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Binding affinities of cucurbit[*n*]urils with cations†

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High binding constants of 19 inorganic cations with the cucurbit[*n*]uril homologues (CB*n*, *n* = 5, 6, 7, 8) in water were determined and the far-reaching consequences and interferences of the high affinities (millimolar to micromolar) are discussed.

The design of artificial receptors for inorganic cations has underpinned the development of supramolecular chemistry.[1,2] Through advances in the understanding of non-covalent interactions,[3] carefully designed supramolecular receptors for (earth) alkaline cations emerged in the 1980's such as Cram's spherands[4,5] displaying high selectivity for Li^+. Conversely, Lehn's cryptands[6,7] and the more recently introduced orthoester cryptands by von Delius[8,9] can strongly and selectively bind (earth) alkali cations. In contrast to these designer hosts, other classes of macrocyclic receptors were originally tailored for organic guests but later also found to be interesting metal ion binders, for example, *p*-sulfonatocalix[4]arene (CX4). For supramolecular applications, this behavior is often undesirable because alkali ion binding competes for the binding of the target guests (*e.g.*, choline) in biologically relevant, aqueous saline media.[10,11] Likewise, cucurbit[*n*]uril (CB*n*, Fig. 1) macrocycles were long known to interact with (earth) alkaline cations, leading to an increase in their aqueous solubility but a decrease in their organic–guest binding affinities.[12–17] CB*n* have found widespread use as solubility enhancers,[18] for materials applications,[19,20] and for assay development,[17,21] which often take place in buffered aqueous media.[12,16,22,23] The influence of metal cation binding to CB*n* macrocycles is therefore a critical factor. However, while the interaction of CB6 with Ca^{2+} was inferred already a century ago,[24] and

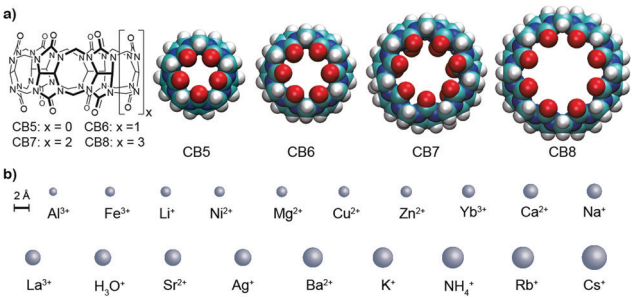

Fig. 1 (a) Chemical structures and their 3D representation (space filling model) of CB*n* and (b) of the metal cations investigated in this study, with sizes drawn to scale with respect to CB*n*; the portal diameters are 2.4 Å for CB5, 3.9 Å for CB6, 5.4 Å for CB7, and 6.9 Å for CB8.

while absolute affinity values for some ions such as Na^+ have been reported,[23,25–29] (see also Table S1 in the ESI†) binding affinities for metal cations to CB*n* in water have not been systematically studied across the homologous series and associated structure-affinity relationships are unknown.

We now provide a comprehensive data set for the complexation of 19 inorganic cations with CB5, CB6, CB7, and CB8 in water. The determination of cation–CB*n* binding is challenging because their interaction does not provide a diagnostic signal change in conventional NMR or UV-vis absorption spectra and phase-extraction protocols[30] are inapplicable for water-soluble hosts such as CX4 or CB*n*. As alternatives, fluorescence displacement titrations and isothermal titration calorimetry (ITC) experiments came to mind for the determination of macrocycle-cation binding constants (K_a).[10,31,32] The experimental details are shown in the ESI† and the aggregated log K_a values are shown in Table 1, along with the ionic radii and their hydration free energies in order to correlate with size fitting and desolvation effects. Additional reference data for classical cation–receptor macrocycles such as 18-crown-6,[2,33,34] *p*-sulfonatocalix[4]arene (CX4),[10,32,35,36] and Cryptand [2.2.2][6,31,37,38] are also shown. Monovalent alkali metal ions, Ag^+, NH_4^+, and H_3O^+, divalent earth alkaline ions, transition metal ions, and trivalent cations were investigated. It transpires from Table 1 and from the

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Table 1 Binding constants (log K_a values) of cations with CB*n* (*n* = 5–8) and reported values with 18-crown-6, CX4, and Cryptand [2.2.2] in water

Cations	r^a (Å)	$-\Delta G_{hyd}{}^b$ (kJ mol^{-1})	CB5[c]	CB6[d]	CB7[e]	CB8[f]	18-Crown-6[g]	CX4[h]	Cryptand [2.2.2][i]
H_3O^+	1.12	—	≤0.5	1.06[k]	2.22	—	1.46	1.6	(9.66)[l]
NH_4^+	1.48	285	2.59	3.79	2.82	—	1.23	2.22	
Li^+	0.69	475	2.02	2.41	2.34	1.69	~ 0	2.14	1.3
Na^+	1.02	365	3.94	3.89	3.41	2.49	0.8	2.26	3.9
K^+	1.38	295	4.73	3.81	3.46 (3.29)	2.66	2.03	2.33	5.3
Rb^+	1.49	275	3.22	4.30	3.43 (3.42)	2.64	1.56	2.40	4.2
Cs^+	1.70	250	2.61	5.31	3.50 (3.51)	2.55	0.99	2.88	1.4
Ag^+	1.15	430	—[j]	3.87	3.54	2.32	1.50	2.43	9.6
Mg^{2+}	0.72	1830	2.50	2.57	3.24	2.72	~0	3.58	<0
Ca^{2+}	1.00	1505	2.64	4.22	4.25 (4.01)	3.31	1.26	3.89	4.4
Sr^{2+}	1.13	1380	5.16	4.91	4.79 (4.31)	3.63	2.72	3.66	8.0
Ba^{2+}	1.36	1250	6.44	5.29	5.28 (4.78)	3.95	3.87	3.83	9.5
Ni^{2+}	0.69	1980	2.73	2.59	3.50	2.73	—	3.75	<2.0
Cu^{2+}	0.73	2010	—	2.88	3.75	2.86	—	3.75	6.8
Zn^{2+}	0.75	1955	—	2.45	3.40	2.67	—	3.75	<2.5
Al^{3+}	0.53	4525	—[j]	3.81	2.90	2.90	—	—	—
Fe^{3+}	0.65	4265	3.66	5.17	4.18	3.0[m]	—	—	—
Yb^{3+}	0.87	3570	3.71	3.50	4.42	3.44	—	3.81	—
La^{3+}	1.05	3145	4.17	4.16	5.28	3.76		4.23	6.45
Mean log K_a value[n]			3.42 (3.74)	3.68 (4.03)	3.66 (3.93)	2.90 (2.85)	1.58 (1.65)	3.09 (3.18)	4.67 (4.22)
StDev of log K_a value[n]			1.42 (1.27)	1.10 (0.92)	0.82 (0.87)	0.55 (0.64)	0.97 (1.13)	0.80 (0.75)	2.97 (2.94)

[a] Ionic radius, from ref. 39. [b] Hydration free energy, from ref. 39. [c] By ITC experiments with desalinated CB5 and the respective nitrate salts at 283 K; error ± 0.10. [d] By fluorescence displacement titrations with the reporter pair CB6·DSMI at 298 K; error ± 0.10. [e] By fluorescence displacement titrations with the reporter pair CB7·BE at 298 K; error ± 0.10; values in parentheses determined by ITC experiments with CB7 at 298 K; error ± 0.10. [f] By UV-vis displacement titrations with CB8·PDI at 298 K; error ± 0.10. [g] From ref. 10, 34 and 40. [h] From ref. 10, 32, 35 and 36. [i] From ref. 6, 31, 37 and 38. [j] Large heats of dilution compared to binding heats prevented determination of log K_a by ITC. [k] Derived from ref. 41 by using the affinity for cyclohexylmethylamine from ref. 42 extrapolated for neat water. [l] Cryptand [2.2.2] is being protonated. [m] From fluorescence displacement titrations, error ± 0.3. [n] Mean log K_a values and standard deviations for each host type; values in parentheses are for subset of alkali and earth alkaline metal ions.

comparison to the data for the established hosts that CB*n* are highly competitive inorganic cation receptors, although they are much better known for their tight binding of organic guests. Correlations of the log K_a values with ionic cation radius are shown in Fig. 2; the interconnected lines for the alkali and alkaline earth ions reveal a general trend in favour of a stronger binding for the larger and less strongly hydrated metal ions; only for the smallest CB5 a bell-shaped curve for the alkaline metal ions is obtained, which points to an ideal size matching as an additional determinant (see Fig. 1 for size comparison). Within a homologous series, the most rigid macrocycle, CB5 in our series, is in general known to display a pronounced peak selectivity.[6,31] Rb^+ and Cs^+ appear to be too large to penetrate into the carbonyl portal region of CB5, where the dipolar interaction with the oxygen lone pairs is most effective. Ag^+ follows the trend for the alkaline metal ions, but NH_4^+ and H_3O^+ fall binding-wise below the correlation for (similarly sized) monovalent ions (Fig. 2), presumably because they engage as potent hydrogen bond donors in bulk water. This demonstrates that the formation of hydrogen bonds to the cucurbituril portals presents no significant driving force for organic guest complexation. Instead, the enhanced binding of organic guests upon introduction of cationic groups can be satisfactorily explained by ion–dipole interactions with the portals. Cationic centers that do not act as hydrogen-bond donors and that are less strongly hydrated, such as quaternary ammonium groups, present accordingly the better choice if optimized binding to CB*n* is desired.[43] Hydrogen-bond proximity patterns, that are frequently observed in crystal structures of CB*n*,[14,44,45] do not reflect on the driving force of host–guest binding in aqueous solution.

Ion–dipole interactions are supported by the dependence on metal ion valency, with the general trend trivalent > divalent > monovalent at comparable ionic radius, see colour coding in Fig. 2. The three divalent transition metal ions Ni^{2+}, Zn^{2+}, and Cu^{2+} bind with similar affinity, as expected from their similar

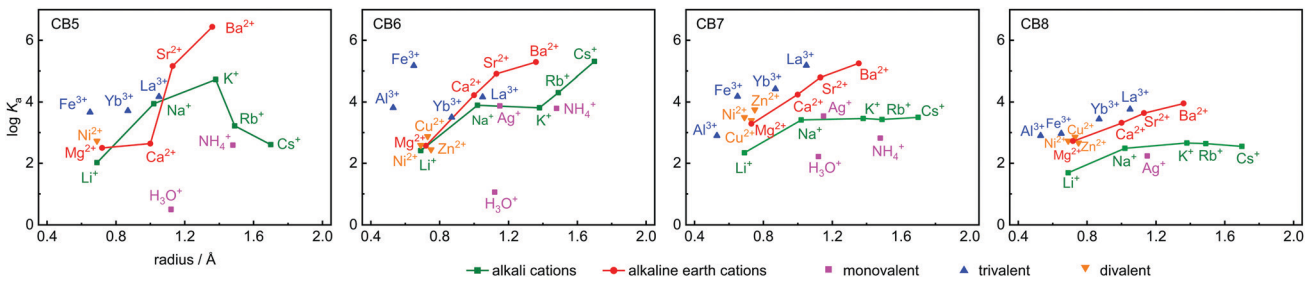

Fig. 2 Plots of log K_a *vs.* cation radius for different CB*n*.

cation size and hydration free energy, but the trivalent Fe^{3+} binds more tightly, regardless of its higher hydration free energy. Within the series of trivalent ions, the hydration free energies ($Al^{3+} > Fe^{3+} > Yb^{3+} > La^{3+}$) are a good indicator, but CB6 is an outlier here; it binds the lanthanide ions less tightly. CB6 is also the only CB*n* homologue that shows a markedly enhanced affinity to Rb^+ and Cs^+ compared to K^+, which reveals the complex interplay in underlying factors.

To ease quantitative discussion, we define in Table 1 (bottom) also the average binding constants as an overall measure of the "strength" of the different cation receptors and the standard deviation as a measure of their "selectivity". In terms of strength, the sequence Cryptand [2.2.2] > CB6 ≈ CB7 > CB5 > 18-crown-6 > CB8 > CX4 is obtained. In terms of selectivity, the order Cryptand [2.2.2] > CB5 > CB6 > 18-crown-6 > CB7 ≈ CX4 > CB8. As can be seen, within the CB*n* series, the selectivity decreases systematically as the portal size increases, but the intermediary sized CB6 is the strongest inorganic metal ion binder, presumably because its cavity provides the best size match for most of the investigated ions (see Fig. 1 for size comparison). All CB*n* homologues easily outperform the more flexible 18-crown-6, compete with CX4, but cannot reach the goodness of the diligently designed and three-dimensionally optimized receptor Cryptand [2.2.2]. In direct comparison, although CB6 and 18-crown-6 have comparable O···O cross-portal distances (r = 1.4 Å), the rigid portals of CB6 have clear advantages for metal ion binding in terms of preorganization. Similarly, CX4 is usually compared to CB7, because both macrocycles have a comparable preferred organic guest capacity (*ca.* 130 Å^3), but CX4 is conformationally much more flexible, which renders it a less potent inorganic ion receptor, despite its penta- or tetraanionic charge status.[10] As it comes to absolute binding potential, CB5 is the CB*n* homologue of choice for binding of Ba^{2+} and CB6 excels in binding Cs^+ in aqueous solution, reaching micromolar affinity in these cases. The binding constant between CB6 and Cs^+ is at least two orders of magnitude higher than that with other hosts (Fig. 2). For CB7 and CB8, La^{3+} and Ba^{2+} emerge as strongest binders.

Apart from potential application aspects related to the (metal) ion binding, *e.g.*, for sensing, the measured affinities of CB*n* with common inorganic ions have important practical implications for the determination of binding constants with (organic) guest molecules. As quantitatively demonstrated earlier,[17,46] the common (and for biological measurements necessary) practice to determine binding constants in buffered solution rather than in neat water[16,22,23] leads to apparent binding constants (K_{app}) of organic guest molecules with CB*n*, because the large concentrations of buffer cations compete effectively with CB*n* binding and, thereby, lower them.

The decrease of the binding constants can be estimated from eqn (1),[10] which assumes a direct stoichiometric competition, similar to the competitive displacement principle used to determine the ion binding constants in this study. At high buffer cation concentrations (>10 mM) and with sizable affinities to CB*n* ($\log K_a > 2$), significant reductions of the measured guest binding constants result, which are tabulated in Table 2 by using the average binding constants from Table 1 as example (the use of the affinity of Na^+ as the most common buffer cation affords comparable values). As can be seen, the error introduced by measuring binding constants in buffer solution can easily reach factors of 100–1000, and it is only for the largest homologue, CB8, where the variation is much smaller but still significant.

Table 2 Calculated systematic "error" of binding constants at varying concentrations of metal ions and typical metal-ion affinities

	Systematic "error": $K_{CBn\cdot Guest}/K_{app}$			
$[M^+]$/mM	$\log K_{CB5\cdot M^+}$ = 3.4	$\log K_{CB6\cdot M^+}$ = 3.7	$\log K_{CB7\cdot M^+}$ = 3.7	$\log K_{CB8\cdot M^+}$ = 2.9
0	1	1	1	1
5	15	25	25	5
20	50	100	100	20
100	250	500	500	80
200	500	1000	1000	160

$$K_{app} = \frac{K_{CBn\cdot Guest}}{1 + K_{CBn\cdot M^+}[M^+]_0} \quad \text{for } [M^+]_0 \gg [CBn]_0 \qquad (1)$$

The underestimation of the magnitude of the salt effects may account in part for the large variations in affinities of the same guests reported in the literature. The best practice is the determination of CB*n* binding constants in neat water.[17,18,42,47–49] The presence of cations present in the CB*n* samples themselves, *e.g.*, ammonium ions in CB5, also needs to be kept in mind (see also Table S7, ESI†),[50] similar to the omnipresence of Na^+ cations in CX4 samples.[10] For biological measurements, the choice of NH_4^+ instead of Na^+,[21] may reduce the variation, but does not eliminate it. We found that for CB6, where buffers are frequently used to increase its solubility, the smallest variation is obtained when using 1 mM HCl (H_3O^+) as additive,[49] but the resulting pH of 3 must be tolerated. In contrast to the strong influence of buffers on the CB*n*–guest binding affinities (Table 2), the use of D_2O as an NMR solvent is inoffensive because solvent isotope effects on the affinity are only *ca.* 20%.[51]

The situation with using buffers becomes even more intricate when measurements of thermochemical parameters other than the binding constants are concerned, *e.g.*, complexation enthalpies and entropies obtained by ITC. The measured released or absorbed heat in ITC experiments may reflect the heat associated with the decomplexation/displacement of the inorganic cation present in the solution rather than for the host–guest binding process. In these cases, the complexation enthalpies or entropies are invariably incorrect, and in extreme cases host–guest binding events which are intrinsically exothermic may appear as being endothermic, and enthalpically driven reactions may come out as entropically driven ones. In ITC experiments with CX4, we have previously demonstrated such a worst-case scenario, where the binding enthalpies of two competitors canceled out, which gave rise to the impression that there is no binding.[10] For CB5-cation binding, the complexation enthalpies and entropies were found to span a remarkable wide range from very favorable to unfavorable; ΔH varied from +24 kJ mol^{-1} to −35 kJ mol^{-1} and $-T\Delta S$ ranged from +21 to −47 kJ mol^{-1}, see Table S2 (ESI†).

Consequently, thermochemical parameters measured for CB*n* in the presence of buffer cations, including our own measurements, contain a systematic error and are incorrect in absolute terms. Strictly speaking, they do not only report on the host–guest binding event, but on the differences in complexation enthalpies between guest and cation binding.

Another sequential but severe shortcoming is that the apparent binding constants in the presence of buffers have already been used in the literature as benchmark values not only to evaluate the performance of computational methods in so-called challenges,[52–54] but in some cases also for the parameterization and calibration of computational methods.[55] Assuming that the experimental values underestimate the binding by up to three orders of magnitude (Table 2), corresponding to 17 kJ mol^{-1} at 298 K, an incorrect calibration of computational methods, which aim in part for an accuracy better than 8 kJ mol^{-1}, may have resulted. It appears essential that computational challenges that aim at host–guest binding of CB*n* or other ion receptors either explicitly consider the presence of buffer ions or address the true guest affinities, namely those in neat water.

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Conflicts of interest

There are no conflicts of interest to declare.

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