in pH in aqueous solutions that are indicative of protonation of the porphyrin (eq 3). Figure 1 shows spectra of **1**

$$\mathrm{H_2P^{4-} + 2H^+ \rightleftarrows H_4P^{2-}} \qquad K_\mathrm{a} \qquad (3)$$

at pH 3 and pH 6.5. When very dilute pH 3 solutions containing PVP are mixed with dilute pH 3 solutions of **1**, an immediate spectrophotometric change occurs (Figure 1B), indicating that the principal form of **1** in solution is $\mathrm{H_2P^{4-}}$. The apparent decrease in the degree of protonation observed for **1** in the presence of PVP is similar to changes observed for other nitrogen bases covalently attached to PVP and is attributed to modification of water structure within the polymer.[19] The absorption maximum of **1** in the presence of PVP at pH 3, 422 nm, differs from that of **1** in pH 6.5 aqueous solutions, 414 nm. The spectral changes suggest that the porphyrin associates with the polymer and exists within the polymer in an environment of higher pH than the bulk pH of 3. Dipole moment measurements of N-methylpyrrolidone in water suggest that the resonance form **3** is significant.[20] In solutions buffered to pH 3, **4** may also exist. The presence of **4**,



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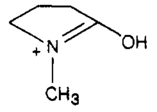

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TABLE I: Association Constants for Binding of 1 with PVP (M = 48 000)[a]

[buffer], M	T, °C	K', M	$1/n^c$	$K_b{}^b \times 10^{-6}$, M^{-1}	$K_a \times 10^{-8}$, M^{-2}
0.1	25	0.165	160	14.0 ± 1.5	1.20
0.1	30	0.188	150	9.7 ± 1.0	1.94
0.1	40	0.213	136	4.4 ± 0.5	4.83
0.1	45	0.228	155	3.1 ± 0.4	7.46

[a] Ionic strength of pH 3 acetate buffers. [b] K'/K_a. [c] All values ±20.

even at very low concentrations in the PVP, cannot, however, explain the uptake of **1** and other anionic dyes by PVP since the degree of association does not decrease above pH 3 (vide infra).

Association of **1** with PVP was examined spectrophotometrically as a function of total porphyrin concentration at pH 3 (Figure 1B). The association can be treated as a Langmuir adsorption process.[15] The concentrations of protonated free porphyrin, $[H_4P^{2-}]_f$, and polymer-bound porphyrin, $[H_2P^{4-}]_b$, were determined from the absorbances of each species at 422 nm (λ_{max} of $[H_2P^{4-}]_b$) and 434 nm (λ_{max} of $[H_4P^{2-}]_f$). The data were analyzed by using Scatchard plots (eq 4) based on the equilibrium expression of eq

$$r/[H_4P^{2-}]_f = K'/[H^+]^2n - K'r/[H^+]^2 \qquad (4)$$

5. In eq 4 r represents the moles of porphyrin bound per mole of polymer monomer units, n is the limiting number of dye moles bound *per monomer unit*, and K' is the binding constant for the association of *free porphyrin at pH 3* with the polymer (eq 5).

$$[H_4P^{2-}]_f + PVP \rightleftarrows \{[H_2P^{4-}]_b,PVP\} + 2H^+ \qquad K' \qquad (5)$$

$$[H_2P^{4-}]_f + PVP \rightleftarrows \{[H_2P^{4-}]_b,PVP\} \qquad K_b \qquad (6)$$

Values of K_b, the pH-independent binding constant, are determined from K' and the porphyrin acid dissociation constant, K_a (eq 3). The above analysis assumes that $[H_4P^{2-}]$ does not associate with the polymer and that binding sites on the polymer do not interact. Attempts to independently measure binding constants by equilibrium dialysis methods were unsuccessful because of strong association of **1** with the dialysis tubing.

Plots of $r/[H_4P^{2-}]$ vs. r are linear over the range of porphyrin concentrations employed. Reeves and co-workers[5] have noted that large deviations from ideality can occur at high dye concentrations, reflecting interaction between separate binding sites; here measurements were performed at low porphyrin/polymer ratios (<3) to avoid specific site interactions. Slopes of the plots yield $-K'/[H^+]^2$; K' is determined directly from the slope of the Scatchard plot since measurements were made in buffered solutions. The value of K_b is determined by using K_a for $[H_4P^{2-}]$, $K_b = K'/K_a$; values of K_a were determined independently. Table I lists data obtained in determinations of K_b and K_a at four temperatures.

The average number of basemoles per mole of bound **1**, 160, for the M_w = 48 000 PVP in 0.10 M buffer at 25 °C, is similar to the binding capacity measured by others for mono- and dianionic dyes such as methyl orange,[23,24] TNS,[5] and the azo dye 4-phenylazo-1-naphthalenedisulfonate.[5] For the PVP used, the limiting number of porphyrins bound per polymer is approximately 2.7. The data of Table I also illustrate that K_b exhibits a strong temperature dependence. After correction for acid dissociation, a van't Hoff plot for K_b yields a ΔH of −60 kJ mol^{-1} and ΔS of −64 J mol^{-1}. The negative value of ΔS is atypical of values obtained for association of amphiphilic dyes with PVP,[4,5] as is the exothermicity, and the thermodynamic data are suggestive of specific porphyrin–polymer interactions for this system.

Metalation of 1 with Cu^{2+}. In buffered (pH < 7) aqueous solutions Cu^{2+} readily inserts into **1** to produce the cupric complex, CuP^{4-} (eq 1a). Hambright has examined the pH dependence of the process in acetate buffer and has found that the acetate ion catalyzes the insertion process.[16] When PVP is added to acetate-buffered solutions of **1**, a significant decrease in the Cu^{2+} insertion rate is observed. Spectrophotometric changes observed during the reaction (Figure 2) exhibit a clean isosbestic point and thus no observable intermediate is formed. Table II lists rate constants obtained from reaction of **1** with Cu^{2+} under pseudo-first-order conditions (excess Cu^{2+}), both in the absence and in the presence of PVP. PVP concentrations were high enough to ensure complete association of **1** with the polymer ([PVP] ≥ 10[**1**]). In the absence of PVP the observed rate constant increases by a factor of 100 between pH 3 and pH 6.4, whereas the rate constant for metalation of the polymer bound porphyrin is unaffected by pH. The most striking feature of the observations is the greater than four order of magnitude decrease in the observed rate constant between free and polymer complexed porphyrin at pHs above 5.

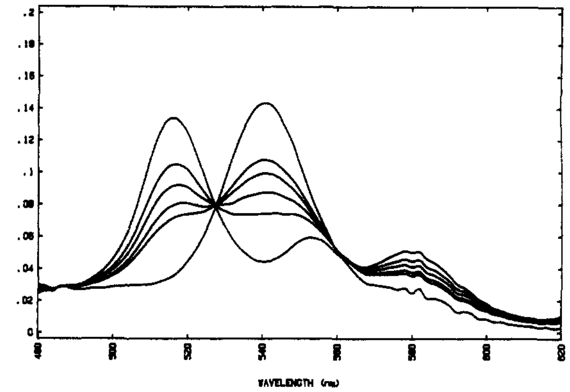

Figure 2. Spectrophotometric changes observed during Cu^{2+} insertion into **1** at pH 6.4. [PVP] = 10^{-4} M, [**1**] = 2×10^{-5} M.

TABLE II: Rate Constants for Cu^{2+} Insertion into 1 in the Absence and Presence of PVP[a]

pH	k_{H_2O}, M^{-1} s^{-1}	k_{PVP},[b] M^{-1} s^{-1}	k_{H_2O}/k_{PVP}
3.0	2.1 ± 0.2	0.013	160
4.0	36.0 ± 4	0.014	2 500
5.1	244.0 ± 20	0.013	19 500
6.4	246.0 ± 25	0.016	15 400

[a] Solutions buffered to 0.1 M with acetate or phosphate. [b] [PVP] = 10^{-4} M, 40000; [Cu^{2+}] = 0.01 M; $[H_2P]_T$ = 1×10^{-5} M. All rates ±0.005.

Very recently Yokoi and co-workers reported complexation of Cu^{2+} with poly(vinyl alcohol) and poly(acrylic acid) at pH > 3.[26] For both polymers spectrophotometric changes in the Cu^{2+} ligand field transitions were observed upon complexation. Since no spectral changes are observed for the Cu^{2+} ligand field transition at 800 nm upon addition of high PVP concentrations to aqueous Cu^{2+} solutions, it is assumed that the rate decrease is not linked to association of Cu^{2+} with the PVP.

In order to evaluate the inhibition of the porphyrin metalation by PVP in greater detail, the reaction was examined as a function of Cu^{2+} and PVP concentrations, maintaining pseudo-first-order conditions with at least a tenfold excess of Cu^{2+}. Initial kinetic measurements were made using pH 6 phosphate buffer to avoid the acetate catalysis in the insertion into H_4P^{2-} observed at lower pH.[16] Varying the Cu^{2+} concentration in solutions having a fixed measured excess of PVP reveals that the reaction is less than first order in Cu^{2+}. Decreases in the metalation rate occur upon increasing the [PVP]. The observed rate constants obtained are consistent with a mechanism involving preequilibrium association of **1** with PVP and metalation of free porphyrin as the rate-determining step (eq 7 and 8). The observed rate constant for the

$$[H_2P^{4-}]_f + PVP \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [H_2P^{4-}]_b \qquad K_b \qquad (7)$$

$$[H_2P^{4-}]_f + Cu^{2+} \xrightarrow[2]{k_{-2}} [CuP^{4-}] + 2H^+ \qquad (8)$$

sequence is given by eq 9 when the number of free binding sites

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[15] Molyneux, P. *Water-Soluble Synthetic Polymers: Properties and Behavior*; CRC Press: Boca Raton, FL, 1984; Vol. II, Chapter 2.

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$$(k_{\text{obsd}})^{-1} = [nK_b[\text{PVP}]_T/k_2[\text{Cu}^{2+}]] + 1/k_{-1} \quad (9)$$

is large relative to the concentration of bound porphyrin. Figure 3 shows a fit of the inverse of the observed rate constant vs. $[\text{PVP}]_T$ for metalation in pH 6.4 phosphate buffer. The slope of fits of $(k_{\text{obsd}})^{-1}$ vs. [PVP] or $[\text{Cu}^{2+}]^{-1}$ yields nK_b/k_2. Since k_2, the aqueous reaction rate, is known to be 250 M^{-1} s^{-1} at pH 6.4, K_b is calculated to be 5.0×10^8 and 1.05×10^8 M^{-1} from fits varying [PVP] and $[\text{Cu}^{2+}]$, respectively. The agreement between kinetic and spectrophotometric measurements (Table I) is poor (a factor of 35 from the fit varying [PVP]). Experimentally, the major difference is the buffer: acetate (pH 3) in the spectrophotometric case and phosphate (pH 6.5) in the kinetic studies. Numerous examples of generic effects on polymer properties such as the cloud point and viscosity have been observed upon addition of high concentrations of solutes. Eliassaf, Eriksson, and Eirich have shown that PVP exhibits no viscosity change in 0.10 M acetic acid whereas a 0.2 M sodium biphosphate solution (pH 8.7) causes a decrease in the viscosity.[17] Since the pH-independent equilibrium constant, K_b, does not account for polymer conformation changes that may occur with changes in pH and chemical composition of the buffer, the observed difference in K_b by the independent measurements may reflect such differences.

The kinetics of Cu^{2+} insertion into **1** in pH 3 acetate buffer are complicated by the fact that equilibria exist for both the association of acetate with Cu^{2+} and the porphyrin protonation (eq 3). Hambright and co-workers[16] have examined the metalation in detail at pH 3; the rate law obtained suggests three paths for Cu^{2+} insertion into the porphyrin (eq 8, 10, and 11).[16] If the

$$\text{CuAc}^+ + [\text{H}_2\text{P}^{4-}]_f \xrightarrow{k_3} [\text{CuP}^{4-}] + \text{HAc} + \text{H}^+ \quad (10)$$

$$\text{Cu}^{2+} + \text{Ac}^- + [\text{H}_4\text{P}^{2-}]_f \xrightarrow{k_4} [\text{CuP}^{4-}]_f + \text{HAc} + 3\text{H}^+ \quad (11)$$

porphyrin–polymer association (eq 6) is included in this proposed mechanistic scheme, a rate law is obtained having an observed rate constant with k_2, k_3, k_4, Q (the copper monoacetate formation constant), and K_b as variable parameters. By using values of k_3, k_4, and Q from ref 16 and fitting the observed rate constants at pH 3 vs. [PVP] using a simplex algorithm, a value of $(8 \pm 4) \times 10^8$ M^{-1} is obtained for K_b which is considerably larger than the value determined from direct spectrophotometric titration (1.4×10^7 M^{-1}). The mechanistic complexity of the porphyrin metalation at pH 3 interferes with the determination of the role of PVP in the reaction under these conditions and the poor correlation with spectrophotometric titration data is not surprising. However, introduction of PVP does inhibit the metalation process in a way similar to that at pH 6.4 (Table II).

Phosphorescence Quenching of **2** *by* MV^{2+}. The Pd(II) complex, **2**, also exhibits a red shift in the Soret absorption upon mixing aqueous solutions of **2** with aqueous solutions containing PVP at equimolar concentrations (Table III). The emission maximum does not change upon addition of PVP (Table III); however, the phosphorescence lifetime of complex **2** increases significantly. Quantitation of the association equilibrium of **2** with PVP by spectrophotometric methods was not possible because of the relatively small shift in the absorption maxima; however, luminescence quenching with methylviologen, MV^{2+}, in solutions having varying PVP concentrations allowed determination of K_b (vide infra).

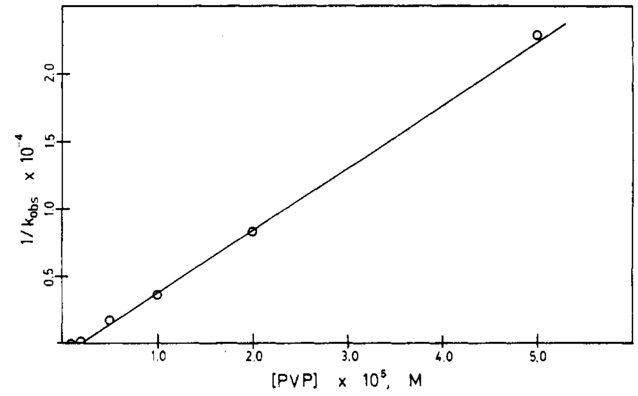

Figure 3. Plot of $1/k_{\text{obsd}}$ vs. $[\text{PVP}]_T$ for Cu^{2+} insertion in **1** at 25 °C. Solid line represents least squares fit to eq 14. $[\text{Cu}^{2+}] = 0.01$ M.

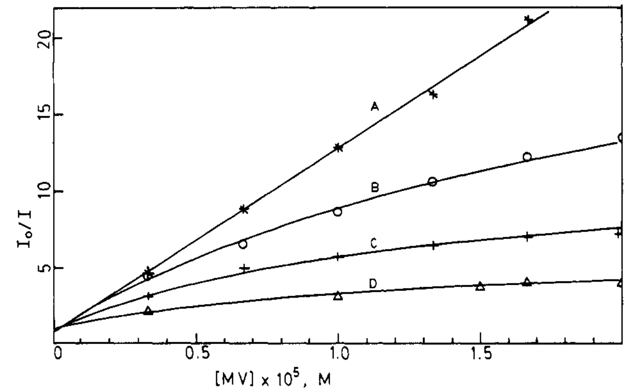

Figure 4. Stern–Volmer plots for quenching of **2** with methylviologen in solutions containing 0 M (a), 5×10^{-9} M (b), 1×10^{-8} M (c), and 2.5×10^{-8} M (d) PVP; pH 7; [**2**] = 10^{-6} M. Solid lines represent fits to eq 16.

TABLE III: Absorption and Luminescence Properties of 2 in Aqueous and Aqueous PVP Solutions

solvent	$\lambda^{\text{abs}}_{\text{max}}$ (log ϵ), nm	$\lambda^{\text{em}}_{\text{max}}$, nm	τ^0_{p}, μs
H_2O, pH 7	408 (5.19)	690	250 ± 10
H_2O, 10^{-5} M PVP, pH 7	420 (5.34)	690	373 ± 18

TABLE IV: Quenching Rate Constants and Association Equilibrium Constants for Quenching of 2 by MV^{2+}[a]

[PVP],[b] M	$k_q \times 10^{-9}$, M^{-1} s^{-1}	$K_b' \times 10^{-6}$, M^{-1}
0	4.64 ± 0.05	
5×10^{-9}	4.6 ± 0.1	5.0 ± 0.3
1×10^{-8}	3.6 ± 0.1	6.0 ± 0.4
2.5×10^{-8}	1.8 ± 0.2	5.2 ± 0.2

[a] Obtained from fits of I^0/I vs. $[MV^{2+}]$ to eq 16. [b] pH 7, phosphate buffer; [**2**] = 1×10^{-6} M.

Quenching of the phosphorescence of **2** by MV^{2+} in aqueous solutions follows Stern–Volmer kinetics and occurs at near diffusion-limited rates (eq 12; PdP^{4-} = **2**; τ_0 = lifetime of triplet state).[12] Quenching results in the oxidation of **2** and reduction

$$[\text{PdP}^{4-}] \xrightarrow{h\nu} [\text{PdP}^{4-}]^* \rightarrow [\text{PdP}^{4-}]^{3*} \xrightarrow{\tau_0} [\text{PdP}^{4-}] + h\nu \quad (12a)$$

$$[\text{PdP}^{4-}]^{3*} + MV^{2+} \xrightarrow{k_q} [\text{PdP}^{3-}] + MV^+ \quad (12b)$$

of MV^{2+}. Figure 4 illustrates the degree of quenching of **2**, I^0/I vs. $[MV^{2+}]$, in solutions having different concentrations of PVP. Strong association of MV^{2+} with PVP can be dismissed since the shape of the quenching curve would reflect little or no quenching at low $[MV^{2+}]$ and observable quenching only after saturation of the PVP with MV^{2+}. At high [PVP] ($>5 \times 10^{-5}$ M), all **2** is polymer bound, and complete suppression of quenching occurs; solutions saturated in MV^{2+} (~0.1 M) have I^0/I values less than 1.1. Thus, even if MV^{2+} does associate with PVP, the associated MV^{2+} does not quench the emission of polymer-bound **2**. As a result, quenching of **2** by MV^{2+} in the presence of PVP can be modeled assuming that only **2** in the aqueous phase is quenched (analogous to metalation of **1** with Cu^{2+}, eq 7 and 8). If excitation is at the isosbestic point of free and polymer-bound **2** and it is assumed that the radiative decay rate of **2** is independent of association, the emission quenching can be described in terms of the equilibrium constant for association of **2** and PVP, K_b', and the aqueous quenching rate, k_q (eq 13), where τ_p and τ^0_{PVP} rep-

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$$I^0/I = \frac{1 + K_b'[\mathrm{PVP}](\tau^0_{\mathrm{PVP}}/\tau^0_{\mathrm{p}})}{(1 + k_q\,\tau^0_{\mathrm{p}}\,[\mathrm{MV}^{2+}])^{-1} + K_b'[\mathrm{PVP}](\tau^0_{\mathrm{PVP}}/\tau^0_{\mathrm{p}})} \tag{13}$$

resent the lifetimes of aqueous and polymer-bound **2**, respectively (Table III). Fits of the data to eq 13 were obtained by using a Marquardt algorithm[18] with K_b' and k_q as variable parameters. Results of the fits, given in Table IV, indicate an average K_b' of $(5.4 \pm 0.3) \times 10^6$ M^{-1}. The total suppression of phosphorescence quenching of **2** in the presence of excess PVP provides kinetic boundaries for association and dissociation of **2** with PVP. Since the decay rate of excited **2** is 2700 s^{-1}, and no quenching is observed when **2** is completely polymer bound, the rate of dissociation of **2** bound to PVP must be significantly less than 2700 s^{-1}. From the measured K_b' an upper limit of 1.3×10^{10} $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ is estimated for the rate of association of **2** with PVP, assuming 2700 s^{-1} represents the rate of dissociation.

Flash photolysis of solutions of **2** and MV^{2+} in the presence of PVP indicates that no solvent-separated radical ions (PdP^{3-} and MV^{+}) are formed. Assuming quenching of **2** occurs only in the aqueous phase, the flash photolysis behavior is consistent with the observation that no PdP^{3-} and MV^{+} ions are observed upon flash photolysis of aqueous solutions of **2** and MV^{2+} where complete ion recombination occurs within the encounter complex.[10] This behavior contrasts that observed for the reaction of excited **2** and MV^{2+} in the presence of poly(vinyl alcohol), PVA,[10] where quenching of **2** bound to PVA does occur and charge separation following quenching is observed.

Since the reactions of both **1** with Cu^{2+} and excited **2** with MV^{2+} represent reaction with cationic reagents, the possibility of electrostatic control of reactivity cannot be dismissed (i.e., PVP may have a small fraction of resonance form **4**). If electrostatic effects do govern reactivity in the above cases then quenching of excited **2** with an anionic quencher should be accelerated in the presence of PVP. The dianionic quinone, anthraquinone-2,6-disulfonate, quenches the luminescence of **2** at diffusion-controlled rates[22] in aqueous solution. When the quenching is examined in the presence of 1 μM PVP and 0.5 μM **2**, all **2** is associated with the PVP and virtually no quenching of **2** is observed ($I^0/I < 2$) at the solubility limit of the dianionic quinone ($\sim 4 \times 10^{-4}$ M). This represents a suppression of the quenching rate by a factor of at least 250 relative to quenching in homogeneous solution. The observed rate suppression in this case serves to discount Coulombic factors as the principal reason for inhibition of the reactions of the bound porphyrins with cationic reagents.

Summary. The light-scattering characterization of the PVP used in this study indicates that the PVP is a narrow distribution macromolecule of $M_w = 47\,600 \pm 4000$ and equivalent hydrodynamic diameter of 123 ± 5 Å. The association of **1** with PVP does not lead to any observable aggregation, nor does it cause any measurable change in the PVP size or net conformation.

The porphyrins **1** and **2** associate with PVP in buffered aqueous solutions. Insertion of Cu^{2+} into **1** and quenching of **2** phosphorescence is slowed when the porphyrin is associated with PVP. Kinetic analysis of both porphyrin reactions is consistent with a rapid equilibrium association of the porphyrin with the polymer and reaction only of aqueous porphyrin. The kinetic inhibition can be rationalized by (1) electrostatic repulsion, if the polymer bears a net positive change or (2) the existence of hydrophobic pockets within the solubilized polymer that serve to exclude Cu^{2+} and MV^{2+}. Since luminescence quenching of **2** with an anionic quinone is slowed relative to aqueous solution, hydrophobic sequestering of the porphyrin by the polymer provides a more consistent explanation for the observed changes in reactivity.

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Registry No. **1**, 39174-47-5; **2**, 79745-32-7; PVP, 9003-39-8; MV^{2+}, 4685-14-7; Cu, 7440-50-8.

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Electron Transfer between Azurin and Metalloporphyrins

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The electron-transfer reactions between azurin and the triplet as well as the radicals of several water-soluble metalloporphyrins (ZnTPPS, ZnTPPC, and ZnTMPyP where TPPS is tetrakis(sulfonatophenyl)porphyrin, TPPC is tetrakis(carboxyphenyl)porphyrin, and TMPyP is tetrakis(*N*-methylpyridyl)porphyrin) have been studied by two different photoexcitation schemes. The second-order rate constant varies from 1.7×10^8 to 5.5×10^8 $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ ($\mu = 0.5$ M, pH 7, 25 °C) within the wide range of driving force (0.49–1.47 eV) covered in the experiment. A reorganization energy λ_{12} of about 1.1 eV and a maximum bimolecular electron-transfer rate $k_{\max}$ of 3.8×10^9 $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ were obtained by analyzing the data according to the theory of Marcus.

Introduction

Recently, we have employed the method of photoexcitation to study the kinetics of electron transfer between cytochrome *c* and metalloporphrins.[2,3] A pulse laser was used to convert porphyrin molecules first into strongly reducing triplet excited states[2] or anion radicals.[3] By studying the subsequent electron transfer between these porphyrin complexes and the protein, we have obtained electron-transfer rates over a wide range of driving force from 0.5 to 1.4 eV. The data have been analyzed according to the

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