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Recognition of protonated aliphatic *α,ω*-diamines by coproporphyrin I tetraanion in water

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ABSTRACT: Interactions of aliphatic α,ω-diamines [$H_2N(CH_2)_nNH_2$, $2 \leq n \leq 8$] with coproporphyrin I tetraanion (CP) were studied by spectrophotometry, fluorimetry and ^1H NMR spectroscopy in the pH range 7–10 and ionic strengths 0.01–0.1 M. Diprotonated diammonium cations induce dimerization of CP by forming 1:1 complexes with CP which undergo much stronger self-aggregation than free CP tetraanions. On increasing the number of methylene units *n* connecting the ammonium groups, the binding constants for the complex formation with monomeric CP (K_L) increase but the dimerization constants of the resulting complexes decrease. A hydrophobic contribution to the binding free energy of -1.6 ± 0.2 kJ mol^{-1} per methylene unit was obtained from the linear correlation of logK_L values extrapolated to zero ionic strength vs the number of methylene units ($n = 2$–6). A model for diammonium-induced porphyrin dimerization is proposed, which involves complexation of diammonium cations with CP monomer via a combination of electrostatic and hydrophobic interactions and subsequent formation of porphyrin face-to-face dimers in which diammonium cations serve as the stabilizing bridges via ion pairing to carboxylate groups of two CP monomeric units. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: diamines; recognition; coproporphyrin I

INTRODUCTION

Low molecular weight aliphatic di- and oligoamines are ubiquitous compounds that play important roles in biological processes.[1] A number of synthetic host molecules have been proposed for their recognition.[2–13] Selective complexation of aliphatic α,ω-diamines of general structure $H_2N(CH_2)_nNH_2$ was achieved by using ditopic receptors containing binding sites for terminal neutral or protonated amino groups. A large number of polymacrocyclic ditopic receptors containing crown or azacrown ethers as binding sites for ammonium groups of protonated diamines have been synthesized.[2–10] Among them, bis-crown bicyclic receptors with single aromatic[7] or peptide[8] linkers as well as the chromogenic dye-linked ditopic receptors[9] were reported. In other instances, bis-metalloporphyrin ditopic hosts, which bind neutral diamines via coordination to metal cations, were described.[2,11,12] Acyclic basket-shaped hosts containing symmetrically positioned poly(ethylene oxide) chains also function as ditopic receptors for protonated diamines.[13] All these receptors work in organic solvents, typically in chloroform or chloroform–alcohol mixtures, where crown ethers have large affinities to ammonium ions with binding constants of the order of 10^5–10^6 M^{-1} and not unexpectedly such receptors show very large binding constants (up to 10^{10} M^{-1}) for diammonium cations.

More relevant to biological systems, complexation of α,ω-diammonium cations in water was studied first with a series of tetraanionic 18-crown-6 derivatives containing four carboxylate groups or four carboxylate amino acid fragments attached to the macrocycle.[14,15] Binding constants for interactions of these hosts with $^+H_3N(CH_2)_nNH_3^+$ dications reached 1×10^4–4×10^4 M^{-1} for $n = 2$ and decreased to $\sim 1 \times 10^3$ M^{-1} for $n = 4$. These hosts also showed a significant affinity to monoammonium cations. A higher charged hexacarboxylate derivative of 27-crown-9 shows stronger binding of $^+H_3N(CH_2)_nNH_3^+$ dications with log$K = 5.30$, 4.78 and 3.86 for $n = 2$, 3 and 4, respectively, and log$K = 3.08$ for $MeNH_3^+$.[16] A tetracarboxylic derivative of a macrocycle containing elements of cyclophane and crown ether structures was designed for complexation of tetramethylammonium cations and showed strong, but non-selective binding of tetramethylated diammonium cations.[17]

Recently, the ion pairing between biological polyammonium cations and anions of different structures in water has been studied in detail.[18–20] The dominant factor

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in ion-pair stability is the total charge of the interacting species. Highly charged anions such as ATP^{4-} or ${P_2O_7}^{4-}$ form very stable complexes with diammonium cations with $\log K \approx 5$ at zero ionic strength. Of course, interactions between such highly charged species are strongly inhibited by added neutral electrolytes, but even at $I = 0.1$ M the association is still fairly strong with $\log K \approx 3$. In the series of $^{+}H_3N(CH_2)_nNH_3^{+}$ cations, $\log K$ decreases by 0.1–0.3 units on addition of each new methylene group and this effect is larger for anions possessing larger negative charge such as pyrophosphate.[19c]

Thus, purely electrostatic recognition of diammonium cations is rather non-specific, but both high affinity and certain selectivity in favor of short-chain diamines may be achieved by using highly charged anions. In the same line, the discrimination ability of negatively charged crown ether receptors in water has a similar direction, i.e. binding decreases with increase in *n*, but the sensitivity to *n* is larger, probably because of generally stronger binding.

In this paper, we explore the possibility of using a receptor, which would bind electrostatically the terminal ammonium groups of a diammonium cation and would interact simultaneously with its hydrocarbon chain hydrophobically. Such a combination of Coulombic and hydrophobic interactions has been reported for the binding of steroidal polyamines to DNA.[21] We chose as a possible host molecule the tetraanion of coproporphyrin I (CP), a naturally occurring porphyrin that possesses four carboxylate groups connected to the apolar porphyrin macrocycle via flexible saturated hydrocarbon chains. Owing to the known sensitivity of porphyrin absorption and fluorescence spectra to the microenvironment, we also expected that such a host would make possible the optical detection of diammonium guests.

Molecular modeling shows that all four carboxylate groups of CP can easily form contacts with both ammonium groups of $^{+}H_3N(CH_2)_nNH_3^{+}$ cations at the van der Waals distances (the calculated mean distance between ammonium and carboxylate groups is 2.55 Å) without inducing any considerable strain for $2 < n < 8$. Figure 1 shows as an example the simulated structure of CP complex with diprotonated 1,4-diaminobutane, which illustrates the expected features of diammonium–CP complexation: formation of four salt bridges with carboxylates and contacts of diammonium methylene groups with the surface of porphyrin. Evidently, flexible attachment of distant carboxylic groups to the porphyrin macrocycle makes CP a rather non-preorganized host. However, the stability of ion pairs formed by dications and dianions of different structures was found to decrease slowly with increase in the flexibility of substituents connecting the charged groups in each ion.[22] Therefore, in spite of the lack of preorganization, CP should still be capable of efficient ion pairing. It should be noted that a related preorganized host, a tetracarboxylic derivative of tetraphenylporphyrin with convergent carboxyls derived from Kemp's triacid, was prepared and used for the selective complexation of 4,4′-bipyridine in non-aqueous medium.[23]

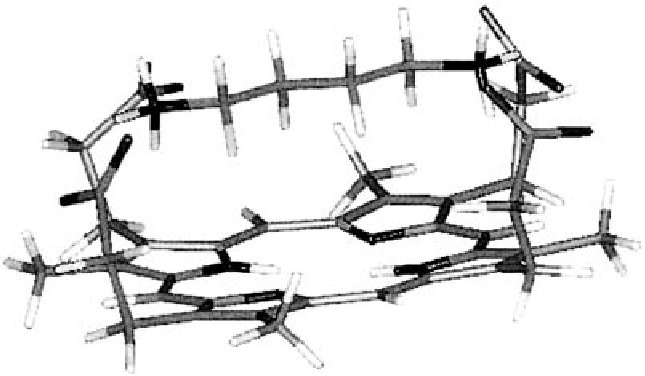


Figure 1. Simulated structure of CP complex with diprotonated 1,4-diaminobutane

On the other hand, the possible contribution of hydrophobic binding of saturated molecules to porphyrins is questionable. The observation of larger stability constants of complexes of charged *meso*-substituted porphyrins with aromatic oppositely charged species than with saturated analogs was interpreted as an indication of the absence of such a contribution.[24] However, studies on the complexation of metalloporphyrins with amino acids[25] demonstrated a noticeable binding increment of ca $-3\,\mathrm{kJ\,mol^{-1}}$ per methylene group. Furthermore, dissociation of CP dimers promoted by organic co-solvents was explained in terms of specific solvation driven by hydrophobic interactions with contributions of $-1.7\,\mathrm{kJ\,mol^{-1}}$ per methylene unit.[26] Finally, recent results on the complexation of coproporphyrin-I-atoiron(III) with histidine-containing peptides demonstrated a considerable hydrophobic contribution of alanine residues.[27] As will be shown below, a significant hydrophobic contribution exists in the interaction of CP with aliphatic diammonium cations, leading to the opposite trend in binding of diammonium cations with different chain lengths compared with that mentioned above for the usual ion pairing, namely better binding is observed for dications with longer chains.

RESULTS AND DISCUSSION

The solution behavior of CP is complicated owing to mutually dependent protonation and self-association processes, the latter being promoted by additions of electrolytes. The equilibrium constants of CP dimerization (K_D) have been determined under a wide range of reaction conditions (pH, electrolyte and organic co-solvent concentrations).[26] Based on these results, we chose for the study of CP–diamine interaction pH 8 and

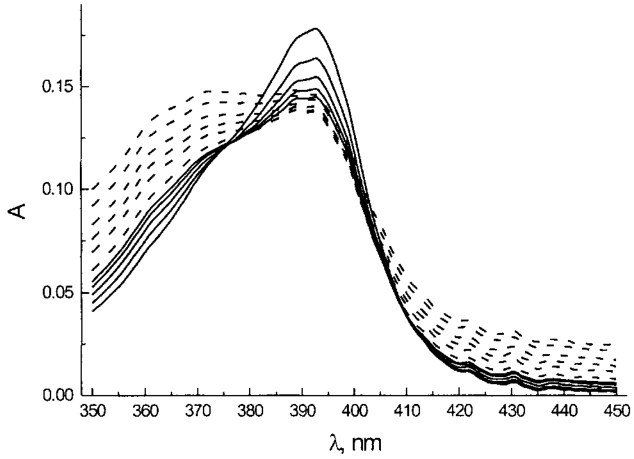


Figure 2. Absorption spectra of 1 μM CP solution in the region of the Soret band at pH 8.0 in the presence of 0.1 M NaCl and increasing concentrations of 1,4-diaminobutane: 0, 3.0, 5.9, 8.9, 12.0 mM, solid lines; 20.4, 28.9, 37.1, 45.1, 55.6, 68.2 mM, dash lines

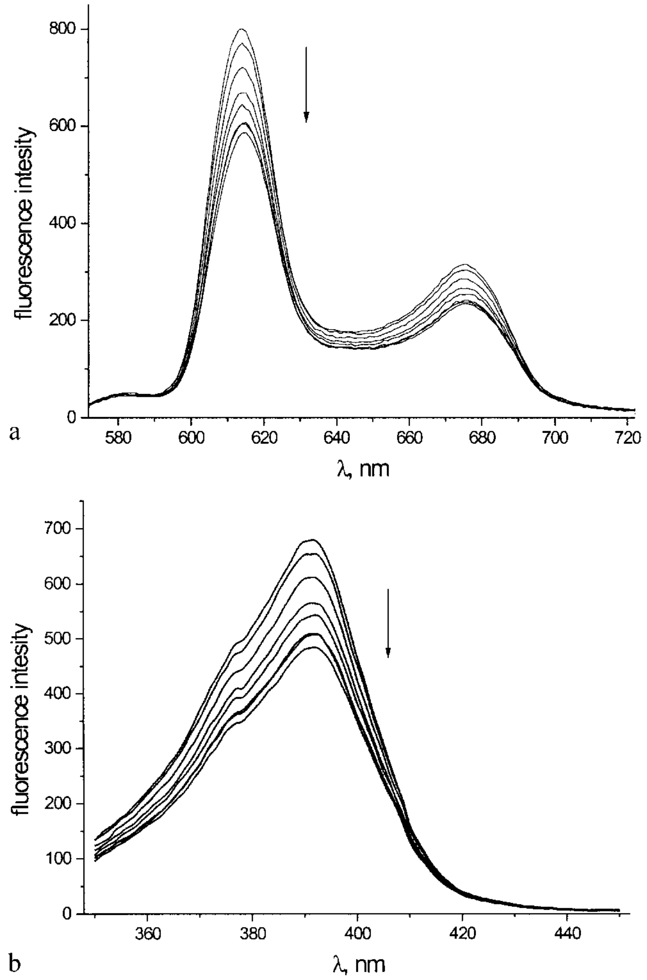


Figure 3. (a) Emission and (b) excitation spectra of 0.25 μM CP in the presence of 1,4-diaminobutane at low diamine concentrations (pH 8.0, 0.1 M NaCl, diamine concentrations 0, 2.12, 4.2, 6.23, 8.23, 10.19, 14 and 17.67 mM)

ionic strength 0.1 M (NaCl). Under these conditions, $K_D = 8.0 \times 10^4\ \mathrm{M}^{-1}$ and at [CP] = 1 μM ca 90% of the porphyrin remains in the monomeric tetraanionic form. The use of a sufficiently high ionic strength is necessary to minimize non-specific salt effects of added diammonium cations on CP dimerization. For all diamines studied, with the exception of less basic ethylenediamine and 1,3-diaminopropane, the dominant form (>95%) at pH 8 is the dication. With these shorter diamines interactions with CP were studied in the pH range 7–9 and the respective equilibrium constants for dications were estimated from the pH dependence. Below pH 7 noticeable spectral changes due to CP protonation ($\mathrm{p}K_a = 6.0$)[26] were observed.

Addition of all diamines to ca 1 μM CP solution produced similar spectral changes in the region of the Soret band, as illustrated in Fig. 2 for 1,4-diaminobutane. In dilute solutions at [diamine] <0.02 M a decrease in absorption with band broadening and an isosbestic point at 374 nm are observed. At higher diamine concentrations much stronger broadening occurs with increase in absorption and no isosbestic point is seen. We assume that the second type of spectral changes are due to the formation of highly aggregated CP forms, but at low diamine concentrations there are only two forms of CP at equilibrium. The character of the spectral changes in this concentration range is similar to those observed for CP dimerization.[26] Therefore, one possibility is that diammonium cations only induce CP dimerization. Alternatively, these changes may reflect the formation of differently absorbing CP–diammonium complexes. It is worth noting that additions of monoammonium cations ($\mathrm{MeNH_3^+}$, n-Pr-$\mathrm{NH_3^+}$) at concentrations up to 0.1 M did not change the CP absorption spectra at all. Hence the observed effects are specific for diammonium cations and cannot be attributed to an increase in total electrolyte concentration.

We did not attempt to establish the type of aggregates formed at higher diamine concentrations. Their formation was not observed with ethylenediamine and 1,3-diaminopropane even on addition of 0.1 M diamine, but with 1,4-diaminobutane it was observed already at concentrations above 0.02 M and with 1,7-diaminoheptane and 1,8-diaminoctane above 0.01 M. Probably both electrostatic and hydrophobic interactions of CP with diammonium cations are involved in the formation of these aggregates. In order to clarify the nature of the spectral changes at low diamine concentrations, we studied the interaction of CP with diamines by fluorescence using both emission and excitation spectra. Dimerization of CP leads to complete quenching of its fluorescence.[26] Thus, if the absorption spectra observed in the presence of diamines belong to a mixture of monomers and dimers, the excitation spectra should always be the spectra of

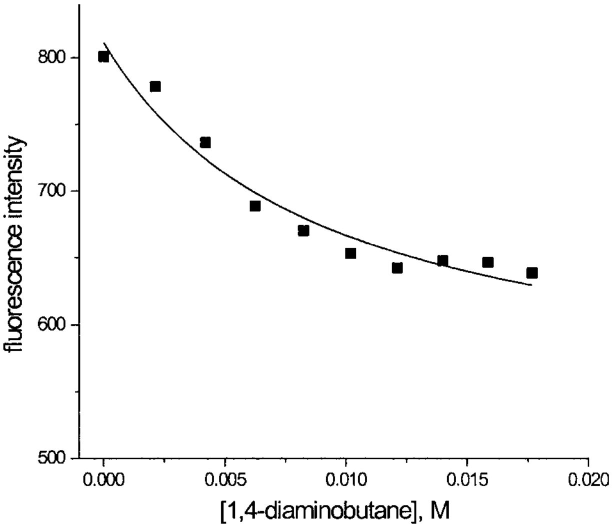


Figure 4. Fluorescence intensity of 0.25 μM CP at the emission maximum at 614 nm as a function of 1,4-diaminobutane concentration (pH 8.0, 0.1 M NaCl)

monomeric CP and, therefore, only a decrease in the fluorescence intensity upon addition of diamines should be observed in both the emission and excitation spectra. Conversely, if the absorption spectra belong to a mixture of monomer and its complex with diamine, the excitation spectra should show the same changes as the absorption spectra. Figure 3(a) and (b) show the emission and excitation spectra of CP in the presence of 1,4-diaminobutane at low diamine concentrations. Evidently, only a decrease in the fluorescence intensity is observed in both types of spectra. However, it is worth noting that the profile of the fluorescence intensity vs 1,4-diaminobutane concentration does not go to zero, but rather tends to 'saturate' at a certain level (Fig. 4). Such behavior agrees with a model that involves association of CP with diamine and subsequent dimerization of this complex, with a dimerization constant larger than that for the free CP. The CP–diammonium association by itself does not induce noticeable spectral changes, as should be expected for the interaction involving principally ion pairing of a diammonium cation with the carboxylate groups separated from the porphyrin ring by two methylenes.

The following equilibria should be taken therefore into account:

(i) dimerization of the monomeric form (M) of CP:

$$2M \rightleftharpoons M_2 \tag{1}$$

(ii) association of monomeric CP with diammonium (L):

$$M + L \rightleftharpoons ML \tag{2}$$

(iii) dimerization of the complex, which involves two equilibria:

$$ML + M \rightleftharpoons M_2L \tag{3}$$

$$2ML \rightleftharpoons M_2L_2 \tag{4}$$

The respective equilibrium constants are defined by the following equations:

$$K_D = [M_2]/[M]^2 \tag{5}$$

$$K_L = [ML]/[M][L] \tag{6}$$

$$K_{D1} = [M_2L]/[M][ML] \tag{7}$$

$$K_{D2} = [M_2L_2]/[ML]^2 \tag{8}$$

The observed molar absorptivity of CP in a solution containing a mixture of monomeric and dimeric forms in the absence of diamines is given by the equation[28]

$$\begin{aligned}\varepsilon_{obs} = 0.5\varepsilon_D + (2\varepsilon_M - \varepsilon_D)\\ ((1 + 8K_D[CP]_T)^{1/2} - 1)/8K_D[CP]_T\end{aligned} \tag{9}$$

where ε_M and ε_D are the molar absorptivities of the monomer and dimer, respectively, and $[CP]_T$ is the total concentration of CP. Analysis of the scheme represented by the set of equilibria (1)–(4) shows that in the presence of a diamine a similar equation [Eqn. (10)] can be used assuming that the species M and ML have similar molar absorptivities ε_M and the species M_2, M_2L and M_2L_2 also have similar molar absorptivities ε_D:

$$\begin{aligned}\varepsilon_{obs} = 0.5\varepsilon_D + (2\varepsilon_M - \varepsilon_D)\\ ((1 + 8K_{D,app}[CP]_T)^{1/2} - 1)/8K_{D,app}[CP]_T\end{aligned} \tag{10}$$

where the apparent dimerization constant $K_{D,app}$ is given by the equation

$$K_{D,app} = (K_D + K_{D1}K_L[L] + K_{D2}K_L{}^2[L]^2)/(1 + K_L[L])^2 \tag{11}$$

Equations (10) and (11) were combined and used for fitting the absorbance vs diamine concentration profiles assuming $[L] = [L]_T$ and using previously determined ε_M, ε_D and K_D values as fixed parameters.[26]

Figure 5(a)–(c) illustrate the shapes and the fitting of the spectrophotometric titration plots obtained at the monomer absorption maximum of 392 nm. Similar plots were obtained at 10 wavelengths both above and below the maximum and the equilibrium constants K_L, K_{D1} and K_{D2} calculated from the fittings of all plots were averaged. A certain interdependence existed between the K_{D1} and K_{D2} values, which allowed us only an approximate estimate of these parameters generally with ±30% relative uncertainty. The results obtained for all

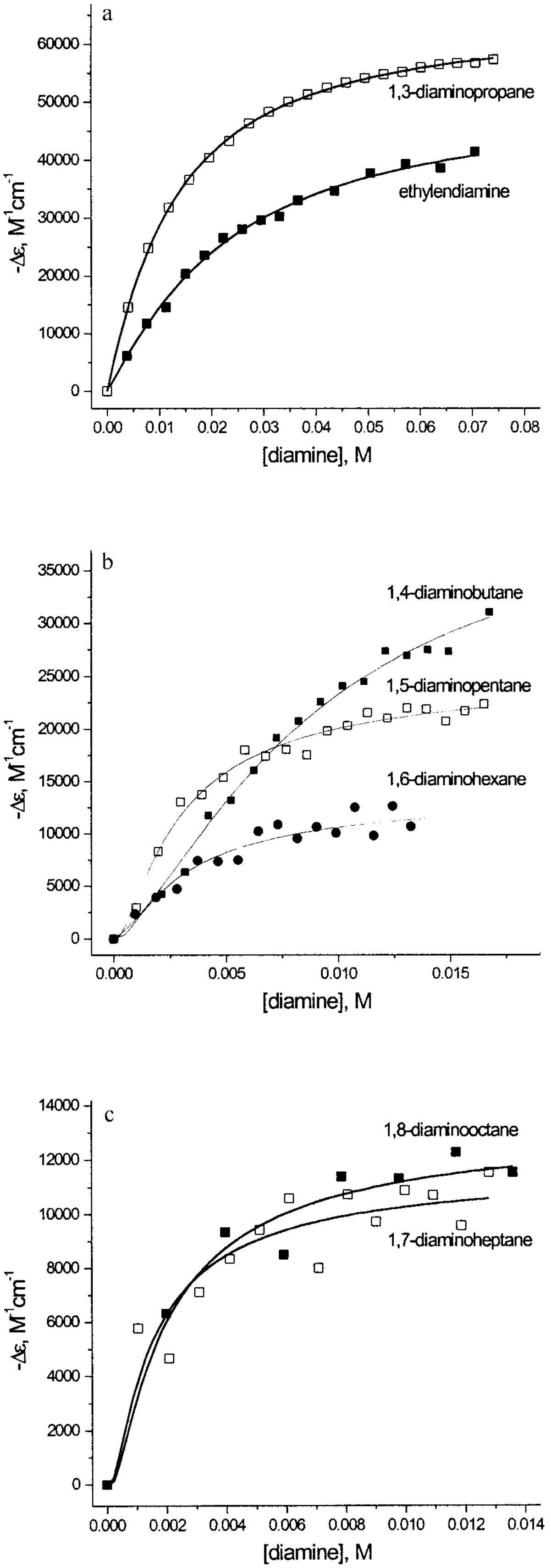


Figure 5. Spectrophotometric titration plots for CP and diamines at 392 nm (pH 8.0, 0.1 M NaCl). Solid lines are the fitting curves in accordance with Eqns (10) and (11)

diamines at different pH values and ionic strengths are given in Table 1. In general, binding constants for interactions of diamines with monomeric CP at a given pH become progressively larger with increase in the number of methylene groups in the diamines, but the dimerization constants for the self-association of CP–diammonium complexes become progressively smaller. Qualitatively these trends are manifested in the shape of titration plots [see Fig. 5(a)–(c)] in such a way that for diamines with longer chains the curves are 'saturated' faster (at lower diamine concentrations), because of stronger complexation, but at a lower level because the observed change in the absorptivity is due to increased CP self-association, which is proportional to the increase in K_{D1} and K_{D2} over K_D.

Let us consider first the pH dependence of K_L. Figure 6 shows the pH dependences of the binding constants for three less basic diamines ethylenediamine, 1,3-diaminopropane and 1,4-diaminobutane, which have the first pK_a values of their diprotonated forms of 7.08, 8.74 and 9.44, respectively.[29] Fitting of these dependences (shown by solid lines) to the respective theoretical equation with literature values of pK_a shows that only dicationic forms of diamines interact with CP, in agreement with the absence of an interaction of CP with monoammonium cations (see above). The dimerization constants K_{D1} and K_{D2} are independent of pH (see Table 1), also in agreement with formation of only one type of monomeric CP–diamonium complexes. Diammonium cations with longer hydrocarbon chains have the first pK_a around 10 and therefore interactions with them were studied only at pH 8 when the degree of formation of the dicationic form is ca 99%.

The next aspect to be considered is the salt effect. All ionic equilibria depend to some extent on the ionic strength, and since this dependence may be different for different reactions, it is always preferable to discuss the equilibrium constants extrapolated to zero ionic strength. It has been shown that for numerous host–guest ionic reactions,[22,30] and also for the CP self-association,[26] the salt effect can be satisfactorily described by a simple Debye–Hückel equation with the apparent charges of reacting species equal to or somewhat lower than the total charges:

$$\log K = \log K^0 + z_A z_P \sqrt{I}/(1 + \sqrt{I}) \qquad (12)$$

where I is the ionic strength, K^0 is the equilibrium constant at $I = 0$ and z_A and z_P are the apparent charges of diammonium and CP, respectively. Although the reasons why the Debye–Hückel equation derived for small spherical ions rather than for large organic ions possessing highly dispersed charges is still formally applicable and what the meaning is of the apparent charged required for the fitting of experimental data to Eqn. (12) remain unclear, the use of this equation just for

Table 1. Binding constants K_L for interactions of diamines with monomeric CP and dimerization constants K_{D1} and K_{D2} corresponding to equilibria (7) and (8), respectively, at 25 °C in water[a]

Diamine	pH	[NaCl] (M)	K_L (M^{-1})	K_{D1}, (M^{-1})	K_{D2}, (M^{-1})	$K_L^{corr\ b}$ (M^{-1})
Ethylenediamine	7.0	0.1	21	6.4×10^6	1.9×10^7	38
	8.0		5.7	5.5×10^6	1.7×10^7	
	8.5		1.4^c	5×10^{6c}	2×10^{7c}	
	7.0	0.05	22.8	3.4×10^6	1.1×10^7	44.7
		0.03	27.3	4.0×10^6	2.2×10^7	55.7
		0.02	38.1	2.2×10^6	1.6×10^7	79.4
		0.01	43.0	2.5×10^6	2.2×10^7	93.5
1,3-Diaminopropane	7.0	0.1	35.0	1.9×10^6	6.4×10^6	35.7
	8.0		23.2	1.5×10^6	8.1×10^6	
	9.0		13.3	1.7×10^6	1.3×10^7	
	10.0		1.4^c	3×10^{6c}	6×10^{6c}	
	7.5	0.03	78.7	1.1×10^6	4.7×10^6	84.6
		0.02	86.9	1.4×10^6	7.6×10^6	93.9
		0.01	89.4	1.7×10^6	9.3×10^6	97.2
1,4-Diaminobutane	7.0	0.1	107	5.0×10^5	2.0×10^6	
	8.0		105	4.9×10^5	2.0×10^6	
	9.0		59.0	5.1×10^5	1.8×10^6	
	10.0		24	5.0×10^5	2.0×10^6	
	8.0	0.05	156	6.2×10^5	2.5×10^6	
		0.03	245	2.4×10^5	1.6×10^6	
		0.02	260	4.0×10^5	1.5×10^6	
		0.01	250	5.0×10^5	2.0×10^6	
1,5-Diaminopentane	8.0	0.1	262	3.0×10^5	4.7×10^5	
1,6-Diaminohexane	8.0	0.1	352	1.8×10^5	2.5×10^5	
1,7-Diaminoheptane	8.0	0.1	370	2.9×10^5	2.1×10^5	
1,8-Diaminooctane	8.0	0.1	400	2.1×10^5	2.7×10^5	

[a] Relative error in K_L is ca ±10% and in K_{D1} and K_{D2} ca ±30%.

[b] Corrected for incomplete protonation of diamines by using pK_a values from Ref. 29.

[c] Relative error ±50%.

extrapolation of binding constants to $I = 0$ is completely justified. The electrostatic contribution to complexation of diammonium cations with CP should be similar for all diamines and therefore the product of apparent charges in Eqn. (12) should be also similar for all guests. Figure 7 shows the values of $\log K_L$ as a function of $\sqrt{I}/(1 + \sqrt{I})$ for three diamines (Table 1; binding constants for ethylenediamine and 1,3-diaminopropane are corrected for incomplete diamine protonation at the pH used). In all cases the linear correlation is reasonably good. The

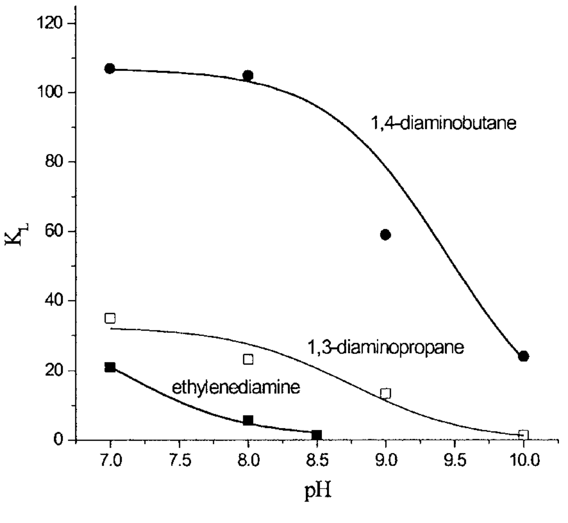


Figure 6. Plots of binding constants for interactions of diamines with monomeric CP in 0.1 M NaCl vs pH

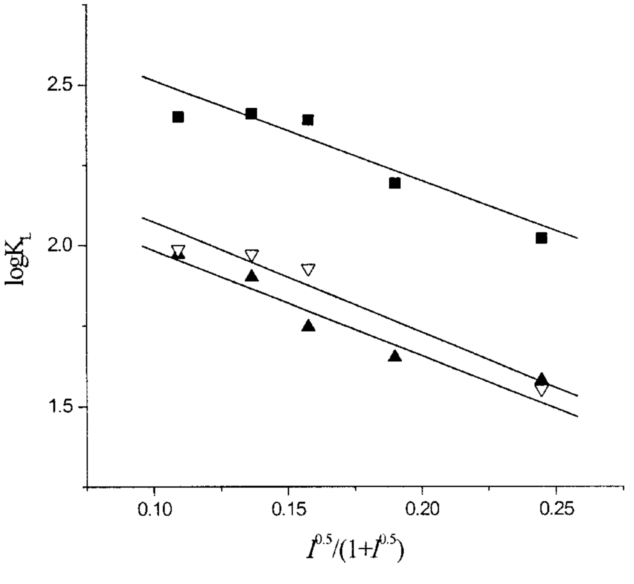


Figure 7. Salt effects on binding constants for interactions of diprotonated diamines with monomeric CP

Table 2. Logarithms of binding constants K_L^0 (M^{-1}) for interactions of diprotonated diamines $^+H_3N(CH_2)_nNH_3^+$ with monomeric CP at zero ionic strength, the products of effective charges of the reactants z_Az_P from the slopes of dependences in Fig. 7 [see Eqn. (12)] and some literature values of binding constants (K^0) for interactions of diammoniums with anions at zero ionic strength

		n							
Anion	Parameter	2	3	4	5	6	7	8	Ref.
CP^{4-}	Log K_L^0	2.31 ± 0.16	2.41 ± 0.12	2.82 ± 0.23	3.15	3.36	3.38	3.41	This work
	$-z_Az_P$	3.3 ± 0.9	3.4 ± 0.8	3.1 ± 1.2	3.3[a]	3.3[a]	3.3[a]	3.3[a]	
	Log K_{D1}^0	6.3 ± 0.1	6.2 ± 0.2	5.7 ± 0.2	5.5	5.2	5.5	5.3	
	Log K_{D2}^0	7.2 ± 0.1	6.9 ± 0.1	6.3 ± 0.1	5.7	5.4	5.3	5.5	
$P_2O_7^{4-}$	Log K^0	5.8		5.8	5.6	5.0		4.4	19[c]
$Fe(CN)_6^{4-}$	Log K^0	4.8		4.3	4.4				19[c]
$H(CHCO_2^-)_4H$	Log K^0	4.2		4.1	4.2				19[b]
$^-O_2C\text{-}R\text{-}CO_2^-$[b]	Log K^0			2.85		2.44		2.27	22
$^-O_2CCH_2CO_2^-$	Log K^0	2.5			2.3	2.0		1.9[c]	18

[a] Mean value of the slopes found for diamines with $n = 1$, 2 and 3.
[b] Aromatic dicarboxylates.
[c] 1,10-Diaminodecane.

values of logK_L^0 and z_Az_P are given in Table 2. As expected, the product of apparent reactant charges is the same within the limits of experimental errors with a mean value $z_Az_P = -3.3$, much smaller in absolute value than the product of total reactant charges, −8. This mean value was used to extrapolate to zero ionic strength the binding constants for other diamines (Table 2).

For dimerization constants K_{D1} and K_{D2} the salt effect is significant only for K_{D1} for ethylendiamine (Table 1). Fitting of the results to Eqn. (12) gives log$K_{D1}^0 = 6.32 \pm 0.08$ and $z_P^2 = 4.9 \pm 1.5$. In other cases we assumed K_{D1} and K_{D2} to be independent of the ionic strength and took as the estimates of their values at zero ionic strength averaged values of these constants obtained under different conditions (Tables 1 and 2).

Table 2 summarizes the equilibrium constants for reactions (6)–(8) and also gives reported binding constants for interactions of diammonium cations with some other anions at zero ionic strength. Comparisons of reported logK^0 values for different tetraanions [$P_2O_7^{4-}$, $Fe(CN)_6^{4-}$ and $H(CHCO_2^-)_4H$] indicate two trends: (1) for a given anion the binding constants decrease with increase in the number *n* of methylenes between nitrogen atoms; and (2) for a given diamine the binding constants decrease with increase in charge delocalization in anions. The degree of negative charge delocalization in CP is, apparently, much higher than even in the most delocalized 1,2,3,4-tetracarboxybutane tetraanion and, not unexpectedly, logK_L^0 values for CP are smaller than logK^0 for the latter, approaching logK^0 for dianions given in the two last rows in Table 2. Interestingly, the apparent charge z_P of CP estimated from the slope of the Debye–Hückel plots in Fig. 7 (Table 2) is ca −1.7 (assuming the charge of diammoniums to be $z_A = +2$), roughly in agreement with the values of binding constants typical for a dication–dianion interaction. However, the trend in binding constants for different diammonium cations with CP is opposite to that for all other anions. Figure 8(a) shows the plot of logK_L^0 vs number of methylenes *n* between the ammonium groups, which is linear for $2 \leq n$

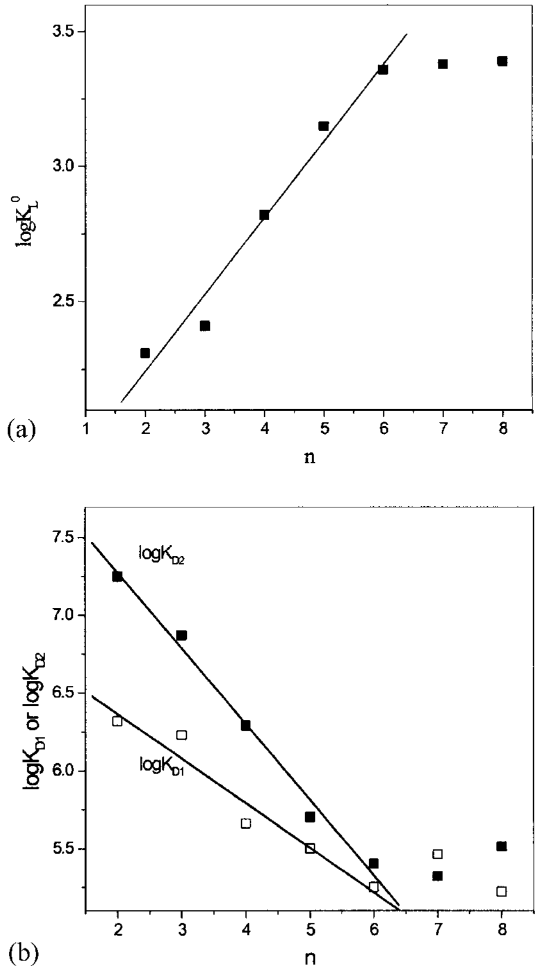


Figure 8. Plots of logarithms of (a) the binding and (b) the dimerization constants at zero ionic strength (Table 2) vs number of methylenes *n* in diprotonated diamines, $^+H_3N(CH_2)_nNH_3^+$

≤ 6 with a slope 0.28 ± 0.03, and levels off for longer chains. This slope corresponds to an increment to the binding free energy of -1.6 ± 0.2 kJ mol^{-1} per methylene and may be interpreted as a contribution of the hydrophobic effect.

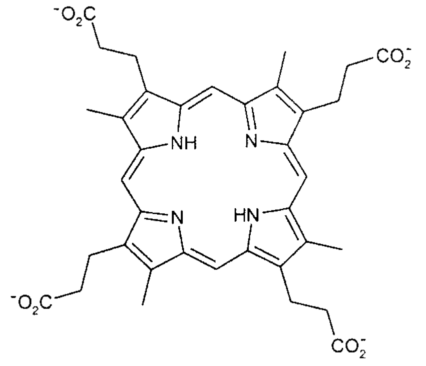
In order to prove the existence of contact between the hydrocarbon chain of a diammonium cation and the surface of porphyrin molecule, a ^{1}H NMR titration of CP by 1,4-diaminobutane was performed. It is well known that the aromatic ring current of the porphyrin ring induces strong upfield shifts of the signals of protons of molecules touching the ring.[31,32] The spectrum of 1 mM CP in D_2O at pH 8 in the presence of 0.05 M NaCl and 25 vol.% CD_3OD added to reduce the degree of CP self-aggregation showed two multiplets at 4.242 and 2.895 ppm corresponding to the α- and β-methylenes of the carboxypropionate chains and two singlets at 3.506 and 10.203 ppm corresponding to methyl groups and *meso* protons, respectively. Additions of higher amounts of CD_3OD did not change the positions of the signals. When the spectrum of CP was recorded in water in the presence of 0.5 M NaCl added to induce the complete dimerization of CP, all signals were displaced upfield to 4.225, 2.812, 3.444 and 9.033 ppm, respectively. Such upfield shifts, which are especially strong for *meso* protons, are typical for the dimerization of porphyrins.[33] On the basis of these results we assume that CP is monomeric under chosen conditions. By the spectrophotometric titration of 1 μM CP with 1,4-diaminobutane in the presence of 25% CH_3OH, 0.05 M NaCl and pH 8, we obtained $K_L = 112$ M^{-1}, $K_{D1} = 7 \times 10^4$ M^{-1} and $K_{D2} = 4.8 \times 10^5$ M^{-1}. Comparison with the results in Table 1 shows that the addition of methanol induces the expected considerable decrease in the dimerization constants, but does not affect significantly the association constant. On addition of 1.4 mM 1,4-diaminobutane to 1 mM CP in 25% CD_3OD–D_2O at pH 8 in the presence of 0.05 M NaCl, the signal for the *meso* protons of CP broadens into the baseline and other signals move upfield by ca 0.1 ppm in accordance with increased dimerization of CP. The reason for the strong broadening of the signal for *meso* protons is unclear. Probably the lifetime of the diamine-stabilized dimer is larger than that for simple CP dimers. At the same time, two resonances of the methylene groups of 1,4-diaminobutane also undergo considerable upfield shifts from 1.557 and 2.841 ppm in the absence of CP to 1.077 and 2.366 ppm in the presence of CP. Further additions of 1,4-diaminobutane up to 4.2 mM caused insignificant shifts in the positions of CP signals, but the diamine signals moved downfield, being equal to 1.251 and 2.535 ppm in the presence of 2.8 mM 1,4-diaminobutane and 1.318 and 2.603 in the presence of 4.2 mM 1,4-diaminobutane. This reversal of the trend in the positions of 1,4-diaminobutane signals reflects, of course, an increase in the fraction of free diamine. The calculated species distribution diagram for the conditions of the NMR experiment with the above equilibrium constants predicts that the fraction of free diamine increases from ca 60% to ca 80% on going from 1.4 to 4.2 mM diamine and that the fraction of the ML complex is always below 3% of total CP. The dominant species are M_2L, the fraction of which decreases from 43% at the lowest diamine concentration to 15.7% at the highest, and M_2L_2, the fraction of which varies from 54% to 81% of total CP in the same concentration range of the diamine. Assuming that 1,4-diaminobutane exchanges between its free and bound states rapidly on the NMR time-scale, one can estimate the chemical shifts of the diamine methylene groups in M_2L and M_2L_2 complexes from the equation

$$\delta_{\mathrm{obs}} = \delta_{\mathrm{f}} x_{\mathrm{f}} + \delta_{\mathrm{M2L}} x_{\mathrm{M2L}} + \delta_{\mathrm{M2L2}} x_{\mathrm{M2L2}} \qquad (13)$$

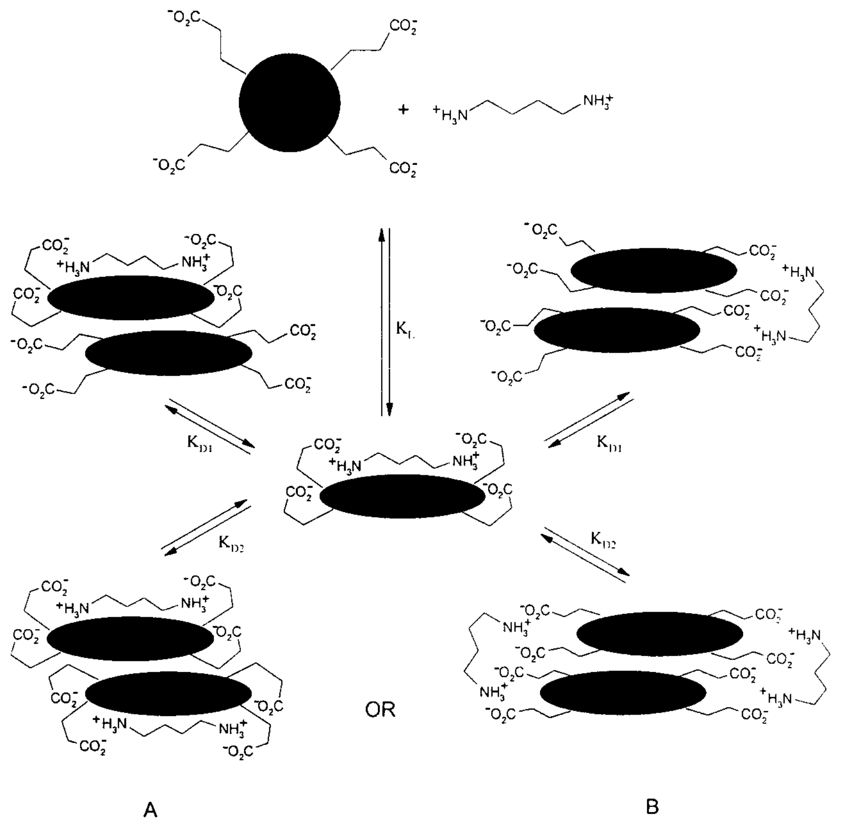
where δ_f, δ_{M2L} and δ_{M2L2} are the chemical shifts of free and bound diamine, respectively, and x_f, x_{M2L} and x_{M2L2} are the fractions of diamine in the respective sates. By using results for all three 1,4-diaminobutane concentrations employed we found $\delta_{M2L} = 0.44$ and $\delta_{M2L2} = 0.35$ ppm for the internal methylene groups of 1,4-diaminobutane and $\delta_{M2L} = 1.79$ and $\delta_{M2L2} = 1.67$ for the external methylene groups with an uncertainty of ± 0.08 ppm. Thus, the resonances of all methylene groups of the diamine undergo an upfield shift of ca -1 ppm, indicating a noticeable aromatic ring current effect on the bound diamine.

Alternatively, stronger binding of diammonium cations with longer chains may be ascribed to geometric factors. First, better complementarity of the ammonium groups to distant carboxylate groups of CP may be achieved with longer diammonium cations. Second, shorter cations may require the carboxylate groups of CP to be closer to each other in the complex, thus inducing stronger mutual repulsion and lower complex stability. Molecular modeling shows, however, that such effects should be insignificant. Comparison of simulated structures of complexes with diprotonated ethylendiamine and 1,4-diaminobutane shows that in both cases the van der Waals contacts between cationic and anionic groups are formed practically withouit strain and the distances between carboxylate groups in the latter complex are larger than in the former by less than 1 Å, which cannot increase significantly the repulsion energy.

Formerly, the dimerization constant of CP in the absence of diamines at zero ionic strength was found to be $\log K_D{}^0 = 3.05$.[26] Strongly increased dimerization constants of CP complexed to diammonium guests (Table 2) are explicable by the decreased total charge of porphyrin-containing complexes. The importance of the total porphyrin charge for the self-aggregation is well documented. For example, tetracationic porphyrins do not show any tendency for self-aggregation in the concentration range 1–100 μM in water, but dicationic porphyrins are significantly aggregated under these conditions.[34] If diammonium–CP complexes have a structure such as that shown in Fig. 1, their dimerization



Tetraanion of coproporphyrin I (CP)



Scheme 1

probably occurs via stacking between porphyrin planes opposite to those occupied by the guest, as shown on the left in Scheme 1 (mechanism A). In this mechanism the role of the diammonium cation is to neutralize partially the negative charge of CP and to move the carboxylate groups of interacting porphyrins at the largest possible distance. However, in terms of such a mechanism it is difficult to explain the strong dependence of K_{D1} and K_{D2} on the length of the diamine hydrocarbon chain. The logarithms of dimerization constants are plotted against number n of methylene groups of diamines in Fig. 8(b). Clearly, the opposite trend to that observed for K_L [Fig. 8(a)] is seen in this case. The slopes of the lines in Fig. 8(b) are −0.29 and −0.49 for K_{D1} and K_{D2} respectively. Hence it is evident that the favorable hydrophobic contribution for the binding of diammoniums becomes unfavorable for dimerization.

A reasonable explanation of the unfavorable effect of the extension of the diamine chain on dimerization constants may be proposed based on the assumption that diammonium-stabilized dimers have a different structure, shown on the right in Scheme 1 (mechanism B), with diammonum cations serving as bridges between two CP monomers. In this case diammonium cations are located outside the porphyrin plane forming ionic contacts with carboxylate groups. Therefore, in order to form such a dimeric structure the diammonium cation must lose its hydrophobic binding contribution existing in the monomeric complex and the energetic cost of this loss should be approximately the same as the energetic gain upon the formation of the monomeric complex. Indeed, the slopes in Fig. 8(a) and (b) are similar in absolute vaues, but have the opposite signs. In connection with this model of diammonium-induced CP dimerization, it should be noted that polyelectrolytes are known to induce very efficiently the aggregation of oppositely charged porphyrins by templating the stacking between porphyrin planes.[34,35] It seems that the diammonium-induced CP dimerization represents the simplest type of such templated aggregation process.

In conclusion, we note that the hydrophobic contribution observed in diamine–CP complexation may be relevant to polyamine–protein interactions, making them stronger than would be expected on the basis of purely electrostatic binding. Thus, the binding constant reported for interaction of spermine (a tetraamine) with protein kinase CK2 equals $1 \times 10^6\,\mathrm{M}^{-1}$ at an ionic strength of 0.045 M.[1c] The binding site of protein kinase CK2 involves four glutamic residues and therefore this constant may be compared with that determined for interaction of spermine with a tetraanion 1,2,3,4-butanetetracarboxylate, which equals $7 \times 10^4\,\mathrm{M}^{-1}$ at the same ionic strength.[19a] Even the binding constant of spermine to highly charged polyanion of DNA at this ionic strength ($K = 4.4 \times 10^4\,\mathrm{M}^{-1}$)[36] is much smaller than that for protein kinase. In the last two systems the binding of spermine is purely electrostatic and the higher affinity to the protein host may be due to an additional hydrophobic contribution. In line with this, the binding of 1,12-diaminododecane to protein kinase CK2 is much stronger than for 1,4-diaminobutane and even slightly stronger than for a triamine spermidine.[1c]

EXPERIMENTAL

Coproporphyrin I chlorohydrate (Aldrich), diamines and inorganic salts of reagent purity (Merck or Aldrich) were used as supplied. All solutions were prepared in purified water (Milli-Q Reagent Water System).

Spectrophotometric measurements were performed using a Hewlett-Packard Model 8452A diode-array spectrophotometer and fluorescence spectra were recorded on a FluoroMax SPEX spectrofluorimeter equipped with thermostated cell holders. 1H NMR spectra were recorded on a Varian Gemini 300 NMR spectrometer. 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 Microcal Origin (version 5) program. The binding and dimerization constants were calculated from the experimental results by non-linear least-squares fitting to the respective theoretical equations. Species distribution diagrams were calculated by using Species Ver. 0.8 Academic Software 1999.

Molecular modeling was performing by using the Biosym/MSI San Diego 1995 program package for docking calculations. The force field used was the consistent-valence force field CFF91. The minimization algorithms used were steepest descents for initial steps and Newton–Raphson for final steps of minimization. The dielectric constant for docking calculations was set equal to 78.5. No cutoffs were defined. The strategy selected was fixed docking, which freezes the CP ring and leaves the rest of the binding set totally flexible. Fixed docking for each ligand–receptor system studied produced 10 structures. The lowest energy structure was selected for further refinement. The scheme of atomic charges used as input for docking calculations was obtained through Hartree–Fock molecular orbital calculations with a $6\text{–}31+\mathrm{G}$ basis set. The structures of diammonium cations were fully optimized and for CP the single-point calculations were performed. The atomic charges selected were based on fits to the molecular electrostatic potential. The Hartree–Fock calculations were performed with Spartan version 4.0 from Wavefunction.

REFERENCES

1. (a) Tabor CW, Tabor H. *Annu. Rev. Biochem.* 1984; **53**: 749–790; (b) Morozova L, Dasmet J, Joniau M. *Eur. J. Biochem.* 1993; **218**: 303–309; (c) Leroy D, Fihol O, Deleros JG, Pares S, Chambaz EM, Cochet C. *Biochemistry* 1997; **36**: 1242–1250; (d) Cohen SS. *A Guide to the Polyamines*. Oxford University Press: Oxford, 1998.
2. (a) Sutherland IO. *Chem. Soc. Rev.* 1986; **15**: 63–91; (b) Sutherland IO. *Pure Appl. Chem.* 1989; **61**: 1547–1554; (c) Voyer N. Lamothe J. *Tetrahedron* 1995; **51**: 9241–9284; (d) Gokel GW, Abel E. In *Comprenhensive Supramolecular Chemistry*, vol. 1, Gokel GW (ed). Elsevier: Oxford, 1996; 511–620.
3. Fages F, Desvergne J-P, Kampke K, Bouas-Laurent H, Lehn J-M, Meyer M, Albreht-Gary A-M. *J. Am. Chem. Soc.* 1993; **115**: 3658–3664.
4. Hamilton A, Lehn J-M, Sessler JL. *J. Am. Chem. Soc.* 1986; **108**: 5158–5167.
5. Kotzyba-Hibert F, Lehn J-M, Saigo K. *J. Am. Chem. Soc.* 1981; **103**: 4266–4268.
6. Boudon C, Cross M, Kotzyba-Hibert F, Lehn J-M, Saigo K. *J. Electroanal. Chem. Interfacial Electrochem.* 1985; **191**: 210–221.
7. De Silva AP, Sandanayake KRAS. *Angew. Chem., Int. Ed. Engl.* 1990; **29**: 1173–1175.
8. Voyer N, Deschénes D, Bernier J, Roby J. *J. Chem. Soc., Chem. Commun.* 1992; 134–136.
9. Fuji K, Tsubaki K, Tanaka K, Hayashi N, Otsubo T, Kinoshita T. *J. Am. Chem. Soc.* 1999; **121**: 3807–3808.
10. Tsubaki K, Tanaka H, Furuta T, Kinoshita T, Fuji K. *Tetrahedron Lett.* 2000; **41**: 6089–6093.

11. Hayashi T, Nonogushi M, Aya T, Ogoshi H. *Tetrahedron Lett.* 1997; **38**: 1603–1606.
12. Ema T, Misawa S, Nemugaki S, Sakai T, Utaka M. *Chem Lett.* 1997; 487–488.
13. (a) Smeets JWH, Sijbezma RP, Van Dalen L, Spek AL, Smeets WJJ, Nolte RJM. *J. Org. Chem.* 1989; **54**: 3710–3717; (b) Smeets JWH, Van Dalen L, Kaats-Richter VEM, Nolte RJM. *J. Org. Chem.* 1990; **55**: 454–461.
14. Behr JP, Lehn J-M, Vierling P. *J. Chem. Soc., Chem. Commun.* 1976; 621–623.
15. Behr JP, Lehn J-M, Vierling P. *Helv. Chim. Acta* 1982; **65**: 1853–1867.
16. Lehn J-M, Vierling P, Hayward RC. *J. Chem. Soc., Chem. Commun.* 1979; 296–298.
17. Dhaenens M, Lacombe L, Lehn J-M, Vigneron JP. *J. Chem. Soc., Chem. Commun.* 1984; 1097–1099.
18. Cascio S. De Robertis A, De Stefano C, Foti C, Gianguzza A, Sammartano S. *J. Chem. Eng. Data.* 2000; **45**: 717–723.
19. (a) De Robertis A, De Stefano C, Gianguzza A, Sammartano S. *Talanta* 1999; **48**: 119–126; (b) De Stefano C, Gianguzza A, Maniaci R, Piazzese D, Sammartano S. *Talanta* 1998; **46**: 1079–1084; (c) De Robertis A, De Stefano C, Gianguzza A, Sammartano S. *Talanta* 1998; **46**: 1085–1093.
20. De Stefano C, Giuffré O, Sammartano S. *J. Chem. Soc., Faraday Trans.* 1998; **94**: 1091–1095.
21. Muller JG, Ng MMP, Burrows CJ. *J. Mol. Recognit.* 1996; **9**: 143–148.
22. Hossain MA, Schneider H-J. *Chem. Eur. J.* 1999; **5**: 1284–1290.
23. Lindsey JS, Kearney PC, Duff RJ, Tjivikua PT, Rebek J Jr. *J. Am. Chem. Soc.* 1988; **110**: 6575–6577.
24. Schneider H-J, Wang M. *J. Chem. Soc., Chem. Commun.* 1994; 413–415.
25. (a)Tabata M, Tanaka M. *Inorg. Chem.* 1988; **27**: 203–205; (b)Tabata M, Shimada A. *Bull. Chem. Soc. Jpn.* 1990; **63**: 672–676.
26. Morales-Rojas H, Yatsimirsky AK. *J. Phys. Org. Chem.* 1999; **12**: 377–387.
27. Huffman DL, Suslick KS. *Inorg. Chem.* 2000; **39**: 5418–5419.
28. (a) Das RR, Pasternak RF, Plane RA. *J. Am. Chem. Soc.* 1970; **92**: 3312–3316; (b) Pasternak RF, Huber PR, Boyd P, Engasser G, Francesconi L, Gibbs E, Fasella P, Venturo GC, Hinds LdeC. *J. Am. Chem. Soc.* 1972; **94**: 4511–4517; (c) Pasternak RF, Francesconi L, Raff D, Spiro E. *Inorg. Chem.* 1973; **12**: 2606–2611.
29. Smith RM, Martel AE. *Critical Stability Constants*. Plenum Press: New York, vol. **6**, 1989 and vol. **2**, 1975.
30. Schneider H-J, Kramer R, Simova S, Schneider U. *J. Am. Chem. Soc.* 1988; **110**: 6442–6448.
31. Abraham RJ, Bedford GR, McNeillie D, Wright B. *Org. Magn. Reson.* 1980; **14**: 418–425.
32. Mikros E, Gaudemer F, Gaudemer A. *Inorg. Chem.* 1991; **30**: 1806–1815.
33. (a) Abraham RJ, Burbidge PA, Jackson AH, Macdonald DB. *J. Chem. Soc. B* 1966; 620–626; (b) Abraham RJ, Barnett GH, Hawkes GE, Smith KM. *Tetrahedron* 1976; **32**: 2949–2956; (c) Abraham RJ, Evans B, KM. *Tetrahedron* 1978; **34**: 1213–1220; (d) Abraham RJ, Fell SCM, Pearson H, Smith KM. *Tetrahedron* 1979; **35**: 1759–1766.
34. Pasternak RF, Gibbs EJ. In *Metal Ions in Biological Systems*, Sigel A, Sigel H (eds). vol. **33**, 1996; 367–415.
35. Purrello R, Gurrieri S, Lauceri R. *Coord. Chem. Rev.* 1999; **190**: 683–706.
36. Braunlin WH, Strick TJ, Record MT Jr. *Biopolymers* 1982; **21**: 1301–1314.