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Selective Sensing of Citrate by a Supramolecular 1,8-Naphthalimide/Calix[4]arene Assembly via Complexation-Modulated pK_a Shifts in a Ternary Complex

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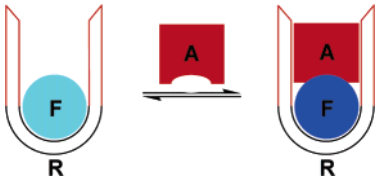
Received February 7, 2007



A water-soluble supramolecular sensing assembly, composed of an imidazolium-substituted calix[4]arene and a fluorescent aminodiacetate derivative of 1,8-naphthalimide, was studied. Addition of citrate led to a large fluorescence enhancement, while tartrate, acetate, as well as selected inorganic anions gave smaller effects. The sensing principle and selectivity for citrate rely on the formation of a ternary fluorophore−host−anion complex and complexation-induced pK_a shifts of an amino group attached to the fluorophore. The complexation of citrate induces a protonation of the amino group, which switches off intramolecular photoinduced electron transfer as the fluorescence quenching pathway, leading to an enhancement of the optical output signal. The intricate sensor principle was corroborated by pH titrations, binding constants, and structural information as obtained by ^{1}H NMR spectroscopy.

Introduction

The development of selective chemosensors for anions is of high significance for biological and clinical applications. In particular, several systems for the detection of carboxylates (e.g., citrate, tartrate, amino acids) and phosphates (e.g., nucleotides) have been reported.[1−5] Many molecular sensor systems[6] are based on fluorescence for detection, due to its high sensitivity down to the single molecule level and the possibility for selective read-out. Commonly, a fluorophore is covalently linked to a receptor site, which is designed for the specific recognition of the target anion. As a communication mechanism between receptor and fluorophore, intramolecular photoinduced electron transfer (PET) is frequently used.[2−5] Accordingly, the recognition event induces a change of the electronic properties of the receptor, which leads to either enhanced or reduced fluorescence by blocking PET or promoting PET, respectively. An alternative strategy in the design of anion sensors involves the displacement of the fluorescent dye from a supramolecular assembly by competitive binding of the anion, which again alters (turns on or switches off) the fluorescence of the reporter group (Scheme 1a).[1,2] A precondition for the latter approach is a sufficient differentiation between the fluorophore properties in its complexed and uncomplexed form.

We now present a citrate sensing assembly based on a water-soluble supramolecular host−fluorophore complex between a calix[4]arene with positively charged upper-rim imidazolium groups (ICX) and the 1,8-naphthalimide dicarboxylate derivative **1** as a noncovalently bound fluorophore (cf., Chart 1). Originally, our work was motivated by (i) the comparably scarce use of

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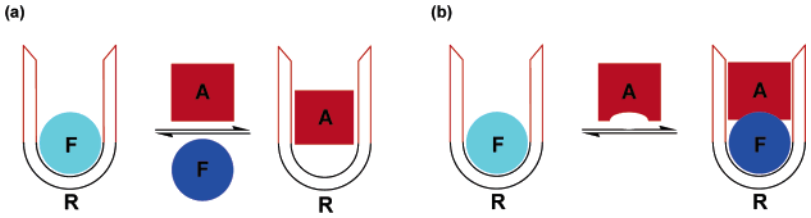
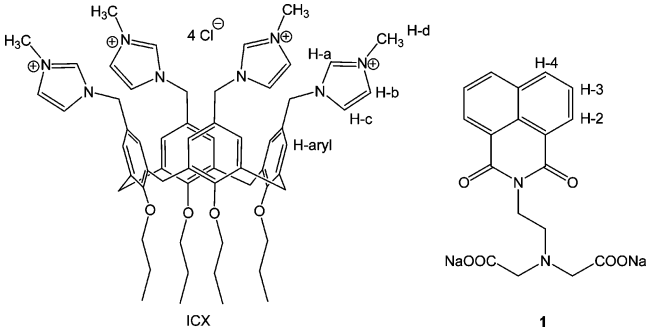
10.1021/jo070268+ CCC: $37.00
Published on Web 04/14/2007

SCHEME 1. Comparison of Fluorescence Sensing Principles Based on (a) Displacement and (b) Ternary Complex Formation[a]

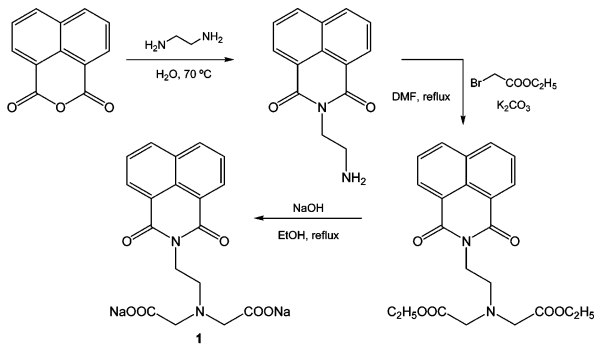


[a] R, receptor; A, analyte; F, fluorophore.

CHART 1. Structures of ICX and 1 with Proton Assignments Used for the Discussion of ^{1}H NMR Data



SCHEME 2. Synthesis of 1



calixarenes for analyte sensing based on the above principles,[7−10] and (ii) the continued demand for improved and selective sensors for the citrate anion,[11−20] which plays a central role in biochemical processes like the Krebs cycle, as universal chelating and buffer agent, and as a ubiquitous food and in particular beverage additive.[21,22]

Close inspection of the fluorescence titration profiles upon addition of citrate revealed that the host−fluorophore−analyte interaction cannot be described by the conventional displacement model (Scheme 1a), but rather involves the formation of a ternary complex as depicted in Scheme 1b. Consequently, the fluorescence response of the supramolecular sensing assembly cannot be understood in terms of differential fluorescence of the free and complexed forms of the dye, but instead in terms of a fluorescence variation in a binary and ternary complex. In detail, we observed that the binding of citrate causes a pK_a shift of the dye; that is, the amino functionality of the dye becomes protonated upon citrate binding, which enhances its fluorescence by reducing intramolecular PET as quenching mechanism. Although the present ternary supramolecular sensing assembly is practically appealing (water solubility, operation near neutral pH, convenient synthetic access to both host and dye), our study was driven by the conceptual novelty of the sensing principle (ternary complex with positive cooperativity) and the challenge to mechanistically understand the interplay of intermolecular interactions, in particular the complexation-induced pK_a shifts.

Results and Discussion

The 1,8-naphthalimide derivative **1**, used as fluorophore in our chemosensing ensemble, was synthesized in three steps by condensation of 1,8-naphthalic anhydride with an excess of 1,2-diaminoethane to yield an aminoethyl-substituted naphthalimide according to a recently reported procedure.[23] This compound was further reacted with bromoacetic acid ethyl ester, and the resulting diester was saponified to yield the target compound **1** (Scheme 2). The calixarene derivative ICX was obtained as chloride salt according to a known literature procedure;[24] the host content was checked by ^{1}H NMR and found to be higher than 95%. The ^{1}H NMR shifts of the ICX protons did not vary with concentration in the range of 0.5−4.0 mM, which suggests that the host does not aggregate under the relevant experimental conditions; this is in agreement with the previously reported critical micelle concentration of 5 mM.[24]

The 1,8-naphthalimide derivative **1** is an amino diacid and should therefore exist as a monoanion near pH 7 in water

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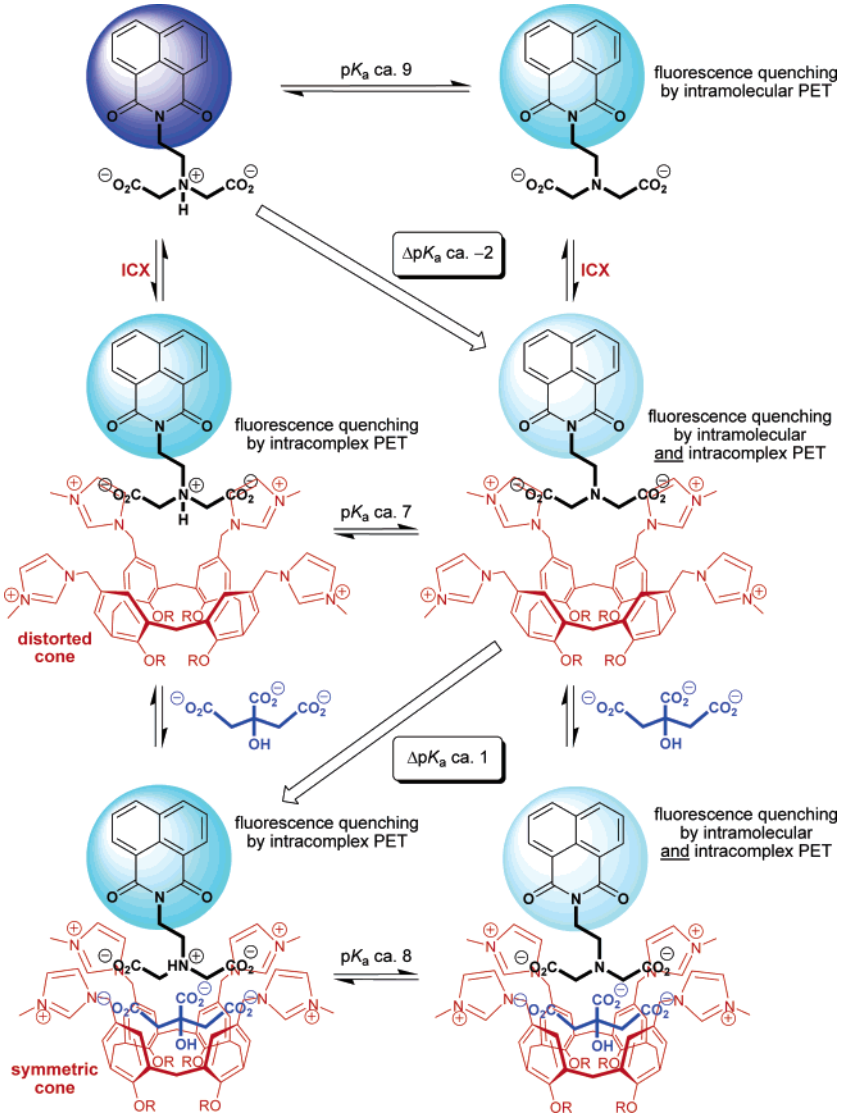
SCHEME 3. Proposed Interaction Mechanisms in the Citrate Sensing Assembly of 1 and ICX, Concomitant pK_a Shifts, Changes in Complex Geometries, and Effects on Dye Fluorescence (Color-Coded)



(Scheme 3, top left structure). At this pH, **1** showed a blue and broad fluorescence with λ_{max} = 398 nm (Φ_f = 0.19) upon excitation at 350 nm. The pH titration profile, monitoring the fluorescence of **1**, is shown in Figure 1 (○). In the pH range between 3 and 7, the fluorescence intensity of the dye remained high and constant. At alkaline pH, strong fluorescence quenching was observed (>95%), which is related to the conversion of the ammonium group of the dye to an amino group (pK_a = 9.0, see top right structure in Scheme 3); the latter causes fluorescence quenching by intramolecular PET from the nitrogen lone pair. This process is thermodynamically favorable, as can be readily estimated with the Rehm−Weller equation[25] (ΔG_{et} = −1.06 eV).[26] As will be seen in the following, the pK_a value of **1** shifts considerably in its supramolecular complex ICX·**1**, and also upon binding of citrate, which constitutes the fascinating working principle of the new sensing assembly.

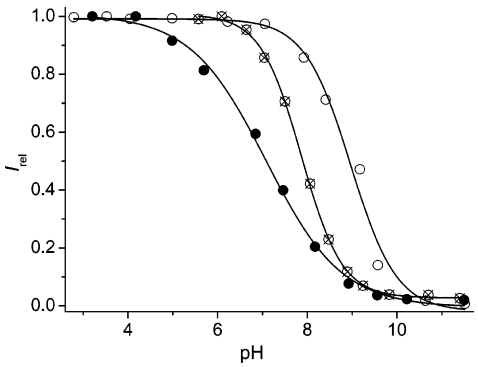


FIGURE 1. pH titration profiles for the fluorescence intensity (λ_{exc} = 350 nm, λ_{obs} = 398 nm) of 5 μM **1** (○), 5 μM **1** in the presence of 4 mM ICX (●), and 5 μM **1** in the presence of 4 mM ICX as well as 20 mM citrate (⊗).

Host−Dye Complex Formation. As has been demonstrated for various supramolecular architectures,[24,27,28] positively charged imidazolium residues interact with anions via Coulombic interactions and hydrogen bonding. Therefore, we expected good binding properties of ICX with the bis-carboxylate **1**. Indeed, complexation by ICX caused sizable ^{1}H NMR upfield shifts of the naphthalimide protons of **1**, which are indicative for the

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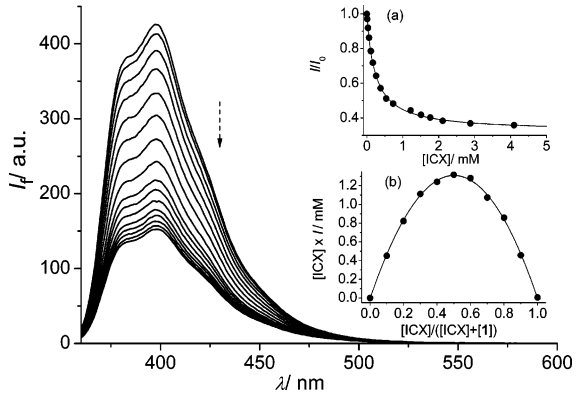

FIGURE 2. Fluorescence titration (λ_{exc} = 350 nm) of **1** (5 μM) with ICX at pH = 7.0. The insets show (a) the titration curve and the fit according to 1:1 complexation and (b) the Job's plot, both at λ_{obs} = 398 nm.

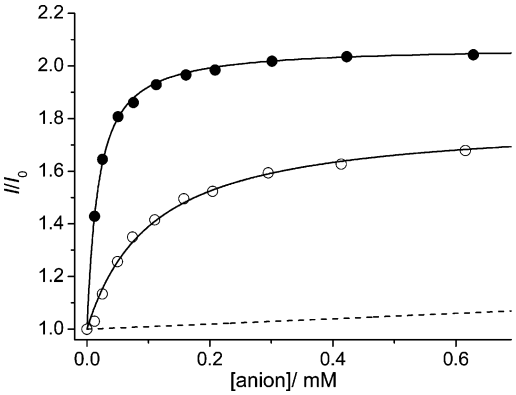

FIGURE 3. Fluorescence titrations (λ_{exc} = 350 nm, λ_{obs} = 398 nm, pH = 7.0) of a solution containing 5 μM **1** and 1 mM ICX with citrate (●) and tartrate (○) fitted according to a model for ternary complexation (Scheme 1b). The dashed line shows a simulation for a titration expected for displacement (Scheme 1a, taking $K_{ICX\cdot 1}$ = 4200 M^{-1} and $K_{ICX\cdot citrate}$ = 800 M^{-1}).

formation of a host−guest complex. For the H-4 and H-2 protons (see Chart 1), upfield shifts between 0.5 and 0.6 ppm were detected (0.5 mM **1**, 4 mM ICX, pD = 7.4). The H-3 protons showed similar shifts, but overlapped with host signals in the course of the titration. In addition, ROESY cross-peaks between **1** and CX4 were observed (see below), which confirmed complex formation as well.

Complexation was also monitored by optical spectroscopy. When a 5 μM solution of **1** was titrated at pH = 7.0 with ICX (Figure 2), a fluorescence quenching by ca. 70% was observed toward the endpoint of the titration (ca. 4 mM ICX). The absorption spectrum of **1** (corrected for a residual absorption of added ICX) did not change notably in the same concentration range. Fitting of the fluorescence titration curve with a 1:1 complexation model (inset a) led to a binding constant of $K_{ICX\cdot 1}$ = 4200 ± 150 M^{-1}. The formation of a binary 1:1 complex (ICX·**1**) was independently confirmed by a Job's plot (inset b), which showed a maximum at a mole fraction of 0.5. At pH = 5.0, a smaller 1:1 binding constant was measured ($K_{ICX\cdot 1}$ = 1700 ± 200 M^{-1}), and the fluorescence quenching (extrapolated to quantitative complexation) was less pronounced, only 50%.

Fluorescence Quenching Mechanisms. The observed fluorescence quenching of **1** upon addition of ICX can be rationalized by invoking two pathways: (a) intramolecular PET triggered by a calixarene-induced pK_a shift, that is, deprotonation, of the amino group, and (b) intracomplex (intermolecular) PET from the electron-rich alkoxybenzyl units of the calixarene to the strongly electron-accepting dicarboximide (ΔG_{et} = −0.46 eV).[26] With respect to pathway (a), it has recently been documented that charged calixarenes can lead to a pronounced pK_a shift (up to 2 units) of included guest molecules.[29] In our case, it can be inferred that the positively charged imidazolium residues will favor the binding of the dianionic form of the dye (Scheme 3, top right) over that of the monoanionic form (Scheme 3, top left) on grounds of Coulombic interactions. The net result is a pK_a shift of the amino group of **1**, which results in a (partial) deprotonation of the ammonium group upon complexation and opens the channel of intramolecular PET as a fluorescence quenching mechanism. In quantitative terms, the pH titration under conditions where the ICX·**1** complex dominates (95% complexation for 5 μM **1** and 4 mM ICX, Figure 1, ●) afforded a pK_a of 7.1 (read from the half-point of the titration). This corresponds to a shift by ca. 1.9 units as compared to uncomplexed **1**. Hence, at pH = 7.0, ca. 50% of the complexed 1,8-naphthalimide guest are deprotonated.

Upon lowering the pH from 7 to 5, the amino group of **1** in the binary complex is reprotonated, which accounts for the lower binding constant (4200 versus 1700 M^{-1}, see above) and also for the reduced quenching (70% versus 50%, see above) in the binary complexes (Scheme 3). The "residual" fluorescence quenching at pH ≤ 5, where no significant (<1%) deprotonation of the complexed dye is expected, is accordingly assigned to an additional fluorescence quenching pathway by intracomplex PET from the electron-rich alkoxybenzyl groups of the host, pathway (b).

The fluorescence titrations and the retrieved pK_a shifts establish a thermodynamic cycle between the complexed and uncomplexed, as well as the protonated and unprotonated forms of the dye **1**, all of which differ in their fluorescence properties. The combined protonation equilibria and associated fluorescence quenching effects are shown in Scheme 3.

Anion Sensing through Formation of a Ternary Complex. The supramolecular assembly ICX·**1** can be used as chemosensing ensemble for anions. We tested three carboxylates with varying numbers of negative charges (acetate, tartrate, and citrate) as well as three inorganic anions (nitrate, chloride, sulfate). The addition of citrate and tartrate led to a strong

(26) The thermodynamics of intramolecular PET from the amino *N* to the naphthalimide can be calculated with the Rehm−Weller equation [$\Delta G_{et} = E_{ox} - E_{red} - E^* + \Delta G(\epsilon)$]. The following electrochemical potentials (versus SCE, MeCN) and spectroscopic data were used: E_{ox} = 0.96 V for triethylamine as model (cf., Arbogast, J. W.; Foote, C. S.; Kao, M. *J. Am. Chem. Soc.* **1992**, *114*, 2277−2279), E_{red} = −1.45 V for *N*-methyl-1,8-naphthalimide as model (cf., Ramachandram, B.; Saroja, G.; Sankaran, N. B.; Samanta, A. *J. Phys. Chem. B* **2000**, *104*, 11824−11832), and E^* (S_1) = 3.37 eV, calculated from the intersection of normalized absorption and emission spectra (at neutral pH). $\Delta G(\epsilon)$ includes the Coulombic term and the different solvation energy in water versus MeCN. This term was taken as −0.10 eV (cf., Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541−5553). For the calculation of intracomplex PET from the alkoxybenzyl units of the calix[4]arene, an oxidation potential of E_{ox} = 1.56 V (versus SCE, MeCN) for *p*-methylanisole as model was used (cf., Allonas, X.; Jacques, P. *Chem. Phys.* **1997**, *215*, 371−378).

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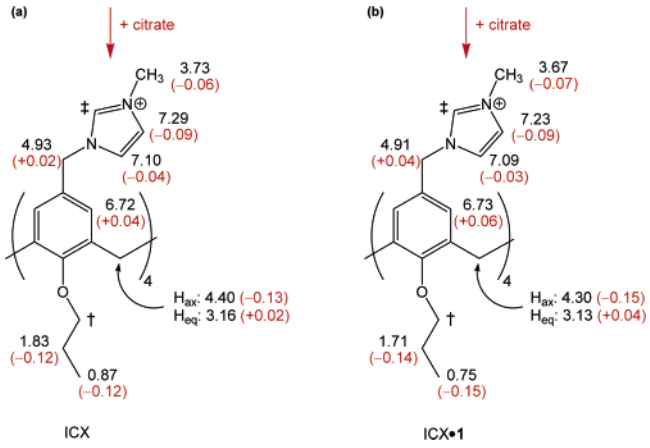
fluorescence enhancement with factors of 1.9 and 1.4 (110 μM anion, Figure 3), respectively, while acetate as well as the inorganic anions showed only small effects ($\leq$10%). At lower concentrations (13 μM), the differentiation was even larger, with a 9−14 times stronger fluorescence enhancement for citrate than for tartrate or acetate.

From the slope and shape of the titration curves, under consideration of the experimental conditions (large excess of host), it became obvious that the fluorescence recovery could not be treated by a conventional displacement model (Scheme 1a); a sigmoidal curve with a flat onset in the examined concentration range (dashed line in Figure 3) is expected for such a case, because the analyte would initially bind to the large excess of vacant host. In this simulation, we employed the actual binding constant of citrate to ICX in the absence of dye **1** ($K_{ICX\cdot citrate}$ = 800 ± 200 M^{-1}), which was independently determined from a ^{1}H NMR titration of 1.0 mM ICX upon successive addition of citrate (see the Supporting Information). The simplest mechanistic alternative to a displacement is the formation of a ternary 1:1:1 host−dye−analyte complex according to Scheme 1b, and, indeed, the data could be satisfactorily fitted by such a model (Figure 3).

Note that the literature is replete of supramolecular ternary complexes, those which employ coordinating transition metal ions or lanthanides as auxiliary additives.[2,10,12−14,30,31] In part, the formation of the ternary complexes has been facilitated by allosteric effects, for example, when the complexation of the metal ion changes the conformation of the host and thereby assists the complexation of a second guest.[32] However, there are relatively few systems incorporating fluorescent dyes for signaling,[33] and even less which incorporate two organic components as guests, for example, as in the recently reported 2:1 complexes of cucurbit[8]uril.[34,35] Specifically, the present sensing assembly appears to be the first of its kind involving two organic components[36] (dye and analyte) in combination with a water-soluble calixarene as macrocyclic host.

In line with the ternary complexation model, the fluorescence titrations can be understood as a preferential binding of the analyte to the host−dye complex, and the fitting of the fluorescence recovery curve was performed according to a 1:1 complexation stoichiometry between citrate and the preformed ICX·**1** binary complex. This direct fitting was made possible by neglecting the low affinity of citrate to the free host (800 M^{-1}, see above) and by adjusting the experimental conditions to predominant dye complexation (5 μM **1**, 1 mM ICX, corresponding to ca. 80% complexation). The apparent binding constants for complexation of citrate and tartrate to the ICX·**1** complex were found to be 62 600 ± 1700 and 9800 ± 900 M^{-1}, respectively.[37] This difference accounts nicely for the observed selectivity and micromolar sensitivity of the supramolecular assembly toward citrate. As noted above, the fluorescence enhancement by citrate is also pH dependent, which allows principally for a fine-tuning. Incidentally, it turned out that the optimal window (with largest enhancement factors) lies around neutral pH, which is also desirable for most biologically relevant analytes.

SCHEME 4. ^{1}H NMR Shifts and Complexation-Induced Shift Changes upon Addition of Citrate (in Red) in D_2O (pD = 7.4) for (a) ICX (1 mM), [citrate] = 12 mM and (b) ICX·1 (1 mM 1, 3 mM ICX), [citrate] = 5 mM[a]



[a] A dagger (†) indicates not determined due to overlap with guest protons. A double dagger (‡) indicates not determined due to H/D exchange.

Structural Aspects. To obtain more insights into the structure of the ternary complex, we monitored the ^{1}H NMR spectra of the ICX·**1** complex (1 mM **1**, 3 mM ICX) in the presence of 5 mM citrate. Slight downfield shifts between 0.05 and 0.10 ppm for the aromatic protons of **1** were observed. Important to note, the signals were still ca. 0.3−0.4 ppm upfield as compared to the free dye (e.g., for H-4: 8.30 ppm versus 7.96 ppm for uncomplexed **1** and in the ternary complex, respectively), which excludes the possibility of its displacement (Scheme 1a) and supports the postulate of the ternary complex (Scheme 1b). Strikingly, the protons of the calixarene host experienced also sizable shifts (by ca. 0.1 ppm) upon citrate addition to ICX·**1** (Scheme 4).

The difference between the resonances of the axial and equatorial methylene protons ($\Delta\delta = \delta_{ax} - \delta_{eq}$) is considered to be diagnostic for the conformation of the calixarene cone.[38,39] Interestingly, $\Delta\delta$ decreased substantially upon citrate addition (cf., opposite shifts of the H_{ax} and H_{eq} protons in Scheme 4). This provides an indication that the free host ICX and the binary complex ICX·**1** prevail in a distorted cone conformation ($\Delta\delta$ = 1.24 and 1.17 ppm, respectively),[39] while citrate addition under formation of the ternary complex induces a symmetrical C_4 cone conformation with a typical $\Delta\delta$ value of 0.98 ppm.[39] An induced fit[32] of the calixarene conformation[40−43] applies, which is depicted in Scheme 3. However, there was no

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TABLE 1. ROESY Cross-peaks (250 ms Mixing Time) between the Guests (10 mM Citrate and 2 mM 1) and the Host (4 mM ICX) at pD 7.4

		host proton (increasing depth of inclusion) →			
guest	guest protons	H-d	H-b	H-c	H-aryl
1	H-2	++	−[a]	+	
	H-4		−[a]	+	
citrate	CH_2	++	+	+	
citrate + **1**	H-2		++	++	
	H-4			++	
	CH_2	+			+++

[a] Not assigned due to overlap with intramolecular cross-peak (H-3).

indication for an allosteric effect;[32] that is, the complexation of either citrate or the dye **1** alone did not result in a comparable change of the host conformation (as judged by ^{1}H NMR).

Unique evidence for the formation of a ternary complex in the presence of citrate and the dye **1** was obtained by ROESY NMR measurements,[44] which were recorded at fixed concentrations (2 mM **1**, 10 mM citrate, 4 mM ICX) in the presence and absence of each guest (Table 1). In the respective binary 1:1 complex of either citrate or **1**, the strongest cross-peaks were detected with the imidazolium methyl protons (H-d in Chart 1), suggesting a peripheral complexation near the upper rim of the host, presumably driven by weak electrostatic and hydrogen-bonding interactions between the carboxylate and imidazolium groups (Figure 4a). Strikingly, in the presence of both guests, these peripheral interactions disappeared, and, instead, strong cross-peaks with the more deeply positioned aromatic imidazolium protons (H-b and H-c, for **1**) or even the calixarene aryl proton (H-aryl, for citrate) were observed (Figure 4b). This reveals a deeper immersion of both co-included guests (sketched in Scheme 3), although no mutual cross-peaks between the two guest molecules could be detected. Apparently, the two guests reinforce their binding to the host by forming a new guest−guest aggregate with improved Coulombic, hydrophobic, and/or space-filling properties. We consider the variations of the ROESY spectra upon addition of the second guest (Table 1 and Figure 4) as particularly conclusive, because they provide structural evidence that the two guests must be positioned in the same host cavity.

A possible explanation for the selective binding of citrate to the ICX·**1** complex relative to tartrate, acetate, and the inorganic anions may be found in structural details of the resulting ternary complex and, again, in the role of the protonation equilibrium of the amino functionality of the dye. Presumably, citrate undergoes three attractive Coulombic interactions with the two "vacant" imidazolium groups and the ammonium group of the dye to form an overall neutral assembly (bottom left structure in Scheme 3). The latter interaction is important, because it provides a driving force for the observed co-complexation (schematically illustrated in Scheme 1b). The dramatic increase of the binding constant of citrate in the presence of the dye **1** (62 600 versus 800 M^{-1} in the absence of **1**) also reflects the positive cooperativity (factor of ca. 80) for the formation of the ternary complex.

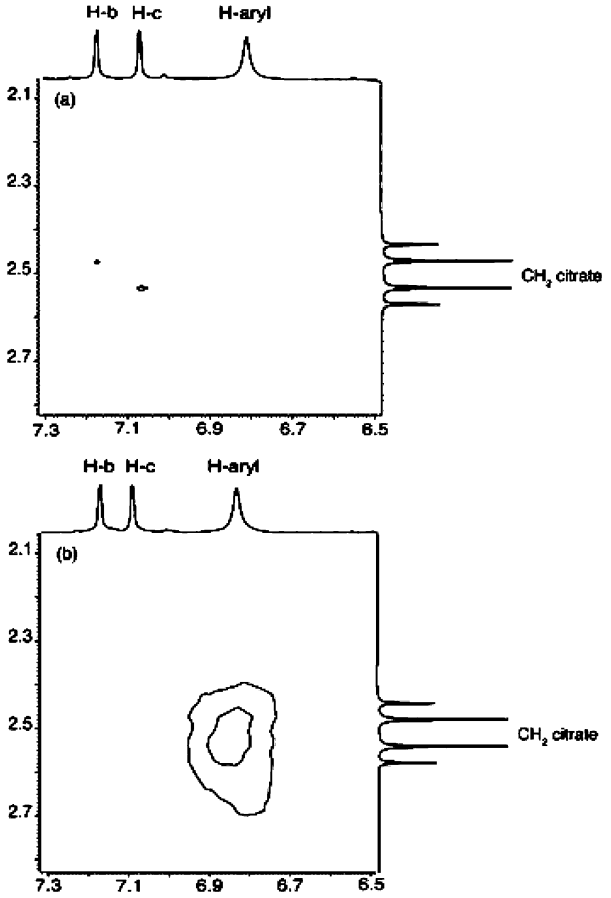

FIGURE 4. Characteristic variation of the ROESY cross-peaks between the protons of citrate (10 mM) and ICX (4 mM) at pD 7.4 (a) in the absence and (b) in the presence of the fluorescent dye **1** (2 mM).

Near the working pH of 7, the formation of this ternary complex will effectively displace the equilibrium toward the bottom left structure in Scheme 3, that is, toward the more strongly fluorescent ammonium form of the dye.[45] Accordingly, one expects again a pK_a back shift (and partial fluorescence recovery) upon addition of citrate, which we were in fact able to verify experimentally. In detail, the pH titration of a solution containing 5 μM **1**, 4 mM ICX, and 20 mM citrate, where the ternary complex dominates, yielded a pK_a of 7.9 (Figure 1, crossed circles). This is nearly one unit above the value determined for the binary complex ICX·**1** (●), but more than one unit below that measured for the uncomplexed **1** (○). The combined results based on the pH titrations, fluorescence titrations, and NMR measurements do therefore corroborate the complexation model advanced in Scheme 3.

Conclusions

In summary, we have investigated a novel supramolecular sensing assembly for citrate, which operates by a unique sensing principle involving the formation of a ternary complex and a sequence of pK_a shifts responsible for differential fluorescence quenching. In essence, the binding of the citrate trianion is partially reversing the pK_a shift induced by the macrocyclic host, which facilitates the protonation of the amino group of the dye,

(43) For comparison, acetate, which gave the smallest fluorescence enhancement effect, showed only very small downfield shifts for the aromatic protons of **1**, that is, 0.02−0.04 ppm, and provided also no indications for a significant change of the host conformation under comparable conditions (1 mM **1**, 3 mM ICX, and 10 mM acetate).

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(45) The ternary complex of citrate with the amino form of the dye (bottom right structure in Scheme 3) may also form, but lacks the additional Coulombic interaction and is therefore disfavored.

thereby blocking intramolecular PET as quenching mechanism and enhancing the fluorescence. The underlying photophysical effects and interaction pathways at the working pH of 7 can be simplified as follows (note the sequence of block arrows in Scheme 3): Complexation of the ammonium form of uncomplexed **1** by ICX yields significant amounts of the amine form of the dye as a consequence of repulsive Coulombic interactions; this causes a strong fluorescence quenching ("switching-off", Figure 2). Addition of citrate and formation of the ternary complex leads to a recovery of the ammonium form of the dye as a consequence of attractive Coulombic interactions; this causes a fluorescence enhancement ("switching-on", Figure 3), which can be used to detect the tricarboxylate citrate with good selectivity and sensitivity at neutral pH.

To which degree the presently exposed sensing principle (ternary complexes and pK_a shifts) can be transferred to other host/dye/analyte combinations cannot be projected at present. However, the results demonstrate that addition of analytes to a host−dye complex may cause a significant fluorescence response not only for common dye displacement (Scheme 1a), but also in cases of an incorporation of the analyte into a ternary complex (Scheme 1b). This broadens the general applicability of this supramolecular approach to analyte sensing. With respect to the design of suitable host−dye pairs, it appears to be advisable to choose dyes with pK_a values near the operating pH for analyte binding, for which complexation-induced pK_a shift effects are expected to be most pronounced.

Experimental Section

Materials and Methods. All chemicals for the synthesis of **1** were from Aldrich and used as received. D_2O, DCl, and NaOD were from Fluka. The anions were used as sodium salts or, in case of tartrate, as mixed sodium/potassium salt (all from Fluka). Fluorescence measurements were performed with a Varian Eclipse spectrofluorometer. NMR measurements were done on a Jeol JNM-ECX 400 spectrometer. All experiments were carried out at ambient temperature (24 °C).

The fluorescence quantum yield of **1** was measured using *N*-propyl-1,8-naphthalimide as standard ($\Phi_f = 0.21$ at pH = 7.4 in water).[46] Fluorescence as well as ^{1}H NMR experiments were performed with maximum ICX concentrations below the reported critical micelle concentration (5 mM).[24] pH values were adjusted by addition of NaOH or HCl. ^{1}H NMR and ROESY spectra were recorded in D_2O at pD = 7.4 (adjustment by addition of DCl and NaOD).[47] In the course of the titrations, the titrand concentration and the pH were kept constant throughout the entire experiment.

Synthesis of 1. *N*-(Aminoethyl)-1,8-naphthalimide (1.5 g, 6.2 mmol) and K_2CO_3 (3.5 g, 25 mmol) were suspended in dry DMF (40 mL) and stirred for 10 min. Subsequently, bromoacetic acid ethyl ester (2.6 mL, 24 mmol) was added, and the mixture was heated to reflux overnight. The solvent was evaporated under reduced pressure, and the residue was redissolved in chloroform. The organic phase was washed repeatedly with water and then dried over $MgSO_4$. The crude product obtained after evaporation of the solvent was purified by column chromatography on silica gel using chloroform/*n*-hexane (gradient 10/1 to 3/1) as eluent to yield 1.9 g (74%) of the diester as pale yellow oil.

To NaOH (0.5 g, 12.5 mmol), dissolved in hot ethanol (40 mL), was added a solution of the diester (1.0 g, 2.4 mmol) in the same solvent (10 mL). The mixture was heated to reflux overnight. After cooling, a precipitate was formed; this was recrystallized from wet ethanol to yield **1** as pale yellow crystals (0.92 g, 95%).

^{1}H NMR (D_2O, 5.5 mM **1**, MeOH as internal standard, 400 MHz) δ 2.89 (t, J = 7.3 Hz, 2H), 3.38 (s, 4H), 4.13 (t, J = 7.3 Hz, 2H), 7.72 (t, J = 7.8 Hz, 2H), 8.21−8.36 (m, 4H). ^{13}C NMR (D_2O, 5.5 mM **1**, MeOH as internal standard, 100 MHz) δ 38.3, 52.0, 58.9, 121.7, 127.7, 127.8, 131.7, 132.2, 135.8, 166.3, 179.0 Anal. Calcd for $C_{18}H_{14}N_2Na_2O_6 \cdot 2.5H_2O$: C, 48.54; H, 4.30; N, 6.29. Found: C, 48.90; H, 4.22; N, 6.25.

Acknowledgment. The financial support by the bilateral Portuguese−German collaboration program GRICES-DAAD, the Jacobs University Bremen (visiting professorship for U.P. within the International Center for Transdisciplinary Sciences), and the Spanish Ministry of Education and Science, Madrid (Ramón y Cajal grant for U.P.), is gratefully acknowledged. Further, we thank Marek Kluciar for help in the synthesis of **1**.

Supporting Information Available: ^{1}H NMR spectrum of compound **1**, ^{1}H NMR spectra for the titration of **1** with ICX, and ICX with citrate, ^{1}H NMR spectra for **1**/ICX in absence and presence of citrate, and ROESY spectra for **1**/ICX, citrate/ICX, and **1**/ICX/citrate. This material is available free of charge via the Internet at http://pubs.acs.org.

JO070268+

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