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Medium effects on the dimerization of coproporphyrin-I free base

H. Morales-Rojas and Anatoly K. Yatsimirsky,*

Universidad Nacional Autónoma de México, Facultad de Química, 04510 México D.F., México

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ABSTRACT: Equilibrium constants of dimerization of the tetraanion of coproporphyrin-I free base were determined spectrophotometrically in aqueous solution at pH 9.0 in the presence of nine electrolytes, including salts of alkali metal, tetraalkylammonium and Ca(II) cations. Additions of all electrolytes promoted dimerization and the effect increased in the order tetraalkylammonium $\ll$ alkali metals $\ll$ Ca(II) salts. The Effects of alkali metal salts at concentrations below 0.1 M were quantitatively described in terms of Debye–Hückel model assuming an effective charge of the porphyrin monomer of -3. At higher salt concentrations salting-out/in effects must be taken into account. Addition of tetraalkylammonim salts to solutions containing 0.1 M NaCl produced the monomerization effect attributed to strong salting-in behavior of these salts. The effect of Ca(II) was described by complexation-induced dimerization of the porphyrin. Addition of organic co-solvents (methanol, ethanol, acetone, dioxane) to aqueous electrolyte solutions of the porphyrin produced a strong monomerization effect. Logarithms of dimerization constants extrapolated to the zero ionic strength in each mixed solvent roughly correlated with inverse dielectric constant with a negative slope considerably exceeding, however, that expected on the basis of a purely electrostatic model. Correlations with empirical solvent parameters (δ_H, E_T^N, S_P) were less satisfactory than the correlation with $1/\varepsilon$. A satisfactory description of solvent effects on dimerization constants was found in terms of a specific solvation model, which takes into account association of porphyrin monomer and dimer with several co-solvent molecules. Logarithms of the association constants were linear functions of the partial molar volume of the hydrocarbon portion of co-solvent molecules. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: coproporphyrin-I free base; dimerization; medium effects

INTRODUCTION

Hetero- and self-association reactions of porphyrins and structually related phthalocyanines have attracted considerable attention in connection with the significant role they play in biochemistry,[1,2] photochemistry,[3] catalysis[4] and, more recently, in the study of structural and thermodynamic aspects of different types of intermolecular interactions (electrostatic, π–π, hydrophobic, etc.) in solution.[5–9] An important and thoroughly explored porphyrin association reaction is the dimerization of water-soluble peripherally charged porphyrins.[10–22] Although the main factors which control the degree of porphyrin dimerization are well established by now, their interpretation remains largely at a qualitative level.

It is known that increased concentrations of inorganic salts promote porphyrin dimerization in water[10,13a,16] and methanol.[6] For salts of alkali metal cations from Li^+ to K^+ this effect is nonspecific and is attributed to a reduction of mutual repulsion of charged porphyrin monomers upon dimerization. A quantitative model of the salt (NaCl, LiCl) effect on dimerization of uroporphyrin-I and its metal derivatives was proposed.[13a] The model assumes a complexation of porphyrin monomer with one or more cations followed by dimerization of this complex. Another version of the cation complexation model, which assumed a one-step association equilibrium between two porphyrins and *n* salt cations, was proposed for the interpretation of porphyrin dimerization reactions induced by alkali metal salts in the presence of crown ethers.[16] In both cases the models were applied for the fitting of absorbance vs salt concentration profiles which were of a more or less pronounced sigmoid shape, in apparent agreement with a cooperative porphyrin–cation association. On the other hand, salt effects on the kinetics of metal incorporation reactions between various divalent metal cations and charged porphyrins were interpreted in terms of classical Debye–Hückel model.[23] Although the Debye–Hückel model is not appropriate for large non-spherical ions such as peripherally charged porphyrins, it often gives satisfactory results beyond the limits of its applicability[24,25] and therefore should not be ignored in the analysis of salt effects on porphyrin reactions. Porphyrins employed for aggregation studies in aqueous

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**Correspondence to:* A. K. Yatsimirsky, Facultad de Química, Universidad Nacional Autónoma de México, 04510 México D.F., México.
E-mail: anatoli@servidor.unam.mx

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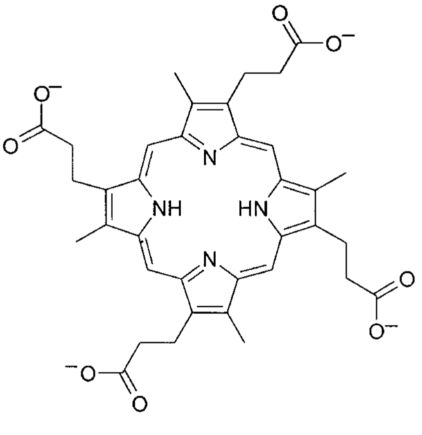

Tetraanion of coproporphyrin I (CP-I)

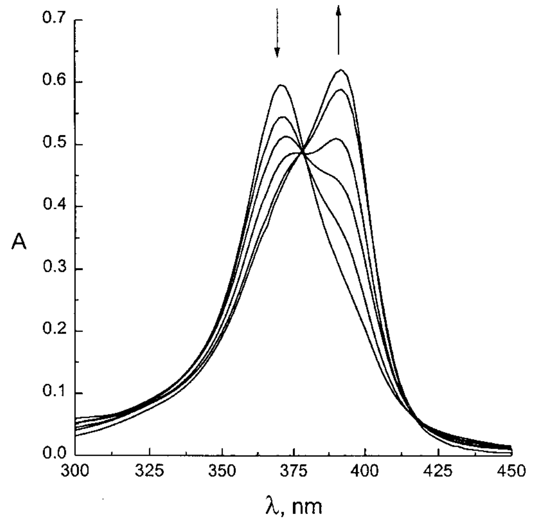

Figure 1. UV–visible absorption spectra in the Soret region of 4.2 μM CP-I in 0.1 M NaCl at pH 5.8, 6.4, 6.9, 7.4, 8.2 and 9.0. Arrows show the order of spectra recorded with increasing pH value

solutions are highly charged species bearing 4–8 ionogenic groups. Evidently, their activity must be strongly dependent on the ionic strength and the respective activity correction must be introduced before any association or other model can be considered.

Porphyrin dimerization is suppressed in organic solvents.[6,10] For charged porphyrins this effect can be due, at least partly, to increased repulsion of monomers in media of lower dielectric constant. However, uncharged porphyrins possess very small dimerization constants in organic solvents,[7,8] which is indicative of the importance of other factors, e.g. the hydrophobic effect. The available data on solvent effects are only qualitative, however, and it is not clear which solvent characteristics are important. A more systematic and quantitative study of solvent effects on porphyrin dimerization would help to identify the factors involved.

The purpose of this work was to perform a systematic study of salt and solvent effects on the dimerization of a single water-soluble porphyrin in order to develop a quantitative model of medium effects on thermodynamics of porphyrin self-association. We chose for this study coproporphyrin-I (CP-I), first because surprisingly little is known about dimerization of natural porphyrins and CP-I is a naturally occurring porphyrin without *meso*-substituents and second because its dimerization constants in aqueous electrolyte solutions appeared to be inside the interval 10^4–10^6 dm^3 mol^{-1} suitable for spectrophotometric measurements.

EXPERIMENTAL

Coproporphyrin-I chlorohydrate (Aldrich), organic solvents (Mallinckrodt), tetraalkylammonium and inorganic salts of reagent purity (Merck or Mallinckrodt), were used as supplied. All solutions were prepared in purified water (Milli-Q Reagent Water System) and contained 0.1 mM EDTA to prevent complexation of the porphyrin with traces of transition metals.

Spectrophotometric measurements were performed by using a Hewlett-Packard Model 8452A diode-array spectrophotometer and fluorescence spectra were recorded on a SPEX FluoroMax spectrofluorimeter equipped with thermostated cell holders. The pH was measured by using an Orion 710-A pH-meter. All measurements were made at 25° C.

Mathematical treatment of experimental data and the regression analysis were performed using the Origin (version 3.15) program. The porphyrin pK_a value, dimerization constants and formation constants of Ca(II) complexes were calculated from experimental results by non-linear least-squares fitting to the respective theoretical equations. Fitting of absorbance (A) vs porphyrin concentration profiles to Eqn (2) with three adjustable parameters, ε_M, ε_D and K_D, did not give convergent results and therefore the monomer molar absorptivity (ε_M) was determined by extrapolation of A/[porphyrin] vs [porphyrin] plots to [porphyrin] = 0. Fitting with only two adjustable parameters, ε_D and K_D, gave satisfactory results.

RESULTS AND DISCUSSION

Protonation of CP-I anion

Protonation reduces the charge and promotes the association of porphyrins in solution[13] and therefore must be taken into account in any study of porphyrin dimerization equilibria. Figure 1 shows UV–visible absorption spectra of CP-I in the Soret region at different pH values in 0.1 M NaCl. The isosbestic points at 378 and

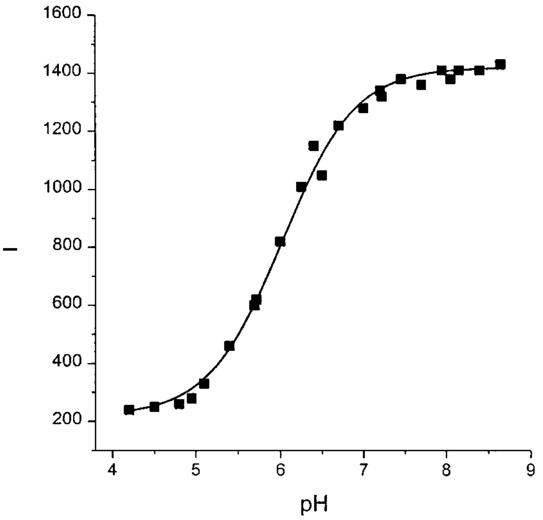

Figure 2. Plot of fluorescence intensity *I* (counts s^{-1}) vs pH. Experimental conditions: 0.055 μM CP-I, excitation wavelength 492 nm, emission wavelength 612 nm

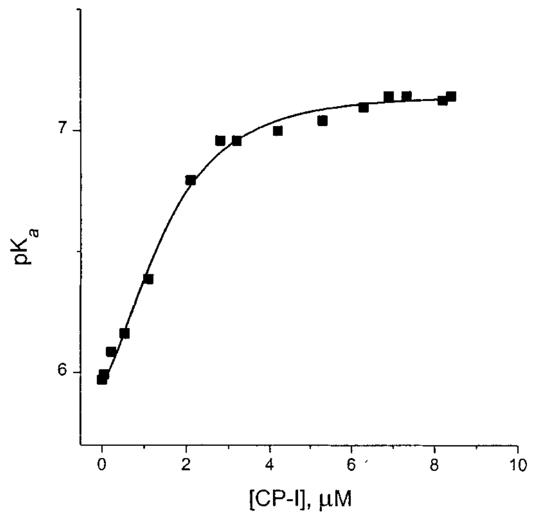

Figure 3. pK_a values of CP-I in 0.1 M NaCl at different concentrations of CP-I

420 nm indicate the presence of only two absorbing species in the pH range 5.8–9.0. Plots of absorbances (*A*) at fixed wavelengths (taken both above and below the isosbestic point at 378 nm) vs pH were of typical sigmoid shape and fitted perfectly Eqn (1) for a monoprotonation equilibrium:[26]

$$A = (A_{PH} + A_P K_a/[H^+])/(1 + K_a/[H^+]) \qquad (1)$$

where A_P and A_{PH} are the absorbances of deprotonated and protonated forms of CP-I and K_a is the acid dissociation constant of the protonated form. Under the conditions of Fig. 1 we found pK_a = 6.95 ± 0.05.

Protonation of CP-I caused considerable quenching of its fluorescence (CP-I possesses two emission maxima, one at 670 and a more intense one at 612 nm)[27] and we also calculated pK_a from the plot of fluorescence intensity (exemplified in Fig. 2) vs pH, which obeyed an equation similar to Eqn (1) with fluorescence intensities instead of absorbances. The value of pK_a calculated from these results was, however, considerably lower: pK_a = 6.00 ± 0.05. An important difference in reaction conditions for spectrophotometric and fluorimetric titration experiments was that the former employed a considerably higher CP-I concentration (4.2 μM) than the latter (0.055 μM). Since CP-I undergoes dimerization (see below), the observed difference in pK_a values may be attributed to a different degree of dimerization under conditions of the titration experiments. To test this possibility we determined pK_a values of CP-I by spectrophotometric titration at different concentrations (Fig. 3). Extrapolation of this plot to zero gives pK_a = 6.0, which can be attributed to monomeric CP-I, and the extrapolation to high concentrations where pK_a tends to ‘saturate’ gives *p*K_a = 7.15, which can be attributed to the dimer. A higher basicity of the dimer probably results from its higher negative charge.

Possible protonation sites of CP-I are pyrrole nitrogens and carboxylate groups. Considerable protonation-induced spectral changes are indicative of the protonation of pyrrole nitrogens. To prove this we performed spectrophotometric and fluorimetric titrations of the Zn(II) complex of CP-I in which pyrrole nitrogens were protected from protonation by coordination to the metal ion and, as expected, did not observe any spectral changes in the pH range 5–9. The basicity of the monomer is close to that of CP-I tetramethyl ester in sodium dodecyl sulfate solution (pK_a = 5.5)[28] and in both cases is considerably higher than is typical for porphyrin pyrroles. The most probable explanation of these increased pK_a values is the effect of the large negative charge from four carboxylates in the case of CP-I or from the surface of micelles of the anionic surfactant in sodium dodecyl sulfate solution.

Salt effects on dimerization of CP-I

The study of salt effects on the dimerization of CP-I was carried out at pH 9.0, where it is deprotonated in both the monomeric and dimeric forms and, consequently its dominant forms are the tetraanion for the monomer and the octaanion for the dimer. Figure 4(a) shows the absorption spectra of CP-I in the Soret region at increasing concentrations in the presence of 0.1 M NaCl. The gradual decrease in the molar absorptivity at 392 nm on going from low to high CP-I concentrations is indicative of an auto-association process. There is also a broadening of the Soret band as the concentration of CP-I increases: the calculated bandwidth at half-height changes from 36 nm for 1.5 μM to 46 nm for 10 μM

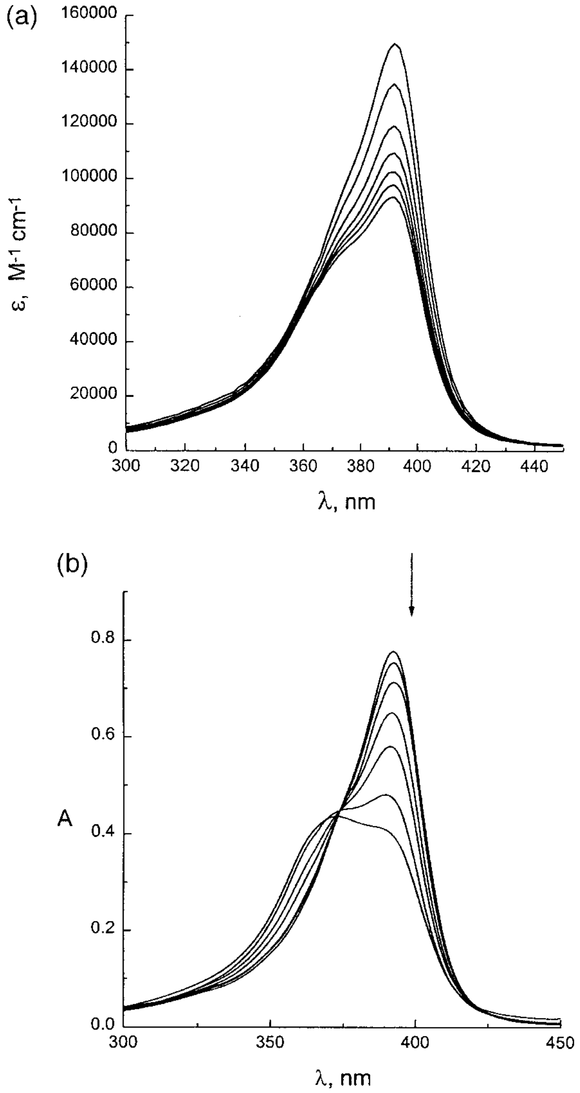

Figure 4. (a) Absorption spectra of CP-I in the Soret region with increasing concentrations in the presence of 0.1 M NaCl at pH 9.0 (borate buffer solution). Concentrations of CP-I (from bottom to top): 10, 8.6, 7.2, 5.7, 4.3, 2.6 and 1.5 μM. (b) Absorption spectra of 4.4 μM CP-I at pH 9.0 (borate buffer solution) in the presence of different concentrations of NaCl. The arrow shows the order of spectra recorded at increasing NaCl concentrations: 0, 0.01, 0.05, 0.1, 0.3 and 0.5 M

porphyrin concentration. Figure 4(b) shows the spectra of CP-I at fixed concentration in the presence of different NaCl concentrations. All spectra except that at [NaCl] = 0.5 M pass through an isosbestic point at 374 nm, indicating the presence of only two absorbing species at lower NaCl concentrations. In the presence of 0.5 M NaCl higher oligomers are probably also formed, although even at this ionic strength the absorbance vs CP-I concentration profile fitted the dimerization model well (see below). Similar concentration and salt-induced changes were observed for the visible bands in the range 450–650 nm.

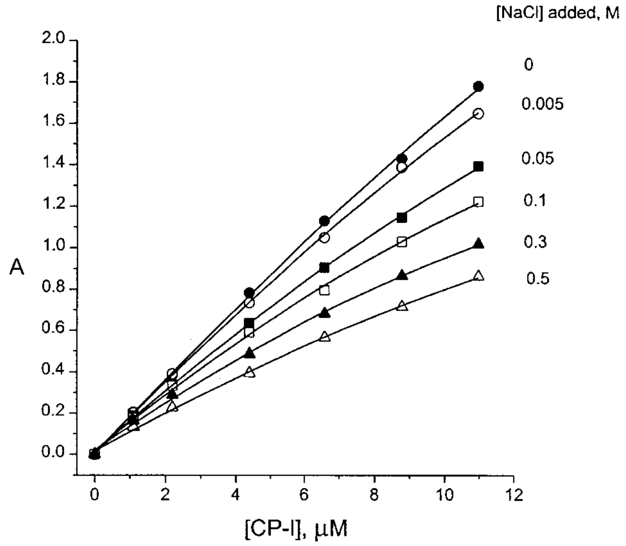

Figure 5. Plots of the absorbance at 392 nm vs CP-I concentration obtained with increasing concentrations of NaCl at pH 9.0. Solid lines are theoretical fits to Eqn (2)

Plots of the absorbance at 392 nm vs CP-I concentration obtained at increasing concentrations of NaCl are shown in Fig. 5. At the lowest ionic strength (*I*) in 5 mM borate buffer without added NaCl the plot is nearly linear, but becomes progressively more curved on going to higher NaCl concentrations. All curves obeyed Eqn (2) derived for the dependence of the observed absorbance (*A*) at fixed wavelength on the porphyrin concentration assuming a single dimerization equilibrium:[10]

$$A = 0.5\varepsilon_{\mathrm{D}}[\mathrm{CP\text{-}I}]_{\mathrm{t}} + (2\varepsilon_{\mathrm{M}} - \varepsilon_{\mathrm{D}}) \times((1 + 8K_{\mathrm{D}}[\mathrm{CP\text{-}I}]_{\mathrm{t}})^{1/2} - 1)/8K_{\mathrm{D}} \qquad (2)$$

where ε_M and ε_D are the molar absorptivities of monomer and dimer, respectively, $[\mathrm{CP\text{-}I}]_t$ is the total concentration of CP-I and K_D is the dimerization constant. The constants calculated by nonlinear least-squares fitting of results in Figure 5 to Eqn (2) (see Experimental for details) are collected in Table 1. Plots similar to those in Figure 5 were obtained also at four maxima of CP-I in the visible region (500, 536, 556 and 608 nm) in 0.1 M NaCl. All of them followed Eqn (2) and the fitting of the results produced the K_D values coinciding with that from the concentration dependence in the Soret band within experimental error.

Dimerization constants in the presence of other salts were found by using a simplified approach based on calculation of the ratios of monomer and dimer concentrations, [M]/[D], directly from the absorption spectra. Evidently, with known [M]/[D] and total concentration of CP-I one can easily calculate the dimerization constant. As is seen from Fig. 4, the monomer and dimer possess different absorption maxima and the ratio of absorbances at 392 and 372 nm can serve

Table 1. Salt effects on the dimerization constant of CP-I at 25°C and pH 9.0[a]

Added salt	Concentration (M)	Log K_D[b]
None		3.62
NaCl	0.005	3.79
	0.01	3.93
	0.05	4.60
	0.1	4.92
	0.3	5.34
	0.5	5.82
KCl	0.01	3.95
	0.05	4.49
	0.1	4.81
	0.3	5.46
	0.5	5.68
$NaNO_3$	0.01	3.97
	0.05	4.45
	0.1	4.78
	0.3	5.23
	0.5	5.56
KI	0.01	3.86
	0.05	4.43
	0.1	4.88
	0.3	5.20
	0.5	5.46
KSCN	0.01	3.91
	0.05	4.46
	0.1	4.76
	0.3	5.04
	0.5	5.42
NEt_4Br	0.005	3.96
	0.01	4.04
	0.05	4.11
	0.1	4.18
NBu_4Cl	0.005	3.89
	0.01	3.98
	0.05	4.15
	0.1	4.18
$Ca(NO_3)_2$	0.002	4.63
	0.005	5.02
	0.01	5.30
	0.05	5.76
	0.1	5.91
	0.2	5.99

[a] Borate buffer solution, ionic strength 0.005 M.
[b] Relative error in K_D ($dm^3\ mol^{-1}$) $\leq \pm$ 10%.

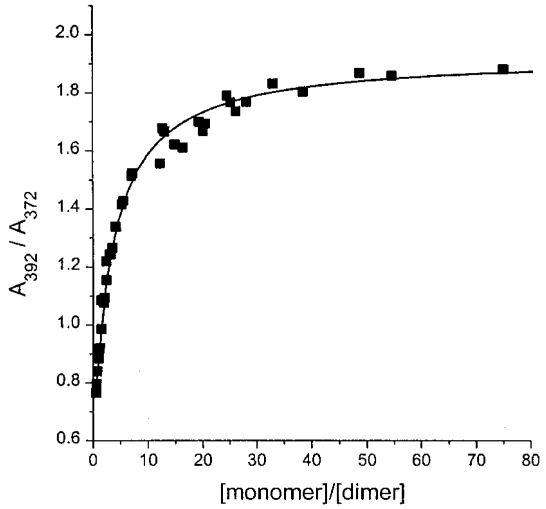

Figure 6. Ratio of absorbances of CP-I at 392 and 372 nm (A_{392}/A_{372}) plotted vs the ratio of monomer and dimer concentrations calculated at different NaCl and total porphyrin concentrations from the respective values of dimerization constants. The solid line is the theoretical fit to Eqn (4)

as a sensitive measure of the degree of dimerization. Indeed, this ratio is given by the equation

$$\begin{aligned} A_{392}/A_{372} &= (\varepsilon_M{}^{392}[M] + \varepsilon_D{}^{392}[D])/ \\ &\quad (\varepsilon_M{}^{372}[M] + \varepsilon_D{}^{372}[D]) \\ &= (\varepsilon_M{}^{392}[M]/[D] + \varepsilon_D{}^{392})/ \\ &\quad (\varepsilon_M{}^{372}[M]/[D] + \varepsilon_D{}^{372}) \\ &= (a[M]/[D] + b)/(c + [M]/[D]) \end{aligned} \tag{3}$$

where *a*, *b* and *c* are the ratios of molar absorptivities $\varepsilon_M{}^{392}$, $\varepsilon_D{}^{392}$ and $\varepsilon_M{}^{372}$ to $\varepsilon_D{}^{372}$. In order to obtain the best evaluations of the parameters of Eqn (3), we used all spectra obtained in the presence of NaCl. From each of them the ratio A_{392}/A_{372} was taken and plotted vs the ratio [M]/[D] calculated for the given conditions from total concentration of CP-I and the respective dimerization constant (Fig. 6). This plot was fitted to Eqn (3) and the resulting equation took the form

$$\begin{aligned} A_{392}/A_{372} = {} & \{(1.926 \pm 0.013)[M]/[D] + (1.984 \\ & \pm 0.255)\}/\{(3.382 \pm 0.261) \\ & + [M]/[D]\} \end{aligned} \tag{4}$$

Equation (4) allows one to determine the ratios [M]/[D] in the interval 0–40 with a relative error $\leq\pm$ 10%. At higher ratios the slope of the plot becomes very small and the use of Eqn (4) gives large errors. This equation was used to calculate the dimerization constants of CP-I in the presence of eight additional salts (Table 1).

The results in Table 1 show that, as expected, additions of electrolytes increase the dimerization constant of CP-I. The effects of 1:1 salts of alkali metal cations are similar at concentrations below 0.1 M, but in more concentrated solutions salts with larger anions ($NO_3{}^-$, I^- and SCN^-) are noticeably less effective as promoters of dimerization. We assume that the non-specific salt effect in diluted ($\leq$0.1 M) solutions can be treated in terms of classical Debye–Hückel model (see below), whereas in more concentrated solutions more specific interactions of CP-I with electrolyte ions must be taken into account. In particular, the difference between chlorides and salts of other anions may be due to different 'salting-out/in'

Table 2. Effects of tetraalkylammonium salts on the dimerization constant of CP-I at 25°C and pH 9.0 in the presence of 0.1 M NaCl

Added salt	Concentration (M)	LogK_D[a]
None		4.92
NEt_4Br	0.0012	4.86
	0.0025	4.86
	0.01	4.74
	0.02	4.62
	0.05	4.44
NBu_4Cl	0.0012	4.63
	0.0025	4.49
	0.01	3.98
	0.02	3.92
	0.05	3.95

[a] Relative error in K_D (dm^3 mol^{-1}) $\leq \pm$ 10%.

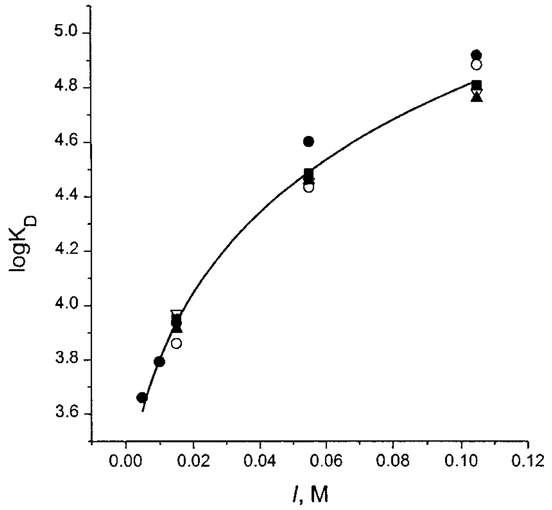

Figure 7. Dependence of the logarithm of the dimerization constant (K_D) on the ionic strength (I) in the range of $I \leq 0.1$ M. Data from Table 1. (●) NaCl; (■) KCl; (○) KI; (▽) $NaNO_3$; (△) KSCN. The solid line is the theoretical fit to Eqn (6)

effects since small anions usually cause salting-out, but larger anions often cause the salting-in effect.[29] The salting-out/in coefficients are usually small and this effect is noticeable only at sufficiently high electrolyte concentrations. It is expected that for chlorides the salting-out effect should promote the dimerization but the salting-in effect possible with other salts will probably oppose the dimerization owing to stabilizing interactions of large anions with the surface of porphyrin monomers.

Repulsion of anions by the negative charge of the CP-I tetraanion should considerably weaken their salting-in effect. In contrast, a similar salting-in effect typical of cations of tetraalkylammonium salts[29] can be enforced by electrostatic attraction. Indeed, alkylammonium salts cause a very small increase in the dimerization constant (Table 1). Moreover, addition of these salts to CP-I solution in 0.1 M NaCl causes its monomerization (Table 2). In the last experiment a weak effect of alkylammonium salts as promoters of dimerization becomes undetectable with the background of a much stronger effect of NaCl and one can see the manifestation of only their 'salting-in' effect.

Addition of $Ca(NO_3)_2$ caused a much stronger increase in the dimerization constant than all 1:1 electrolytes studied (Table 1). This effect cannot be attributed to an increased ionic strength of solution since already at $I = 0.02$ M with calcium nitrate K_D is larger than at $I = 0.105$ M with salts of alkali metal cations. As will be shown later, the effect of the calcium salt can be quantitatively described by the complexation-induced dimerization model.

Let us consider now the non-specific salt effect of dilute electrolytes. New equations for electrolyte activity coefficients developed in recent decades[30a,b] were focused principally on extension of the classical Debye–Hückel model to high electrolyte concentrations. In the range of ionic strengths below 0.1 mol dm^{-3}, a simple Debye–Hückel-type equation [Eqn (5)] usually gives a satisfactory approximation:[30c]

$$\log\gamma = -0.51z^2I^{1/2}/(1 + BI^{1/2}) \qquad (5)$$

The parameter B is usually set equal to 1.5.[30c,d] Applying this equation to the CP-I dimerization equilibrium and assuming that the charge of the dimer equals twice the charge of the monomer, one obtains the following expression for the dimerization constant:

$$\log K_D = \log K_D{}^0 + 1.02z_M{}^2I^{1/2}/(1 + 1.5I^{1/2}) \qquad (6)$$

where $K_D{}^0$ is the dimerization constant at $I = 0$ and z_M is the monomer charge.

Figure 7 shows the plot of logK_D vs I in the range of $I \leq 0.1$ M. Fitting of these data to Eqn (6) gives $z_M{}^2 = 8.85 \pm 0.30$ and log $K_D{}^0 = 3.05 \pm 0.04$. Therefore, CP-I behaves in solution as an anion of effective charge −3, not much smaller than its total charge of −4. Although Eqn (5) and consequently Eqn (6) are generally not valid for higher ionic strengths, the results for NaCl, KCl and to a lesser extent $NaNO_3$ obtained at I values of 0.305 and 0.505 M agree well with Eqn (6), which predicts for these ionic strengths logK_D = 5.36 and 5.64 dm^3 mol^{-1}, respectively, with the above $z_M{}^2$ and $K_D{}^0$ values. The dimerization constants for KI and KSCN at these high ionic strengths are considerably lower.

The results for $Ca(NO_3)_2$ not only disagreed with those for alkali metal salts at the same ionic strength but even did not follow Eqn (6) qualitatively. These results agreed well, however, with the model involving complex formation of the monomer and dimer of CP-I with Ca^{2+} in addition to the dimerization process. Carboxylate complexes of Ca^{2+} possess noticeable stability. Thus, the formation constant of the monoacetate complex is 15 dm^3 mol^{-1} at zero ionic strength.[31] The carboxylate groups of CP-I seem to be too distant from each other to allow the simultaneous coordination of Ca^{2+} to two such groups. Therefore, one may expect a stepwise coordination of up to four calcium cations to CP-I monomer. In the dimer, bis-coordination may be possible with the carboxylate groups of two different porphyrin units in addition to mono-coordination similar to that with the monomer. Owing to the large distances between carboxylate groups, we considered binding of at least the two first Ca^{2+} cations to be independent of each other (that is, the single formation constant K_1 was used for the complexation of the first and second Ca^{2+} cations to the monomer and the single constant K_2 for the first and second complexation of Ca^{2+} to the dimer of CP-I). In order to find an adequate model, we fitted the experimental data first to the simplest scheme which involved only one 1:1 complex of Ca^{2+} with the monomer and one 1:1 complex with the dimer. Then we added another complexation step and repeated the fitting until the addition of a new step did not improve the fitting quality further. Such a minimum scheme involved the following set of equilibria:

$$2P \rightleftharpoons P_2 \qquad (K_D) \qquad (7)$$

$$P + Ca^{2+} \rightleftharpoons P(Ca^{2+}) \qquad (K_1) \qquad (8)$$

$$P(Ca^{2+}) + Ca^{2+} \rightleftharpoons P(Ca^{2+})_2 \qquad (K_1) \qquad (9)$$

$$P_2 + Ca^{2+} \rightleftharpoons P_2(Ca^{2+}) \qquad (K_2) \qquad (10)$$

$$P_2(Ca^{2+}) + Ca^{2+} \rightleftharpoons P_2(Ca^{2+})_2 \qquad (K_2) \qquad (11)$$

where P is the monomer of CP-I, P_2 is the dimer and K_D is the dimerization constant without added calcium salt under the given conditions. The expression for the observed dimerization constant $K_D{}^{obs}$ in accordance with Eqns (7)–(11) takes the form

$$K_D{}^{obs} = K_D(1 + K_2[Ca^{2+}] + K_2{}^2[Ca^{2+}]^2)/ \times(1 + K_1[Ca^{2+}] + K_1{}^2[Ca^{2+}]^2)^2 \qquad (12)$$

Since variation of the $Ca(NO_3)_2$ concentration changes the ionic strength and the dimerization was shown to be sensitive to it, each equilibrium constant in Eqn (12) was corrected for the ionic strength effect by introducing the factors which follow from Debye–Hückel treatment $\{K = 2.3\,K_0 \exp\,[1.02 z_A z_B I^{1/2}/(1 + 1.5 I^{1/2})]$, where K_0 is the respective equilibrium constant at zero ionic strength$\}$ with charges +2, −3 and −6 for calcium cation, CP-I monomer and CP-I dimer, respectively. Figure 8 shows the fitting of the results in Table 1 for $Ca(NO_3)_2$ to Eqn (12) with values of the association constants at zero ionic strength of $K_1 = 11.0 \pm 0.5$ dm^3 mol^{-1} and $K_2 = (6.56 \pm 0.05) \times 10^3$ dm^3 mol^{-1}. The value of K_1 is close to the stability constant of the acetate complex of Ca^{2+} (see above) and therefore can be attributed to the mono-coordination of Ca^{2+} to one of the carboxylate groups of CP-I, whereas K_2 is considerably higher and can be attributed to a chelate complex.

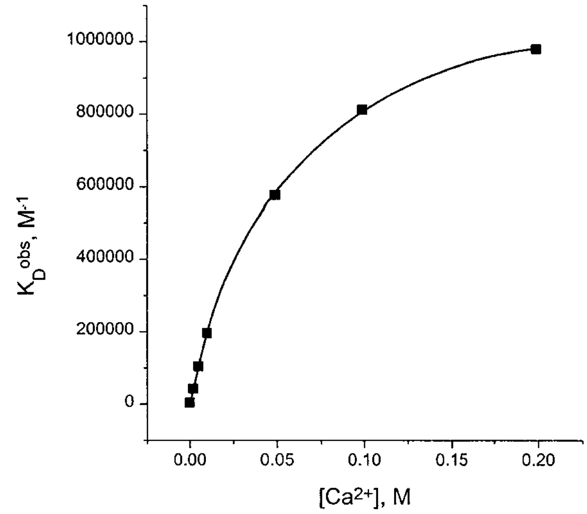

Figure 8. Fitting of the results of Table 1 for $Ca(NO_3)_2$ to Eqn (12)

Temperature effects on dimerization of CP-I

Dimerization constants of CP-I in the presence of 0.1 M NaCl at pH 9.0 were determined in the temperature range 15–60 °C. A good linear plot in the van't Hoff coordinates was observed from which the values of $\Delta H° = -41.0 \pm 0.8$ kJ mol^{-1} and $\Delta° = -45.6 \pm 0.8$ J mol^{-1} K^{-1} were calculated. Thus CP-I shows, typical for many porphyrins,[18] exothermic dimerization due to van der Waals interactions between extended π-electronic systems of monomers.

Solvent effects on dimerization of CP-I

Additions of organic co-solvents (methanol, ethanol, acetone and dioxane) strongly decreased the dimerization. Already in the presence of *ca* 5% (v/v) of any of these co-solvents at low ionic strength created by buffer solution (0.005 M) the dimerization constant was too small for spectrophotometric determination. In view of this, we measured K_D in the presence of 0.1 or 0.5 M NaCl. In preliminary experiments we found that the spectra of monomeric and dimeric forms did not change in the presence of 0–10% (v/v) of acetone and dioxane or 0–20% (v/v) of methanol and ethanol. This allowed us to

Table 3. Effects of organic co-solvents on the dimerization constant of CP-I at 25 °C and pH 9.0 in the presence of 0.1 or 0.5 M NaCl

Co-solvent	Concentration (%, v/v)	[NaCl] (M)	LogK_D[a]	LogK_D^0
None		0.1	4.92	3.05
		0.5	5.82	
Acetone	2	0.1	4.47	2.72
	2	0.5	5.30	2.66
	5	0.1	4.10	2.30
	5	0.5	4.73	2.00
	10	0.5	4.10	1.26
Ethanol	2	0.1	4.59	2.84
	2	0.5	5.39	2.75
	5	0.1	4.35	2.54
	5	0.5	5.09	2.37
	10	0.5	4.62	1.76
	15	0.5	4.21	1.21
Dioxane	2	0.5	5.13	2.50
	5	0.5	4.60	1.84
	10	0.5	4.01	1.09
Methanol	2	0.5	5.43	2.8
	5	0.5	5.24	2.56
	10	0.5	4.90	2.12
	15	0.5	4.59	1.71
	20	0.5	4.29	1.31

[a] Relative error in K_D ($dm^3\ mol^{-1}$) $\leq \pm$ 10%.

calculate K_D by using a simplified procedure based on Eqn (4). In several cases K_D was determined also from the absorbance vs CP-I concentration plots by using Eqn (2) and the results of the two methods agreed very well. All results obtained are given in Table 3.

Addition of organic co-solvents to solutions containing electrolytes changes both solute–solvent and solute–ionic atmosphere interactions. In order to correct for the latter effect we applied Eqn (6) with the above parameters corrected for each mixed solvent from the theoretical Debye–Hückel expressions[32] which predict that numerator in Eqn (5) is proportional to $\varepsilon^{-3/2}$ and B is proportional to $\varepsilon^{-1/2}$, where ε is the solvent dielectric constant (these corrections were, in fact, small and did not exceed 15%). The appropriate form of Eqn (6) for each mixed solvent was used to calculate the values of dimerization constants at $I = 0$ and the respective log K_D^0 values are given in Table 3.

Inspection of the results in Table 3 shows that additions of organic co-solvents in the concentration range 0–20% (v/v) lead to a *ca* 100-fold decrease in K_D^0. This effect can result partially or even completely from the increased repulsion of tetraanionic monomers in media of lower dielectric constants. To test the significance of this contribution we plotted log K_D^0 values vs $1/\varepsilon$ (Fig. 9) and found surprisingly good linear correlations within each solvent mixture and a satisfactory common correlation ($r = 0.95552$, $SD = 0.194$) for all co-solvents employed. However, the slope of common regression line, $-(1420 \pm 90)$, is much larger than one would expect for a purely electrostatic contribution. For example, in terms of the Born equation for the transfer free energy such a slope for an ion of charge -3 (an effective value which fits the salt effect) would be observed if the ion radius were 0.9 Å, which coincides with the crystal radius of Li^+ and is hardly acceptable for the large CP-I anion. Of course, the Born equation is a very rough approximation to solvation free energies. In

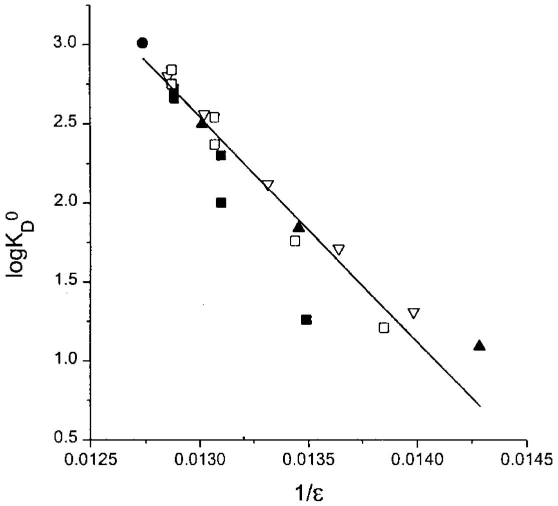

Figure 9. Plot of the logarithm of dimerization constant at zero ionic strength (K_D^0) vs the inverse dielectric constant (ε). Data from Table 3. (●) Pure wate; other points, water–co-solvent mixtures with co-solvents (■) acetone, (□) ethanol, (▽) methanol and (△) dioxane

view of known difficulties in the theoretical treatment of solvation, solvent effects are often treated in terms of correlations with various empirical solvent parameters.[33] Therefore, we attempted to correlate $\log K_D{}^0$ with several such parameters available for aqueous–organic solvent mixtures.

The 'driving force' of porphyrin dimerization in aqueous solutions is a combination of polar (so-called $\pi-\pi$) and hydrophobic interactions.[8] Therefore, we considered first possible correlations with polarity ($E_T{}^N$,[32a,b] and $Y^{32a,c}$ values are available for aqueous–organic mixtures, but only the $E_T{}^N$ scale was used since the parameters are interrelated[33e]) and solvophobicity (S_P)[33d] parameters. Correlations with parameters of both types were less satisfactory than with $1/\varepsilon$: $r = 0.90178$, SD = 0.272 for correlation with $E_T{}^N$ and $r = 0.8202$, SD = 0.36 for correlation with S_P. An attempt to improve the description of the solvent effect by using a two-parameter correlation with both $E_N{}^T$ and S_P did not give a better result. Moreover, we found unexpectedly that an interrelation between these different by their physical meaning parameters exists for the solvent mixtures employed:

$$S_P = -(0.072 \pm 0.107) + (1.08 \pm 0.11)E_T{}^N \qquad (13)$$

with $r = 0.915$, SD = 0.0159. A similar interrelation between these two parameters was reported previously for binary methanol–water mixtures.[9] Evidently, one cannot discriminate the polar and solvophobic contributions to the dimerization free energy with the given set of co-solvents, but what seems to be important is that the empirical parameters do not give better correlation than $1/\varepsilon$.

We also attempted to correlate $\log K_D{}^0$ with the Hildebrand solubility parameter $\delta_H{}^{34}$ and to use linear combinations of δ_H with $E_N{}^T$ or S_P. The correlation with δ_H was of a lower quality ($r = 0.711$, SD = 0.442) than with $E_N{}^T$ or S_P and two-parameter correlations did not give better results than the single-parameter correlations considered above.

It should be noted that the analysis of solvent effects in mixed solvents is especially difficult owing to often observed preferable solvation of the solute by one of the solvent components. To take this phenomenon into account it was suggested to analyze the results in terms of a model which considers solvation of a solute X as an exchange equilibrium between bound and free molecules of two (or more) solvent components S_1 and S_2 with an equilibrium constant β:[35]

$$X(S_1)_i(S_2)_j + pS_2 \rightleftharpoons X(S_1)_{i-q}(S_2)_{j+p} + qS_1$$

At low concentrations of added organic co-sovents used in this study one can consider the concentration of water to be practically constant and substitute simple solute–co-solvent association equilibria for the exact exchange equilibria. Since CP-I is a large symmetrical species, its association equilibria with several first co-solvent molecules can be considered independent and characterized by the same association constant β. In addition, the dimer and monomer of CP-I are chemically similar species and their affinity to co-solvent molecules should be the same. With this assumption the expression for $K_D{}^0$ takes the form

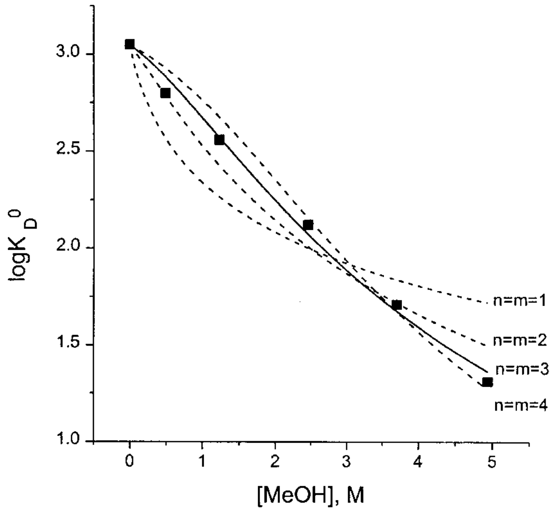

Figure 10. Fitting of the log $K_D{}^0$ vs methanol concentration profile to Eqn (14) at different values of n and m

$$K_D{}^0 = K_D{}^0{}_W(1 + \beta[S] + \beta^2[S]^2 \ldots + \beta^n[S]^n)/$$
$$\times(1 + \beta[S] + \beta^2[S]^2 \ldots + \beta^m[S]^m)^2 \qquad (14)$$

where $K_D{}^0{}_W$ is the dimerization constant in pure water, S is a co-solvent molecule, n and m are solvation numbers of CP-I dimer and monomer, respectively, and β is the association constant which refers to the consecutive additions of cosolvent molecules to the monomer (P) and the dimer (P_2):

$$P + S \rightleftharpoons P(S)\,(\beta), P(S) + S \rightleftharpoons P(S)_2\,(\beta), \text{ etc.}$$

$$P_2 + S \rightleftharpoons P_2(S)\,(\beta), P_2(S) + S \rightleftharpoons P_2(S)_2\,(\beta), \text{ etc.}$$

The values of $K_D{}^0$ in each water–solvent mixture were fitted to Eqn (14) with increasing n and m starting from $n = m = 1$ and in all mixtures no further improvement of the fitting was observed after $n = m = 3$. The procedure is illustrated in Fig. 10 for methanol. The solvation number 3 most probably is a mean value which gives the best fit with the fixed binding constant. One may expect a larger total solvation number with decreasing values of the binding constants, but with the available data the fitting to the respective more general model does not give reliable results.

Table 4. Association constants (β) of organic co-solvents with CP-I calculated from the fitting of $K_D{}^0$ vs co-solvent concentration profiles (data from Table 3) to Eqn (16) with $n = m = 3$ (see Fig. 10 for an example of the fitting) and partial molar volumes ($\overline{V}_H$) of the hydrocarbon portion of the co-solvent molecules calculated from the increments given in Ref. 35

Co-solvent	β (dm^3 mol^{-1})	$\overline{V}_H$ (cm^3 mol^{-1})
Methanol	0.66 ± 0.02	19.06
Ethanol	1.31 ± 0.06	34.86
Acetone	2.36 ± 0.15	47.66
Dioxane	3.56 ± 0.08	56.36

This model thus explains the monomerization effect of co-solvents by a higher solvation of two monomers, which possess the same affinity (probably of hydrophobic nature, see below) to organic co-solvent molecules as the dimer, but can bind larger number of these molecules: six instead of three. The fact that the total solvation numbers of one monomer and one dimer species are the same agrees well with a twofold reduction in the porphyrin surface available for interaction with co-solvent molecules upon dimerization.

The values of β for the co-solvents employed are given in Table 4. Searching for an interrelation of β with structure of co-solvent molecules we found a good correlation between log β and the partial molar volume of the hydrocarbon portion ($\overline{V}_H$) of the co-solvent molecule calculated from the increments given in Ref. 36:

$$\log \beta = -0.56 \pm 0.01 + (0.0200 \pm 0.0003)\overline{V}_H \qquad (15)$$

with $r = 0.9998$ and SD = 0.007. It follows from Eqn. (14) that

$$\beta = 0.275 \exp(0.046\overline{V}_H) \qquad (16)$$

for all co-solvents. Figure 11 illustrates the applicability of Eqn. (14) with $n = m = 3$ and β calculated from Eqn (16) for correlation of $K_D{}^0$ values: the plot of calculated log $K_D{}^0$ vs the experimental values is linear with a slope of 0.98 ± 0.03, r = 0.98909 and SD = 0.09.

It should be noted that the molar volume is now considered to be an important correlation parameter often giving a dominant contribution to linear free energy relationships with transfer free energies, e.g. from water to octanol[37] or from water to surfactant micelles.[38] The correlation in Eqn (15) agrees with the assumption that porphyrin–co-solvent interactions are of hydrophobic (or lipophilic) nature. We suppose that for the given series of co-solvents each carbon atom, methyl or methylene group of a co-solvent molecule is in contact with the plane of the porphyrin anion while co-solvent oxygen atoms remain in contact with surrounding water molecules.

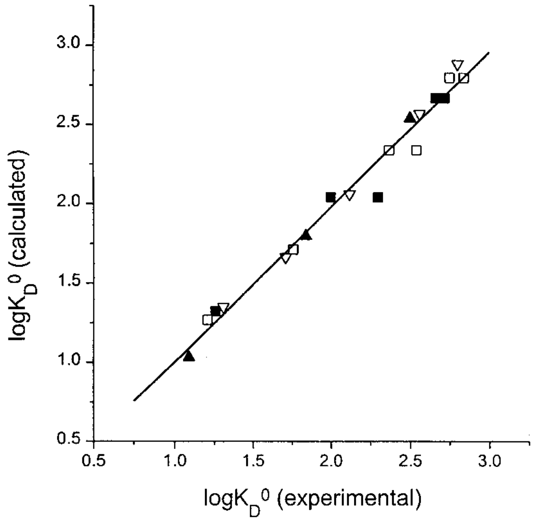

Figure 11. Calculated from Eqn (14) vs experimental log $K_D{}^0$ values [$n = m = 3$, β values for each co-solvent from Eqn (16)]. Points for different co-solvents are shown with the same symbols as in Fig. 9

It follows from Eqn. (15) that each additional methylene group ($\overline{V}_H = 15.8$ cm^3 mol^{-1} for acyclic and 14.09 cm^3 mol^{-1} for cyclic structures)[36] in a co-solvent molecule contributes by approximately -1.7 kJ mol^{-1} to the free energy of association with CP-I. Weak hydrophobic interactions of water-soluble porphyrins with side-chains of aliphatic amino acids have been reported.[39] In accordance with these data, the increment to the binding free energy per methylene group is *ca* -3 kJ mol^{-1}. On the other hand, Schneider and Wang[9b] concluded on the basis of their study of the association of cationic *meso*-substituted porphyrins with anionic species of different structures that hydrophobic interactions do not contribute noticeably to porphyrin–ligand association free energy (ΔG_{ASS}) because saturated ligands lacking π-electrons showed very weak binding, completely explicable by only electrostatic attraction of anionic ligands to cationic porphyrins. However, their data also demonstrate that incorporation of additional aliphatic carbons to anionic ligands always leads to improved binding. Thus, ΔG_{ASS} for 3,5-dimethylbenzoate is by 0.4–1.3 kJ mol^{-1} lower than that for unsubstituted benzoate; also, ΔG_{ASS} for cyclohexanedicarboxylate is 2.3 kJ mol^{-1} lower than that for adipate, which possesses two methylene groups fewer. These results indicate a weak hydrophobic contribution of *ca* -1 kJ mol^{-1} per methylene group, which is not far from the increment found for the co-solvents in the present study.

CONCLUSIONS

The applicability of a Debye–Hückel-type equation to the quantitative evaluation of activity coefficients of large non-spherical ions, provided a reduced effective charge is attributed to such ions, seems to be a general rule. In addition to the present study, the salt effects on stability constants of inclusion complexes of tetracationic and tetraanionic cyclophanes were successfully described in this way assuming the effective charges of the macrocycles to be only slightly lower than their total values.[24] Also, salt effects on the kinetics of metallation of charged porphyrins were described in terms of this model with effective charges of porphyrins ranging between 10 and 80% of their total charges.[23] For salts composed of large anions or large (tetraalkylammonium) cations the salting-in effect must be taken into account. Also, deviations from a simple Debye–Hückel model should be expected with highly charged cations such as Ca(II). The effect if this cation was described in terms of a model which involved a set of complexation equilibria of Ca(II) cation with carboxylate groups of monomeric and dimeric CP-I. Such a model may be generally applicable for salts of cations which form complexes of noticeable stability with peripheral polar groups of porphyrins.

Addition of organic co-solvents causes a considerably larger monomerization effect than one would expect on the basis of a purely electrostatic model which attributes the effect to increased repulsion of CP-I tetraanions in media with decreased dielectric constants. Attempts to correlate the effect with various empirical solvent parameters were unsuccessful. A specific solvation model which assumes stepwise association of monomer and dimer species with co-solvent molecules describes satisfactorily the solvent effect as a result of higher solvation of two porphyrin monomers than the dimer. Logarithms of porphyrin–co-solvent association constants show a linear correlation with the partial molar volume of the hydrocarbon portion of co-solvent molecules, indicating a predominantly hydrophobic type of association.

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