

# Binding of inorganic cations by *p*-sulfonatocalix[4]arene monitored through competitive fluorophore displacement in aqueous solution†

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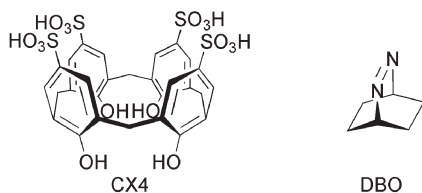
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A new working principle for detecting inorganic cation binding by water-soluble calix[4]arenes involves the displacement of a fluorescent azoalkane as guest. Fluorescence regeneration is observed for various metal ions, and binding of monovalent cations (alkali and ammonium) to *p*-sulfonatocalix[4]arene is detected and quantified for the first time.

Calixarenes are well-established macrocycles with cation receptor properties.<sup>1</sup> When substituted with appropriate tethers, calixarenes with astounding selectivity have been reported.<sup>2</sup> Calixcrown derivatives soluble in organic solvents have been refined to allow an efficient phase-transfer extraction of cations from aqueous solution, which has led to practical applications as relevant as the removal of radioactive caesium from nuclear waste.<sup>3</sup> Highly water-soluble sulfonated calixarenes have become increasingly important,<sup>4–18</sup> among which *p*-sulfonatocalix[4]arene (CX4) is perhaps the most common derivative. Although its complexation with organic ammonium ions<sup>5–7</sup> has been intensively investigated, amongst others due to the potential of acetylcholine sensing,<sup>8–10</sup> relatively little work has been expended towards the study of inorganic cation binding,<sup>14–17</sup> although sensor systems for metal ions in aqueous solution remain high in demand.<sup>19</sup>

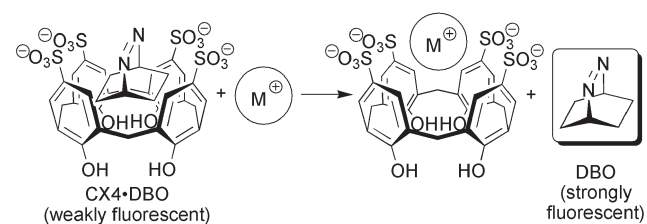


NMR titrations, which have been broadly employed to study binding of organic ammonium ions with CX4,<sup>5–8</sup> are transferable to inorganic cations only in exceptional cases.<sup>15</sup> Recently, microcalorimetry has been successfully employed as an alternative to measure the binding constants of several divalent (alkaline earth) and trivalent (lanthanides) cations with CX4;<sup>16</sup> this technique is principally suitable also for low binding constants (e.g., 10–20 M<sup>-1</sup>), and in addition yields complexation enthalpies and entropies as thermochemical data, but it is intrinsically unsuitable for thermoneutral, i.e., purely entropy-driven complexations. In contrast, binding of inorganic monovalent cations to CX4 has not been quantified yet and has in fact been neglected in

many studies. Moreover, recent microcalorimetric studies have revealed no heat effect upon addition of monovalent cations to CX4, namely ammonium and potassium,<sup>14,16</sup> which would be consistent with a negligible binding. On the other hand, early <sup>1</sup>H NMR coalescence studies have demonstrated that the presence of alkali ions at pD 8.9 leads to a rigidification of the CX4 cone conformation,<sup>4</sup> which implies some form of detectable interaction at least in a qualitative sense and at very high alkali concentrations (0.17–4.7 M).

Herein, we document a sizable affinity of CX4 with monovalent cations, alkali as well as ammonium, which has evaded detection by microcalorimetry, and which has important practical implications, especially when using buffered solutions. The binding constants are on the order of 100 M<sup>-1</sup>, i.e., in the same range as for numerous noncharged guests.<sup>11–14</sup> We applied a fully water-soluble fluorescence regeneration system recently introduced in the context of acetylcholine sensing.<sup>10</sup> It is based on the CX4 complex of 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO), an unconventional nonaromatic and uncharged fluorophore (Scheme 1). The system allows for the first time the application of sensitive optical techniques to conveniently study inorganic cation binding with unsubstituted CX4 and to determine their binding constants through a competitive binding scheme,<sup>20</sup> sufficiently accurate to expose selectivity trends even in a closely related series of metal ions.

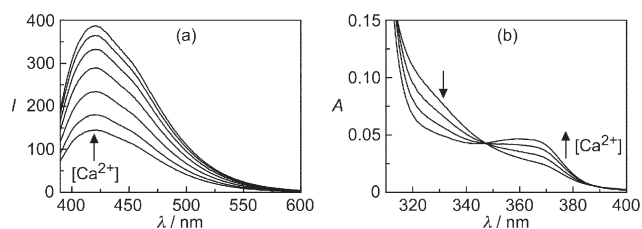
When complexed by CX4, DBO undergoes solvatochromic shifts in the UV absorption spectra, up-field <sup>1</sup>H NMR shifts by up to 2 ppm, and strong fluorescence quenching.<sup>10</sup> The addition of metal ions led to a decomposition of the CX4·DBO inclusion complex as evidenced by the recovery of the spectral features of uncomplexed DBO (Fig. 1), which was most accurately and very conveniently monitored by steady-state fluorescence.† The release was quantitative for some metals, e.g., La<sup>3+</sup>, while other metals such as Na<sup>+</sup> or Mg<sup>2+</sup> displayed smaller effects (Fig. 2). The azoalkane DBO itself is a very poor base<sup>21</sup> and ligand<sup>22</sup> and does not form bimolecular complexes with the investigated metals in



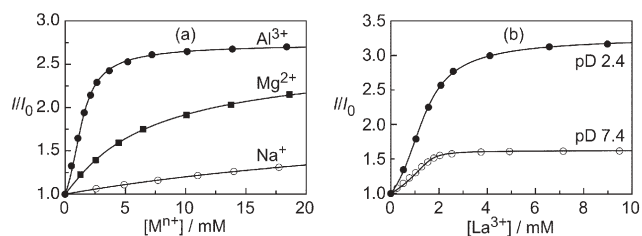
**Scheme 1** Sensor system based on the fluorescence regeneration of DBO by competitive binding of cations with CX4; the lower fluorescence intensity of DBO in the CX4 complex is related to exciplex-induced quenching of singlet-excited DBO by the aryl groups.<sup>10</sup>

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**Fig. 1** Variation of (a) the fluorescence spectra and (b) the near-UV absorption spectra for 1.0 mM DBO in the presence of 1.6 mM CX4 upon successive addition of  $\text{Ca}^{2+}$  in  $\text{D}_2\text{O}$  at pD 2.4.

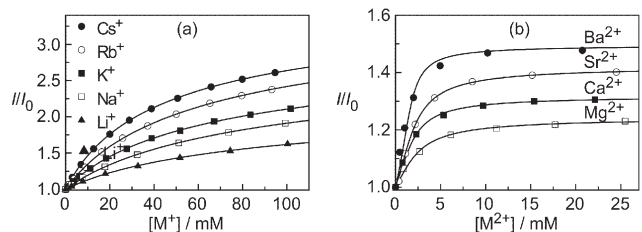


**Fig. 2** Increase in fluorescence intensity of 1.0 mM DBO in the presence of 1.6 mM CX4 illustrating the dependence (a) on cation charge at pD 2.4, and (b) on pD for  $\text{La}^{3+}$ .

water. The effect of the metal must therefore be ascribed to its complexation by the host. This interaction competes with the binding of DBO, thereby leading to a controlled release of the organic guest by competitive binding according to Scheme 1. An impressive case of metal-ion-induced guest release from calixarenes results, which is documented, among others, for other hosts like cucurbit[6]uril in water<sup>23</sup> and for cavitands in organic solvents.<sup>24</sup>

The first data set was obtained at pD 2.4, corresponding to pH 2.0 used in previous studies.<sup>15,16,25</sup> In acidic solution, the tetraanion form of CX4 predominates,<sup>26</sup> such that binding of divalent and trivalent cations has been previously presumed to occur mainly by 1:1 complexation through electrostatic interactions with the upper-rim sulfonato groups (Scheme 1).<sup>15,16,25</sup> The corresponding fitting of the fluorescence titration data (Fig. 2 and 3) afforded the cation binding constants (Table 1). The release of DBO could be alternatively followed spectrophotometrically or by  $^1\text{H}$  NMR to provide very similar binding constants; this was representatively explored for  $\text{Ca}^{2+}$  (values in square brackets in Table 1).

Importantly, binding by alkali ions falls on the order of  $100 \text{ M}^{-1}$ ; binding by  $\text{NH}_4^+$  is also significant and falls into the range expected from the charge and size of the alkali ions, *i.e.*, it is similar to  $\text{K}^+$ . Note that the binding constants of the monovalent cations are 1–3 orders of magnitude smaller than those found for



**Fig. 3** Increase in fluorescence intensity of 1.0 mM DBO in the presence of 1.6 mM CX4 illustrating the dependence on cation size (a) for the alkali ions at pD 2.4, and (b) for alkaline earth metal ions at pD 7.4.

**Table 1** Ionic radii and binding constants<sup>a</sup> of different inorganic ions with CX4 in  $\text{D}_2\text{O}$  determined by fluorescence regeneration

Ion	Radius/ $\text{\AA}^b$	$K_{\text{obs}}/\text{M}^{-1}$	
		pD 2.4	pD 7.4
$\text{NH}_4^+$	1.36	95	165
$\text{Li}^+$	0.59	70	80
$\text{Na}^+$	1.02	75	85
$\text{K}^+$	1.37	100	115
$\text{Rb}^+$	1.52	110	135
$\text{Cs}^+$	1.67	150	280
$\text{Mg}^{2+}$	0.57	1020	2190
$\text{Ca}^{2+}$	1.00	1590 [1720] <sup>c</sup> [1640] <sup>d</sup>	3820 [3730] <sup>d</sup>
$\text{Sr}^{2+}$	1.18	1810	4630
$\text{Ba}^{2+}$	1.35	<sup>e</sup>	6760
$\text{Al}^{3+}$	0.39	20 300	—
$\text{Ga}^{3+}$	0.47	25 200	—
$\text{La}^{3+}$	1.03	23 700	52 600

<sup>a</sup> 10% error. <sup>b</sup> From *Handbook of Chemistry and Physics*, ed. D. R. Lide, CRC Press, Boca Raton, FL, 84th edn., 2003. <sup>c</sup> By UV absorption titration. <sup>d</sup> By  $^1\text{H}$  NMR titration. <sup>e</sup> Precipitation above 1 mM.

quarternary ammonium ions, suggesting that hydrophobic effects are important or dominant for organic cations.<sup>5–8</sup> There is also a weak size selectivity, with the binding constant for the largest cesium being about twice as high as for the smallest lithium (Fig. 3); this is in fact consistent with the early  $^1\text{H}$  NMR coalescence study,<sup>4</sup> according to which the larger alkali metals are able to interact with CX4 at lower concentrations. A similar size selectivity trend is also observed for the alkaline earth metals (for  $\text{Ba}^{2+}$  precipitation interfered above 1 mM at pD 2.4).<sup>4</sup> The trivalent cations  $\text{Al}^{3+}$ ,  $\text{Ga}^{3+}$  and  $\text{La}^{3+}$  displayed the largest binding constants, all close to  $20000 \text{ M}^{-1}$ . Binding constants of inorganic cations at pD 2.4 are therefore on the order of *ca.*  $100 \text{ M}^{-1}$  for monovalent, *ca.*  $1500 \text{ M}^{-1}$  for divalent and *ca.*  $20000 \text{ M}^{-1}$  for trivalent ones (Table 1). The binding constants for the investigated divalent and trivalent cations compare well with the limited available literature data, *e.g.*, reported values for  $\text{La}^{3+}$  at pH 2.0 range from *ca.*  $5000 \text{ M}^{-1}$  (by  $^{139}\text{La}$  NMR titrations)<sup>15</sup> to  $17000 \text{ M}^{-1}$  (by microcalorimetry),<sup>16</sup> while the two binding constants previously determined by microcalorimetry for  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  at pH 2.0 were both *ca.*  $2000 \text{ M}^{-1}$ .<sup>16</sup>

The contrasting experimental findings, namely that the binding of inorganic monovalent cations is readily detectable by competitive fluorophore displacement but not by microcalorimetry ( $\text{K}^+$  and  $\text{NH}_4^+$ )<sup>14,16</sup> furnishes strong circumstantial evidence for a purely entropically driven complexation process, which has been previously considered as a scarce possibility.<sup>14</sup> With respect to the complexation thermochemistry,  $\Delta_r H^\circ \approx 0$  and  $T\Delta S \approx 10 \text{ kJ mol}^{-1}$  results for monovalent cations. For divalent cations,  $\Delta_r H^\circ \approx 4 \text{ kJ mol}^{-1}$  and  $T\Delta S \approx 23 \text{ kJ mol}^{-1}$  have been reported, and for trivalent ones,  $\Delta_r H^\circ \approx 10 \text{ kJ mol}^{-1}$  and  $T\Delta S \approx 32 \text{ kJ mol}^{-1}$ .<sup>14</sup> These thermodynamic trends (all at 298 K and comparable pH) are consistent with the expectation, based on relative hydration enthalpies and entropies of the cations, that the decrease in complexation enthalpy with decreasing charge is more pronounced than the corresponding decrease in complexation entropy.<sup>14</sup> A thermoneutral complexation of monovalent cations with a nonvanishing entropic driving force can therefore be rationalized.

Fluorescence recovery of DBO can also be monitored in neutral aqueous solution, for which no binding constants of the

investigated cations are known. Unlike the situation in acidic solution, the binding modes of inorganic cations with CX4 in neutral aqueous solution, where one phenoxy group is deprotonated,<sup>26</sup> are not as accurately known. In particular, there is a definitive possibility for complexation at the lower phenoxy rim,<sup>15,17,18</sup> in addition to complexation at the sulfonato rim.<sup>6–8,16,25,27</sup> An additional uncertainty arises from the necessary addition of sodium ions (NaOD) to adjust neutral pD, which will affect the observed binding constants (see below). Nevertheless, fluorescence regeneration and competitive binding of the cations was also observed at pD 7.4 (Fig. 2 and 3), and the binding constants resulting from fitting were throughout larger than those in acidic solution (Table 1). This is consistent with an increased binding strength of the cations as a consequence of a higher overall negative charge on the CX4 system and, specifically, with increased cation– $\pi$  interactions for the CX4 pentaanion.<sup>4–7,28</sup>

It transpires from the present study that binding of inorganic monovalent cations to CX4 interferes strongly both in acidic and neutral aqueous solution. The presence of residual cations (mostly alkali) with CX4, however, is unavoidable during purification<sup>26</sup> and for neutral pH adjustment. While possible adverse effects of buffer constituents have, in fact, remained an immanent issue in complexation studies with CX4,<sup>27,29</sup> most studies have implicitly relied on the assumption that the binding of the alkali cations present in the calixarene sample or the cations added to adjust or buffer pH is negligible, which is clearly not the case (Table 1). The accuracy of reported binding constants depends therefore critically on the actual metal ion concentrations present. From simple equilibrium treatment it can be demonstrated that the experimentally observed binding constant of a guest with CX4 ( $K_{\text{obs}}$ ) should fall below the actual value ( $K_{\text{CX4-Guest}}$ ) whenever there is competitive binding with metals ( $K_{\text{CX4-M}^+}$ ). Eqn. (1) applies for a quantitative release of the guest by an excess of metal ion and defines the systematic error in the measurement. Accordingly, since residual cations in any CX4 sample along with buffer ions typically amount to concentrations of at least 10 mM used in most studies, deviations by a factor of 2 may easily result.

$$K_{\text{obs}} \approx \frac{K_{\text{CX4-Guest}}}{(1 + K_{\text{CX4-M}^+} [\text{M}^+]_0)} \text{ for } [\text{M}^+]_0 \gg [\text{CX4}]_0 \quad (1)$$

Since the actual deviation is strongly dependent on the cation content of the sample, and since different buffer solutions have been broadly employed (the use of phosphate buffer concentrations of 0.1 M has not been uncommon!),<sup>6,8,11,13,30</sup> comparison of literature data must be made with great care. To provide a specific pertinent example, the binding constants for several amino acids (Ala, Leu, Val, Phe, His, Tyr, Trp) titrated at high phosphate buffer concentration (0.1 M)<sup>30</sup> were a factor of 20 or more smaller than those measured at lower buffer concentration,<sup>31,32</sup> which can now be accounted for. For the same reasons, thermochemical data determined by microcalorimetry, in particular the complexation entropies, must be used with some caution.

In conclusion, we have introduced a new fluorescence-based method to sensitively monitor and quantify the binding of inorganic cations by CX4 in water at different pH. The method is based on competitive binding and it is noncalorimetric, thereby

allowing also the detection of purely entropically driven complexations, as for inorganic monovalent cations. The latter have a sizable binding (*ca.* 100 M<sup>-1</sup>) which needs to be considered when comparing binding constants at different metal ion and buffer concentrations.

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