DOI: 10.1002/chem.200501479

Analysis of Host-Assisted Guest Protonation Exemplified for *p*-Sulfonatocalix[4]arene—Towards Enzyme-Mimetic pK_a Shifts

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Abstract: The pD dependence of the complexation of *p*-sulfonatocalix[4]arene (CX4) with the azoalkanes 2,3-diazabicyclo[2.2.1]hept-2-ene (**1**), 2,3-diazabicyclo[2.2.2]oct-2-ene (**2**), 2,3-diazabicyclo[2.2.3]non-2-ene (**3**), and 1-methyl-4-isopropyl-2,3-diazabicyclo-[2.2.2]oct-2-ene (**4**) in D_2O has been studied. The pD-dependent binding constants, determined by ^1H NMR spectroscopy, were analyzed according to a seven-state model, which included the CX4 tetra- and penta-anions, the protonated and unprotonated forms of the azoalkanes, the corresponding complexes, as well as the complex formed between CX4 and the deuteriated hydronium ion. The variation of the UV absorption spectra, namely the hypsochromic shift in the near-UV band of the azo chromophore upon protonation, was analyzed according to a four-state model. Measurements by independent methods demonstrated that complexation by CX4 shifts the pK_a values of the guest molecules by around 2 units, thereby establishing a case of host-assisted guest protonation. The pK_a shift can be translated into improved binding (factor of 100) of the protonated guest relative to its unprotonated form as a result of the cation-receptor properties of CX4. The results are discussed in the context of supramolecular catalytic activity and the pK_a shifts induced by different types of macrocyclic hosts are compared.

Keywords: azo compounds · calixarenes · host–guest systems · protonation equilibria · supramolecular chemistry

Introduction

One of the most fascinating aspects of host–guest inclusion complexes is perhaps how the formation of such very simple and discrete supramolecular assemblies is able to modify the chemical reactivity of guests, an important goal in the understanding and mimicking of enzymatic activity.[1,2] One of the simplest ways to alter chemical reactivity is to modify acidity or basicity constants by supramolecular inclusion, which would pave the way for catalytic and biomimetic applications of host–guest complexes and places the focus on the study of water-soluble systems. There are two principal ways to achieve such pK_a shifts, and both are intuitive. The first one involves unspecific hydrophobic interactions resulting from the immersion of an organic water-soluble guest in a nonpolar environment (hydrophobic pocket). This will disfavor ionized sites and states, thereby resulting in reduced and enhanced pK_a values of the ammonium and carboxylic acid groups of amino acids, for example. The second approach requires specific electrostatic interactions with accurate positioning of the acidic or basic functional groups of the complexed guest near regions of negative or positive charge in the host, thereby shifting acidity constants on account of electrostatic repulsions or attractions.

pK_a shifts resulting from unspecific hydrophobic and specific electrostatic effects are well-documented in biological systems,[3–7] and shifts of up to 5 units have been reported, potentially corresponding to a rate enhancement of an acid–base-catalyzed reaction of five orders of magnitude. Although dissection of the various contributing effects is often difficult in enzymes, evidence for predominantly electrostatic effects has been presented in some cases, for example, in acetoacetate decarboxylase.[8] However, relatively little quantitative data have been documented with regard to the pK_a shifts of guests upon binding to water-soluble macrocyclic hosts like cyclodextrins, calixarenes, and cucurbiturils, which could serve as supramolecular models for enzyme–substrate interactions. We have previously observed such pK_a shifts in the complexation of amines by cyclodextrins[9] and cucurbiturils,[10,11] but without realizing their importance

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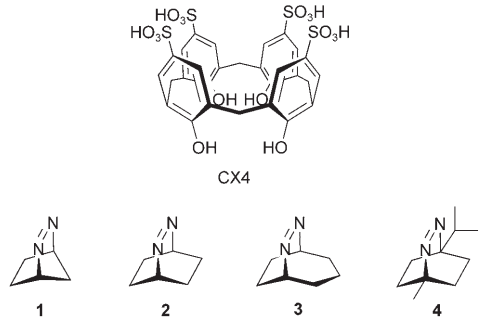
in a more general context or developing this work towards a systematic understanding of these findings.

We recently observed that *p*-sulfonatocalix[4]arene (CX4) shows a pronounced shape complementarity with the non-charged bicyclic azoalkanes **1**–**4** leading to unexpectedly high binding constants.[12] It appeared therefore mandatory



to investigate to what degree the low basicity of these azoalkanes is affected by the complexation with calixarenes. The purpose of this study was not only to qualitatively invoke such effects on protonation equilibria (which would be trivial because one intuitively expects them), but to analyze them quantitatively, to scrutinize their importance for the complexation mechanism of calixarenes,[13] and to predict their absolute magnitude in terms of the cation-receptor properties of the host. Finally, by extending our previous work on cucurbiturils and cyclodextrins to calixarenes, we expected to be able to establish some general trends for enzyme-mimetic pK_a shifts in different supramolecular host–guest systems.

The effects on acid–base equilibria induced by *tert*-butyl-substituted *p*-sulfonatocalixarenes were, in fact, noted earlier by Shinkai et al. for the inclusion of large aromatic dyes,[14] but detailed experimental descriptions and data analyses of the UV spectrophotometric titration data were not provided. In a more recent study, the effect of calixarene complexation on the pK_a values of stilbene dyes was studied by UV spectrophotometry;[15] in this study, a four-state equation for the pH dependence of binding constants was derived, which, however, was not experimentally tested in terms of pH-dependent binding constants. In this paper, we provide data analyses and experimental tests for a refined seven-state model which previous studies did not focus on.

Results

The host-concentration-dependent chemical shifts of azoalkanes **1**–**4** (see Figure 1 for an example) were employed to determine the complex stoichiometry and binding constants at pD 2.4, 7.4, and 13.2 by ^{1}H NMR titrations; the consistent formation of 1:1 host–guest complexes was established (Table 1, inset of Figure 2). The binding constants of the azoalkane 2,3-diazabicyclo[2.2.2]oct-2-ene (**2**) were examined in greater detail over a larger range of pD owing to its importance in recently established fluorescent sensor applications to monitor the competitive binding of choline and carnitine derivatives[16] as well as inorganic cations[17] by fluorescence regeneration. The binding constants reported in this work refer to the concentrations of host and guest rather than to their activity. Note, in this context, that a constant ionic strength at varying pD could not be employed because competitive binding of cations[17] to the host would occur with any added electrolyte.

Bicyclic azoalkanes are very weak bases (pK_a = 1.5 in D_2O, Table 2) and exist in their unprotonated form in neutral aqueous solution. The binding constants of the unproto-

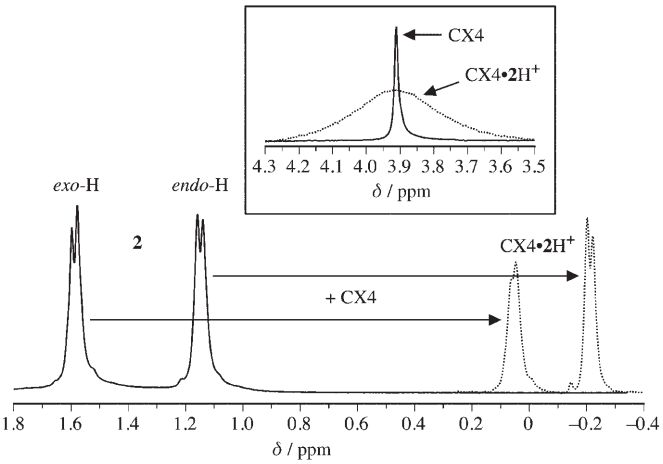


Figure 1. ^{1}H NMR shifts of the *exo* and *endo* protons of **2** (1.0 mM) upon addition of 8 mM CX4 at pD 2.4. The inset shows the ^{1}H NMR peak-broadening of the methylene protons of CX4 (2.0 mM) upon addition of 20 mM **2** at pD 2.4.

Table 1. pD-Dependent binding constants of azoalkanes **1**–**4** with CX4 in D_2O.

Azoalkane	pD[a]	K [M^{-1}][b]
1	2.4	490
	7.4	690
	13.2	470
2	−0.7	530
	0.9	10800
	1.4	12500
	2.4	4300 [4700][c]
	3.4	1200
	7.4	900 [1200][c]
	13.2	570
3	2.4	15000
	7.4	950
	13.2	850
4	2.4	3300
	7.4	480
	13.2	580

[a] The pD was adjusted by addition of DCl or NaOD. [b] An average value for different protons, as determined by ^{1}H NMR titration; 10% error. The values at pD 7.4 are from reference [12]. [c] Determined by UV spectrophotometric titration, taken from reference [16].

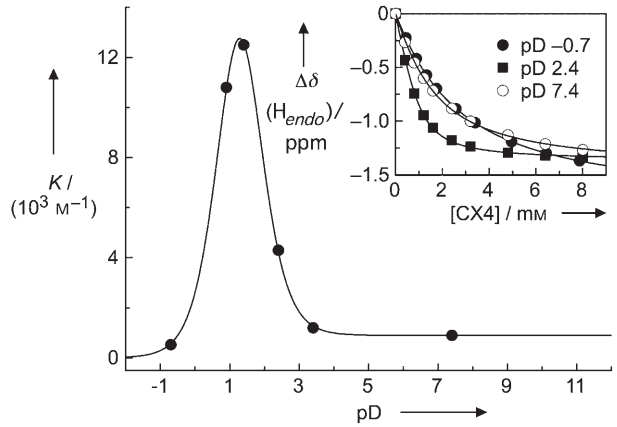


Figure 2. Variation of the binding constants of the CX4·**2** inclusion complex with pD in D_2O and fitted according to Equation (1). The inset shows the ^{1}H NMR titration plots of the chemical shift of the *endo* protons at varying pD.

azoalkanes **2**–**4** in the absence and presence of CX4 was therefore spectrophotometrically monitored to provide information on the acidity constants. The decrease in the near-UV absorbance of uncomplexed **2**–**4** (in the absence of CX4) due to protonation was followed by recording UV spectra at varying pD. The fitting of this titration according to a two-state model afforded pK_a values in the range of 1–1.5 in D_2O (Table 2). Similarly, the decrease in the near-UV absorbance of complexed **2**–**4** with decreasing pD was followed under conditions of significant (60–90%, pD-dependent) complexation (2 mM **2**–**4**, 4 mM CX4). As becomes clear from Figure 3, there are substantial differences in the pK_a values of the uncomplexed and complexed azoalkanes. The fitting of the UV titration data in the presence of CX4 was performed according to a four-state complexation model, that is, by considering absorbance contributions from four different forms of the guest (the complexed and uncomplexed and protonated and unprotonated forms, cf. the Experimental Section); this fitting procedure corrects for partial complexation. The resulting acidity constants are listed as pK_a'(UV) values in Table 2. Based on the UV titrations, the pK_a values of the azoalkanes increase by 1.5–2 units upon complexation. Azoalkane **1**, however, remained unprotonated down to pD 1 even in the presence of CX4, which prevented the determination of a pK_a shift due to complexation by CX4.

Table 2. pK_a values of azoalkanes **1**–**4** in their uncomplexed (pK_a) and CX4-complexed state (pK_a') in D_2O and extrapolated binding constants of the unprotonated and protonated azoalkanes **1**–**4** with the CX4 tetra-anion.

Azoalkane	pK_a	pK_a' (UV)	pK_a' (NMR)	ΔpK_a	$K_{G,1}$ [10^3 M^{-1}][a] (unprotonated)	K_{GH+} [10^5 M^{-1}][b,c] (protonated)
1	[−0.8][d]	–	–	–	[0.69][e]	–
2	1.0[f]	2.5	3.0	1.8 ± 0.3	1.0 ± 0.1[c]	1.0
3	1.5[g]	3.4	3.9	2.2 ± 0.3	[0.95][e]	2.5
4	1.1[h]	2.6	3.1	1.8 ± 0.3	[0.48][e]	0.49

[a] Binding constant for the unprotonated azoalkane with the CX4 tetra-anion, cf. Scheme 2. [b] Binding constant for the protonated azoalkane with the CX4 tetra-anion, cf. Scheme 2. [c] Obtained by fitting the experimental binding constants below pD 8 (Table 1) according to Equation (1), see text; 10% error. [d] Estimated from the value of −1.4 in H_2O from reference [18] by adding 0.6 units as a typical offset for D_2O, cf. reference [19,20]. [e] Taken as the binding constant at pD 7.4, cf. Table 1. [f] This work; the value for **2** in H_2O was determined as 0.5, which compares with a value of 0.4 reported in reference [18]. [g] This work; the value of 3.0 for **3** from ref. [18] in H_2O appears to be in error. [h] This work.

nated azoalkanes (near pD 7.4) with CX4, which were reported previously,[12] are in the range of around 500–1000 M^{-1} (Table 1). Interestingly, the binding constants increased by a factor of 5–15 at pD 2.4, except for **1**. This increase for azoalkanes **2**–**4** was attributed to the binding of the protonated azoalkane, that is, the guest is being protonated when complexed to CX4. Azoalkane **1** did not show the same behavior since its pK_a value is too low (Table 2) to allow protonation even in the presence of CX4. Note that the data in Table 1 also reveal that the binding constant of **2** decreased again at strongly acidic pD values (<1). This results in a characteristic up-and-down feature of the pD-dependent binding constants with a maximum at around pD 1.5 (Figure 2).

The bicyclic azoalkanes **1**–**4** exhibit a characteristic weak near-UV absorption in water with λ_{max} (ε) values of 330 (110) for **1**, 365 (50) for **2**, 377 (70) for **3**, and 372 nm (55 M^{-1}cm^{-1}) for **4**. Protonation of azoalkanes results in a diagnostic hypsochromic shift of their UV absorption band (insets of Figure 3).[16,21] The protonation equilibrium of

Complexation-induced ^{1}H NMR shifts and 2D ROESY NMR measurements at pD 7.4 have previously afforded evidence for the formation of deep inclusion complexes with an equatorial inclusion geometry for **1**–**3** and an axial one for **4**.[12] The complexation-induced ^{1}H NMR shifts in acidic (Figure 1) and alkaline solutions, determined in this study, are very similar to the data obtained at neutral pD and afforded no indication of a major change in the complexation geometry. An exception was azoalkane **4**, for which the bridgehead methyl group showed a significantly larger shift at pD 2.4, which may be indicative of a slightly tilted complex geometry, with the methyl group partially included. Such tilting could improve the centrosymmetric electrostatic interaction of the formal positive charge on the protonated azoalkane with the surrounding sulfonato groups (Scheme 1). ROESY spectra obtained for azoalkanes **2**–**4** at pD 2.4 afforded no significant differences from the spectra at pD 7.4[12] either. We therefore assume that protonation of the azoalkane in acidic solution results in only a minor change in the inclusion geometry from that in neutral solution[12] and propose the complex geometries shown in Scheme 1 for the protonated azoalkanes.

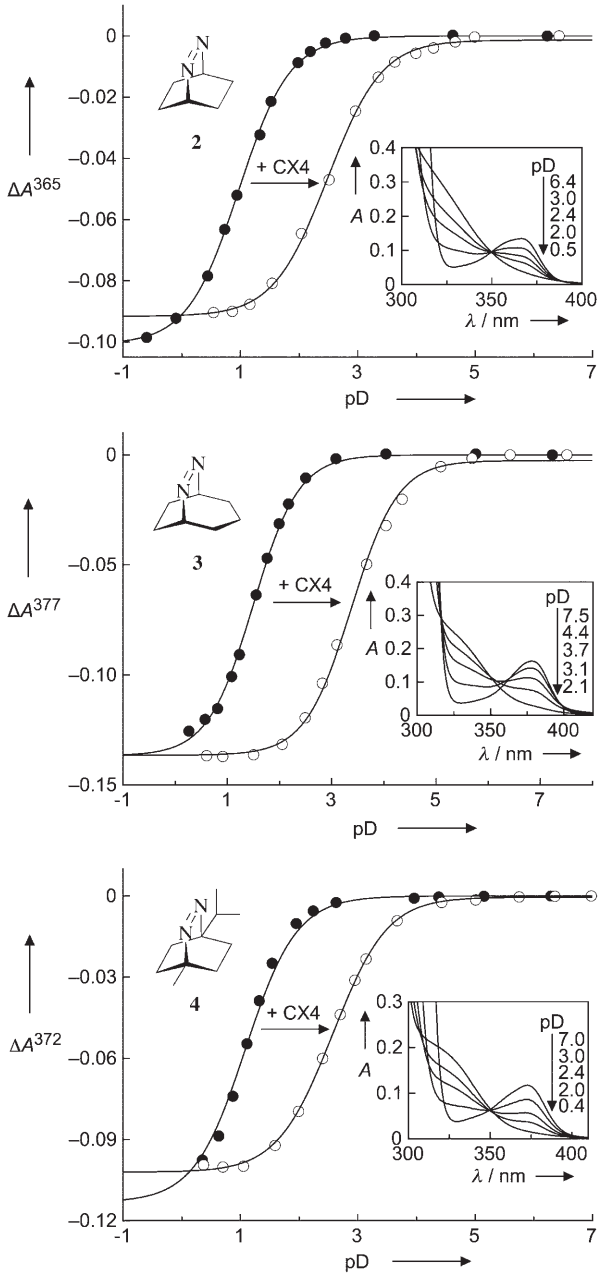


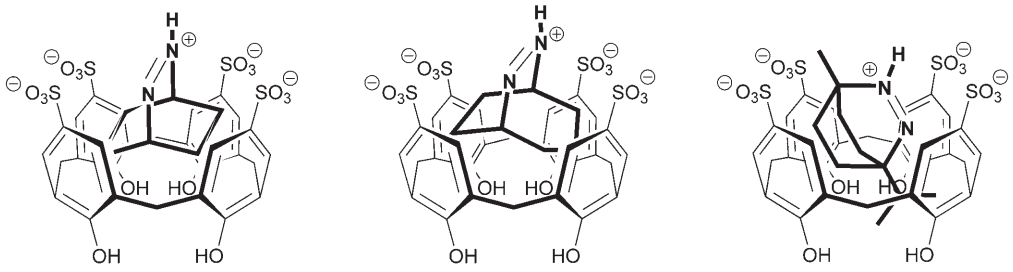
Figure 3. pD titration plots for the UV absorbance of 2 mM **2**–**4** in D_2O in the absence (filled circles) and presence (open circles) of 4 mM CX4. The insets show the corresponding evolution of the UV spectra in the presence of CX4 with pD; note that isosbestic points (approximately observed for **2** and **4** but not for **3**) were not necessarily expected owing to the involvement of a four-state (or seven-state) equilibrium in the presence of CX4.

Discussion

Herein, we describe a quantitative analysis of the pD-dependent binding and host-assisted guest protonation of azoalkanes **1**–**4**, as guests, with *p*-sulfonatocalix[4]arene (CX4). The first part of the discussion is devoted to the understanding of the accurate complexation mechanism, which was a prerequisite for an accurate determination of the acidity constants. The second part focuses on a generalization and comparison of the observed pK_a shifts of different host and guest molecules.

Mechanism of complexation with CX4: Our interpretations will first concentrate on azoalkane **2**. As can be seen from Table 1 and Figure 2, the binding constant of **2** increases from 900 M^{-1} at pD 7.4 to 12500 M^{-1} at pD 1.4. This full order of magnitude variation in the binding of an organic guest by CX4 is substantially larger than, for example, the factor of 2–3 observed for amino acids, in which pH-dependent electrostatic effects related to the charged residues cause a minor modulation.[22] As borne out by NMR line-broadening effects, the conformational rigidity of CX4 increases with pD[23,24] as a consequence of different degrees of ionization of the phenoxy groups.[25] The most flexible tetra-anion (with none of the phenoxy groups being deprotonated) converts with a pK_a of 3.26 (H_2O) to the penta-anion, which is transformed with a pK_a of 11.8 (H_2O) to the hexa-anion;[25] this last species is presumed to dominate in strongly alkaline solution,[26] although an additional ionization may occur, judging by the values of pK_a reported for the third and fourth phenoxy ionizations which are subject to larger uncertainty.[25] Nevertheless, CX4 is presumed to adopt a cone conformation across the entire pD range,[23,24,27,28] such that complexation by different conformations appears an unlikely cause of the observed pD-dependent binding affinity. In addition, complexation by the flexible host CX4 is generally presumed to proceed efficiently by an induced-fit mode of action, which in the case of a virtually spherical guest is best met by the postulated conical cavity.[24,29,30] In fact, line-broadening of the methylene peak of CX4 in the presence of an excess of **2** at pD 2.4 (see inset of Figure 1) strongly suggests that the cone shape of the host is stabilized by the presence of the guest even for the most flexible tetra-anion form.[23,24] Note that a pD-dependent change in the complexation geometry of the guest has previously been observed with the trimethylanilinium ion as guest,[23,24,30] but the underlying reasons held responsible, namely differential cation–π interactions that are dependent on the degree of ionization of the phenoxy moieties, are not relevant to our case. In fact, we have recently demonstrated[16] that cation–π interactions increase the binding at higher pD by a factor of around five as a result of the ionization of the phenolic hydroxy groups of CX4, which produces better electron-donating phenolate aryl sites, yet the observed trends for azoalkanes **2**–**4** are the opposite.

Surprisingly, although uncomplexed **2** is hardly protonated in D_2O near pD 2.4 (pK_a = 1.0 in D_2O), it is clearly being significantly protonated within the supramolecular complex near pD 2–4, as reflected in the characteristic changes in the UV spectra (insets of Figure 3). This establishes a case for host-assisted guest protonation for calixarenes, which can be directly related to a large pK_a shift. From the UV titrations in the absence and presence of CX4 (Figure 3), the pK_a' value for **2** when complexed by CX4 was estimated to be 2.5. The observed protonation of **2** accounts for the en-



Scheme 1. Presumed complexation geometries for the CX4 complexes (tetra-anion, acidic pD) of the protonated azoalkanes **2**–**4**.

hanced binding constant with CX4 at pD 2.4 since, in addition to the hydrophobic interactions,[27,31] there is additional "charge-assisted" binding.[17,30,32] Very similar pK_a shifts (1.5–2) were determined for azoalkanes **3** and **4** by UV titrations (Table 2) despite the fact that the pK_a value of uncomplexed **3** was shifted by approximately 0.5 units (Table 2) and that the binding constant of **4** at pD 7.4 was about a factor of two smaller than that of **2** (Table 1).

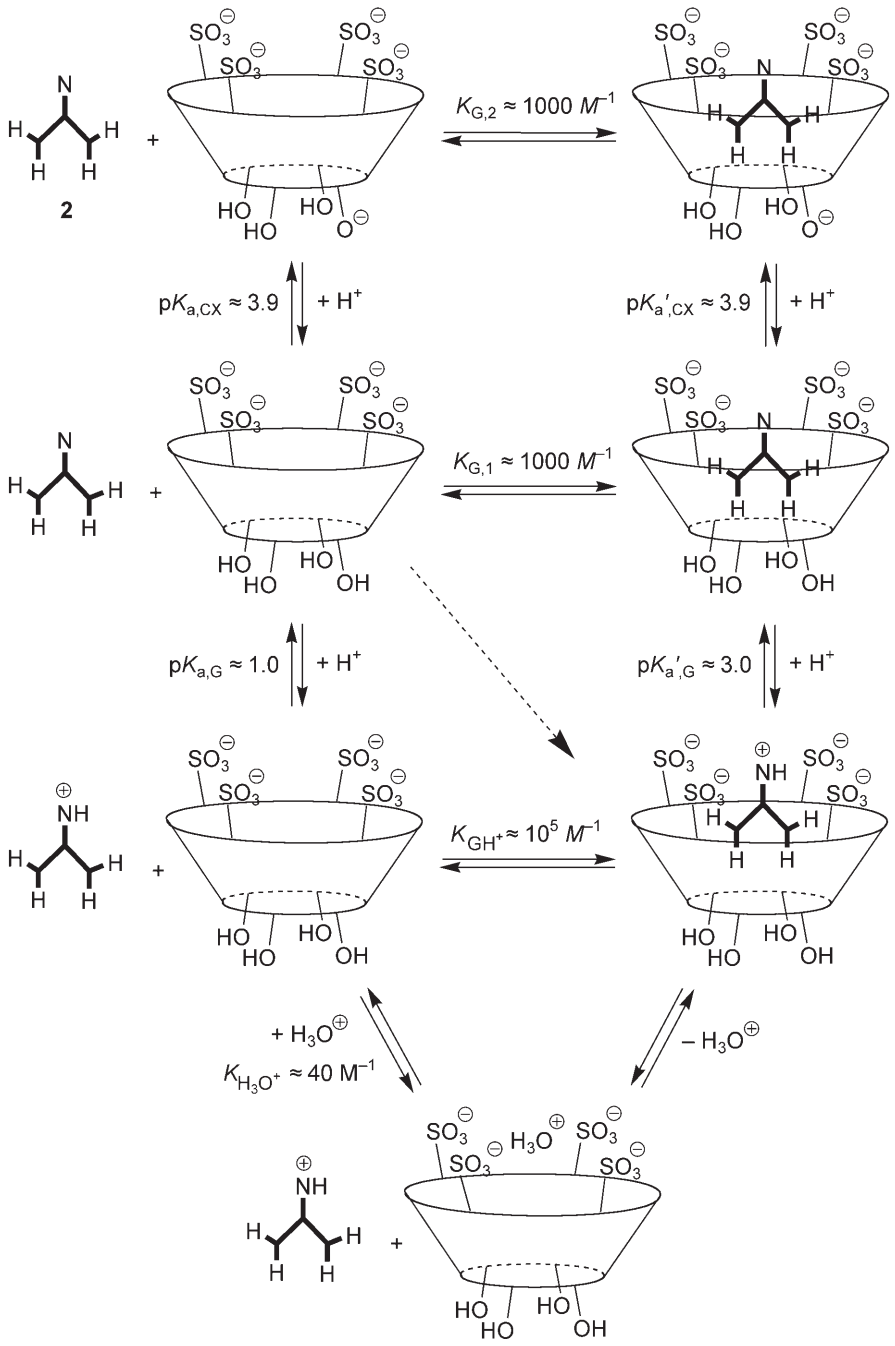
The quantitative understanding of the pD-dependent complexation equilibria in the critical region below pD 8 presented a major challenge. On one hand, CX4 is known to undergo the first ionization of one phenoxy group in this pD range (p$K_{a,CX} \approx 3.9$ in D_2O, assuming a 0.6 unit offset[19,20] relative to H_2O as is commonly found for weak acids).[25] Moreover, the protonation of **2** needs to be taken into account such that at least six species need to be considered. Such a model, however, can only account for an increase in binding at a strongly acidic pD, at which the most stable complex between CX4 and protonated **2** is expected to be formed. Experimentally, however, the binding decreases again at pD values below about 1.4 (Table 1 and Figure 2), such that an additional process needed to be implicated that leads to a destabilization of the complex. Protonation of the sulfonato or phenolic hydroxy groups could be responsible for destabilization of the complex, but this appears unlikely in view of the strongly negative pK_a values of arylsulfonic acids[33] as well as protonated phenols ($ArOH_2^+$).[34] More likely, what is being observed is competitive binding between the deuteriated hydronium ion and the CX4 tetra-anion; such a complexation of inorganic as well as organic cations is expected to lead to competitive binding, a release of **2**, and therefore a lower observed binding constant.[16,17] Consideration of the formation of the hydronium-ion complex of CX4, the existence of which cannot be negated in any case, results then in a seven-state equilibrium (Scheme 2), for which the observed binding constant for complexation between CX4 and **2** can be analytically expressed by Equation (1).

$$K = \frac{K_{a,G}K_{a,CX}K_{G,2} + K_{a,G}K_{G,1}[H^+] + K_{GH^+}[H^+]^2}{(K_{a,G} + [H^+])(K_{a,CX} + [H^+] + [H^+]^2/K_{H_3O^+})} \quad (1)$$

As shown in Figure 2, the fitting of data according to the seven-state model in Equation (1) reproduces the experimental results excellently. The thermodynamic parameters obtained from the data fitting are also of great interest. First, it can be seen that the fitted binding constant of unprotonated **2** with the tetra-anion of CX4 ($K_{G,1} = 1000 \pm 200\ M^{-1}$) is very similar to that with the CX4 penta-anion ($K_{G,2} = 900\ M^{-1}$, the value at pD 7.4). The somewhat lower binding in alkaline solution (pD 13.2; $K_{G,3} = 570\ M^{-1}$), at which the CX4 hexa- or hepta-anion prevails, can be accounted for by the presence of metal cations (required to adjust the pD), which affect the binding adversely.[17] Importantly, there appears to be no significant increase in binding with increasing cone flexibility (lower pD), which suggests that the differently ionized calixarenes bind the neutral guest with comparable strength. This notion is independently supported by the rather pD-independent binding constants of azoalkane **1** with CX4 (Table 1), since this guest does not undergo protonation as a consequence of its negative pK_a value (Table 2). The slightly reduced binding constants for azoalkane **1** in acidic and alkaline solution can again be rationalized by competitive binding by hydronium and alkali ions, respectively.

Secondly, by extrapolation, the binding constant of protonated **2** with the tetra-anion of CX4 [$K_{GH^+} = (1.0 \pm 0.1) \times 10^5\ M^{-1}$] was found to be very high, in the upper range of the binding constants of quaternary ammonium ions.[16,23,24,27,35] As can be seen from comparison of the binding constants of unprotonated and protonated **2**, the electrostatic contribution to the binding is large (factor of 100), but the hydrophobic effect (which accounts for the binding of the unprotonated form) is larger (factor of 1000) owing to the strong binding of noncharged **2** (Table 2).[12] Thus, our case provides an exception to the general conclusion that electrostatic effects dominate over hydrophobic effects in CX4 binding.[36]

Note in Table 1 that the experimental binding constants for **2** at around pD 1 (ca. $10\,000\ M^{-1}$) are nearly one order of magnitude below the extrapolated limit (K_{GH^+}) since at this pD binding by the deuteriated hydronium ion has already become strongly competitive. The absolute binding constant for the deuteriated hydronium ion with CX4 ($K_{H_3O^+} \approx 40 \pm 10\ M^{-1}$) was found to be very low, however, and corresponds to an apparent "first" pK_a value of 1.6 for CX4 in D_2O, which is of interest in view of the known difficulties in determining the pK_a values of CX4.[25,37–41] The binding constant



Scheme 2. Mechanism for the complexation of azoalkane **2** with CX4; data were obtained by using Equation (1) and refer to equilibria in deuteriated water as solvent (exchanged deuterium atoms are not shown for simplicity).

for the hydronium ion is very reasonable in comparison with the binding constants recently determined for other inorganic monocations like alkali (70–150 M^{-1}) and ammonium (95 M^{-1}) at pD 2.4.[17] In particular, if ammonium has a sizable binding constant with CX4, there is absolutely no reason why the hydronium ion should not also form a complex, and this mechanistic intricacy is exactly what is required by the present experimental data. The binding of the hydronium ion with CX4 competes, however, only in strongly acidic media (pD < 3).

The binding constant of the deuteriated hydronium ion with CX4 (obtained for **2**) was subsequently kept fixed to estimate the binding constants for the protonated forms of azoalkanes **3** and **4** with CX4 as well (two-point fittings from NMR data!). The resulting values were again very large (Table 2), in the range of $10^5\ M^{-1}$. By using the relationship $K_{GH+}/K_G = K_{a,G}/K_a'_G$ for the pertinent thermodynamic cycle,[10] the pK_a' values for the complex formed between CX4 and the protonated azoalkanes could be independently projected from the NMR data [see the pK_a'-(NMR) values for azoalkanes **2–4** in Table 2]; these values were slightly larger than those obtained from the UV spectrophotometric titrations (Figure 3) which is presumably related to the use of a four-state model in the latter method. Conservatively, we have provided the pK_a shifts (ΔpK_a values in Table 2) as an average of the determinations by the two independent methods with a considerable uncertainty range. The combined data for the different guest molecules suggest therefore a pK_a shift of around 2 pK_a units (Table 2).[42]

The electrostatic stabilization of the complex, which is responsible for the stronger binding of the protonated form and therefore the pK_a shift, corresponds to about 10 kJ mol^{-1}. Importantly, this extra stabilization is essentially the same as the total stabilization of the CX4 complex with inorganic monocations.[17] We therefore generalize tentatively as follows: The protonation of a noncharged guest molecule increases the binding with CX4 by a factor of around 100; the associated electrostatic stabilization adds to an existing hydrophobic stabilization; for small inorganic cations the binding constants are around 100 M^{-1} because hydrophobic interactions are absent. It should therefore be possible to quite reliably predict from the binding constants of protonated guest molecules those of their conjugate noncharged forms. This is important, because the

binding constants of noncharged guests are typically very small and therefore difficult to determine directly experimentally[14] (with the high binding of azoalkanes **1**–**4** providing a notable exception).[12]

Note that the pK_a shift reveals an interesting peculiarity of the complexation mechanism between azoalkanes **2**–**4** and CX4, which is reminiscent of the situation of amine binding by cucurbit[6]uril between pH 10.5–12:[10] In the narrow pD region between around pD 1 and 3, the protonated complex may not only form by direct complexation of the protonated guest, but, alternatively, the unprotonated guest could be preferably complexed owing to its greater abundance in solution. Once captured the basicity of the uncharged guest increases steeply such that rapid protonation occurs to form the more stable protonated guest complex; the net result of the latter mechanism is a complexation accompanied by protonation, that is, a host-assisted protonation (dashed arrow in Scheme 3).

Complexation-induced pK_a shifts and catalytic activity: As can be seen, CX4 increases the pK_a value of bicyclic azoalkanes by around 2 pK_a units, which is significantly larger than the effect of 1.3 units quantified for the complexation of cyclohexylmethylamine by cucurbit[6]uril,[10] and opposite to the situation for cyclodextrins, which were frequently shown to depress the pK_a value of the conjugate acids of neutral bases by around 0.4–1 units (Scheme 3).[9,43–46] The pK_a shift for cucurbit[6]uril indicates a positioning of the positive charge in the proximity of the ureidocarbonyl groups, such that stabilizing ion–dipole interactions now favor the protonated ammonium ion over the amine form. The larger pK_a shift observed for CX4 can also be rationalized since full anionic charges at the sulfonato groups are now involved which allow stronger ion–ion interactions to select the protonated over the unprotonated guest. The inverse pK_a shift for cyclodextrins is readily accounted for in terms of the relocation of the guest to a hydrophobic environment, which disfavors ionized states in general, and in an unspecific manner. The data for the various host–guest systems are summarized in Table 3. As can be seen, the pK_a shifts in supramolecular host–guest complexes appear to be governed by some systematic trends which may afford design criteria for further optimization. However, the shifts are clearly smaller than the values reported in some biological systems (shifts of up to 5 pK_a units).

Finally, the observed pK_a shifts have immediate implications for the rational use of CX4 in acid-catalyzed reactions.[47] These are predictively (K_a/K_a') accelerated by a factor of around 100 on the basis of the more favorable protonation equilibrium alone; this compares very well with the rate enhancement observed, for example, in the acid-catalyzed methanolysis of *N*-acetylamino acids upon addition of CX4 (factor 12–86).[48]

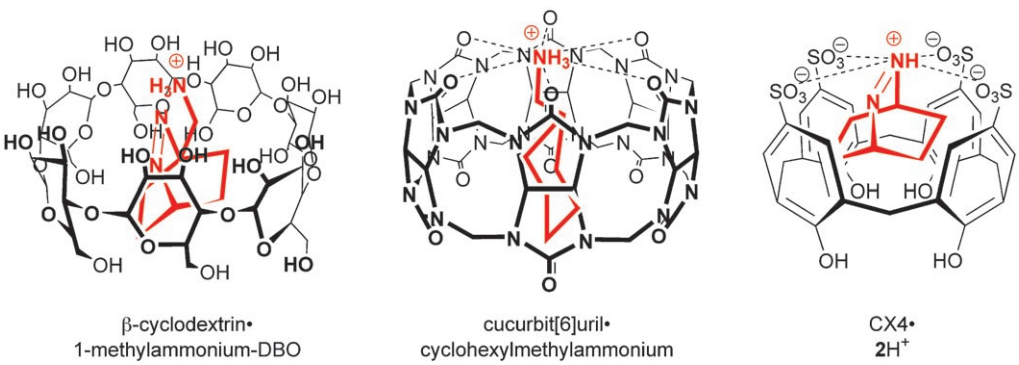
Conclusion

In conclusion, the mechanistic investigations into the complexation of azoalkanes **1**–**4** by the water-soluble CX4 have revealed pK_a shifts of the order of 2, which have been independently determined from the pD-dependent binding constants (by ^{1}H NMR spectroscopy) and from the pD-dependent changes in the UV absorption spectra of azoalkanes **2**–**4**. The pK_a shifts result in larger binding constants for the protonated azoalkanes and therefore in an increased binding constant in acidic solution (pD 2.4). In more highly acidic solutions, the binding constant decreases again. This has been attributed to competitive binding by the hydronium ion ($K \approx 40\ M^{-1}$). The observed host-assisted guest protonation appears to be a rather general phenomenon for macrocyclic cation acceptors and some relationships between the

Table 3. pK_a shifts of guest molecules in macrocyclic water-soluble hosts.

Host	β-Cyclodextrin	Cucurbit[6]uril	CX4
Guest	1-Aminomethyl-DBO[a]	Cyclohexylmethylamine	Azoalkanes **1**–**4**
ΔpK_a	−1.1	1.3	ca. 2
interaction[b]	hydrophobic	ion–dipole	ion–ion
interaction sites	unspecific	6 C=O	4 SO_3^-
titration method	ICD[c]	^{1}H NMR	^{1}H NMR/UV
state model	4-state	4-state	7-state (NMR)/ 4-state (UV)
source	reference [9]	reference [10]	this work

[a] DBO: 2,3-diazabicyclo[2.2.2]oct-2-ene. [b] With protonated guest. [c] Induced circular dichroism.



Scheme 3. Water-soluble host–guest complexes exhibiting pK_a shifts of included guests (DBO = 2,3-diazabicyclo[2.2.2]oct-2-ene).

type of host und guest as well as the interaction topology have emerged. Related pK_a shifts are of course well-recognized in biological systems, in which protein-assisted protonation or deprotonation of substrates is of functional importance.[6,7] The design and understanding of supramolecular or polymeric systems that mimic this enzymatic action presents therefore a great challenge[49–51] which should provide an incentive to study additional cation and anion receptors with respect to their ability to modulate the protonation equilibria of organic guests. Finally, host-assisted changes to the ionization states of guest molecules are not limited to pK_a shifts but should be transferable to shifts in their redox potentials, the understanding of which is of current interest.[52–55]

Experimental Section

p-Sulfonatocalix[4]arene CX4 (>97%) was purchased from Fluka and used as received. Azoalkanes **1**–**4** were available from previous work.[56] All experiments were performed at ambient temperature in D_2O (99.8%, Applichem, Omnilab, Germany). The pD values of the solutions were adjusted by addition of DCl or NaOD. pH readings were taken with a WTW 330i pH meter equipped with a combined pH glass electrode (SenTix Mic) and converted to pD (+0.40 units)[20] where applicable. To obtain pD −0.7 we used 3.2 M DCl and applied an approximate mean activity coefficient of 1.4, based on comparison with tabulated data for HCl.[57] ^{1}H NMR spectra were recorded with a JEOL ECX 400 MHz NMR spectrometer. UV spectra were obtained with a Varian Cary 4000 spectrophotometer (0.2 nm resolution) and were corrected with blank measurements of solutions containing only CX4. All experiments were performed at ambient temperature (25 °C).

The pD dependence of the binding constants was analyzed by using a seven-state model [Equation (1), see text]; the nonlinear fitting procedure of the ProFit software[58] was employed. The pD dependence of the UV absorbance was approximated using a four-state model (by considering a single protonated and unprotonated complex) as a result of the complexity of the analytical expression already at this level; the model assumes that the guest (G) absorbance in the complexes is independent of the degree of protonation of CX4, that is, it is the same for the tetra- and penta-anion complexes.[59] The formula relating to the pD dependence of the UV absorbance was obtained by extension of an expression derived for the fitting of induced circular dichroism data of protonated versus unprotonated CD–guest complexes.[9] Specifically, the UV absorbance contributions of the uncomplexed protonated and unprotonated guest were included in Equation (2), where A is the experimental UV absorbance normalized for the selected path length (d), $\varepsilon_{CX\cdot G}$, $\varepsilon_{CX\cdot GH+}$, ε_G, and ε_{GH+} are the extinction coefficients of the unprotonated and protonated complexed and uncomplexed guest at the particular wavelength, respectively, K_a (known) and K_a' (to be fitted) are the acidity constants of the uncomplexed and complexed guest, K_G is the apparent binding constant of the unprotonated complex (see Table 2), and $[G]_0$ and $[CX4]_0$ are the total concentrations of guest and host. The fitting of the pD titration data for the free guest was performed according to the usual two-state equation (2).

$$A/d = P\left\{\varepsilon_{CX\cdot G}K_a' + \varepsilon_{CX\cdot GH^+}[H^+] + \frac{K_a'}{([CX4]_0 - P\{K_a' + [H^+]\})K_G}\left(\varepsilon_G + \varepsilon_{GH+}\frac{[H^+]}{K_a}\right)\right\} \quad (2)$$

with

$$P = \frac{[G]_0 + [CX4]_0}{2(K_a' + [H^+])} + \frac{K_a'([H^+] + K_a)}{2K_GK_a(K_a' + [H^+])^2} - \frac{\sqrt{(K_GK_a([G]_0 + [CX4]_0)(K_a' + [H^+]) + K_a'([H^+] + K_a))^2 - 4[G]_0[CX4]_0K_G{}^2K_a{}^2(K_a' + [H^+])^2}}{2K_GK_a(K_a' + [H^+])^2}$$

Acknowledgements

This work was supported by the Graduate Program "Nanomolecular Science" of the International University Bremen.

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Received: November 29, 2005
Revised: February 9, 2006
Published online: May 4, 2006

