High-affinity host–guest chemistry of large-ring cyclodextrins

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The host–guest chemistry of large-ring cyclodextrins (LRCDs) has been largely unexplored due to the lack of suitable guest molecules that bind with significant affinities to enable potential applications. Herein, we report their complexation with dodecaborate anions (B12X122−), a novel class of guest molecules. The binding constants of the inorganic guests (104−106 M−1) allow their classification as the first tight binders for LRCDs.

Introduction

Cyclodextrins (CDs, Fig. 1) are native water-soluble macrocyclic molecules that consist of α(1−4)-linked α-D-glucopyranose units. The smallest homologues, α-, β-, and γ-CD with 6, 7, and 8 units, are cone-shaped with a hydrophobic cavity that is capable of encapsulating small organic guests. Larger CD homologues are also available. The first evidence for the existence of large-ring CDs (LRCDs, see δ-, ε-, and ζ-CD in Fig. 1), which are composed of 9 or more glucose units, dates back to the work by Freudenberg and Cramer, which was confirmed by Pulley and French. The structure of LRCDs was found to be different from the annular shape of small CDs. The crystal structures of δ-CD and ε-CD display a distorted elliptic boat-like shape, while even larger rings have more folded conformations. Molecular dynamics studies showed that the distorted shape of LRCDs is induced by steric encumbrance caused by large-ring strain.

A number of potential applications have been proposed for LRCDs, including food-industry and drug-formulation-related ones, owing to their non-toxicity, which is a general asset of CDs. However, even though evidence for the formation of inclusion complexes could be obtained from solubility enhancements of guests with limited water solubility or from crystal structures of the precipitating solids, the host–guest chemistry of LRCDs has received little attention. In particular, their affinities to guest molecules proved to be disappointingly low, which has been related to their large cavities and their high flexibility, which are also known for the large derivatives of other classes of macrocycles such as cucurbiturils and calixarenes. The status-quo of host–guest chemistry of LRCDs can be summed up by Table 1, which includes the guests for which low binding affinities or upper limits have been estimated.

Recently, we have reported the complexation of large dodecaborate cluster dianions (B12X122−, X: H, Cl, Br, and I, Fig. 1) with γ-CD; the binding affinities reached micromolar in

| Host | Guest | K/M−1 | Ref. |
|------|-------|-------|-----|
| δ-CD | Digitoxin | 1700 | 8e |
| δ-CD | Spironolactone | 820 | 8a |
| δ-, ε-, ζ-CD | 4-tert-Butyl benzoate | <50 | 10c |
| δ-, ε-, ζ-CD | Ibufrenen | <30 | 10c |
| δ-, ε-, ζ-CD | Benzoate derivatives | 2−10−3 | 10c |
| δ-CD | 1-Adamantan carboxylate | 4−8 | 10c |
| δ-CD | C70 | n.d. | 10d |
| δ-CD | Cycloundecanone | n.d. | 9 |

*a Measured by the solubility method, while the structural evidence for inclusion was obtained by 1H NMR. b Measured by the solubility method. c Measured by electrophoresis. d The highest affinity was obtained for δ-CD. e The highest affinity was obtained for 3,5-dimethoxy benzoate with ζ-CD. f The highest affinity was obtained for δ-CD and no value was reported for ε-CD. g Spectroscopic evidence for complexation was obtained by UV-Vis titration. h Structural evidence for inclusion was obtained by XRD.
aqueous solution. The driving force for complexation was traced back to the chaotropic effect, based on the super-chaotropic nature of the dodecaborate anions. Additionally, the large polarizability of the clusters contributes to the high stability of the formed inclusion complexes. The binding constant with γ-CD reaches its maximum for B12Br122−, while B12I122− already becomes too large and binds more weakly. We reckoned that these globular clusters, and in particular the largest ones, could serve as ideal guests for LRCDs and now present our results on the binding of dodecaborate clusters with δ-, ε-, and ζ-CD, synthesized and purified as described previously.

Results and discussion
The key-and-lock principle in host–guest chemistry describes a complementarity between the guest size and the cavity size of the host as well as the shape of both. Table 2 lists pertinent structural parameters of common CD homologues, including LRCDs, and dodecaborate anions. The cavity size of CDs spans from 174 to 794 Å³, while the size of the clusters spans from 152 to 520 Å³. The size match between the guest and the host represents a quick estimator for the steric goodness of fit of host–guest complexation. For example, the smallest cluster, B12H122−, is too small to efficiently fill the cavity of large CDs, but matches that of the small CDs, such as α- and β-CD. On the other hand, the larger clusters, such as B12Br122− and B12I122−, are too large to fit inside α-CD and β-CD, but they are expected to lock better into the cavities of γ-CD up to ζ-CD.

The 1H NMR chemical-shift differences among α-, β-, and γ-CD are very small, amounting, for example, to only 0.05 ppm for the H1 proton (Fig. 2). In contrast, for LRCDs, larger differences are observed (up to 0.3 ppm, see Fig. 2), as expected from their distinct, folded structures. Complexation of the clusters by LRCDs was first probed by complexation-induced 1H NMR shifts. The small clusters, B12H122− and B12F122−, showed either no or heavier changes in the 1H NMR spectra. The encapsulation of the perhalogenated clusters inside the large hosts caused significant down-field shifts, selectively of the inner protons, H3 and H5 (Fig. 3 and 4); this confirmed the formation of inclusion complexes. The magnitude of the chemical shifts signals how deeply the

| Table 2  Structural parameters for CDs and dodecaborate anions; see Fig. 1 for geometric parameters |
|---------------------------------|-----------|----------|--------|-------------|----------------|---------------|
| Host   | w/Å     | n/Å      | h/Å    | Vcavity/Å³ | Guest       | d/Å   | V/Å³  |
| α-CD   | 8.0     | 5.0      | 9.0    | 174a      | B12H122−    | 8.0   | 152a |
| β-CD   | 9.7     | 3.6      | 9.0    | 262a      | B12F122−    | 9.0   | 176  |
| γ-CD   | 10.7    | 7.0      | 9.0    | 427a      | B12Cl122−   | 10.5  | 333a |
| δ-CD   | 12.6b   | 7.8b     | 9.0    | 541b      | B12Br122−   | 11.1  | 416b |
| ε-CD   | 13.9b   | 8.8b     | 9.0    | 667b      | B12I122−    | 11.7  | 520b |
| ζ-CD   | 15.3b   | 9.7b     | 9.0    | 794b      |              |       |       |

a From ref. 1. b Linearly extrapolated values by assuming an annular CD shape, cf. Fig. 5. c From ref. 13.
cluster protrudes into the cavity (see $^1$H NMR in Fig. 3). δ-CD showed $^1$H NMR shifts, and, therefore, complexation with all perhalogenated clusters. For B$_{12}$Cl$_{12}$~2~, a large shift was observed for H5, which is located inside the cavity near the lower (narrower) rim, while a smaller shift was observed for H3 (0.2 versus 0.1 ppm), in line with a deep inclusion. For the largest cluster (B$_{12}$I$_{12}$~2~), the H5 proton was significantly shifted, but an even larger shift was observed for H3 (0.2 versus 0.4 ppm), indicating that this large cluster cannot protrude as deeply as the smaller perchlorinated one. ε-CD and ζ-CD showed either no or small changes in the $^1$H NMR upon the addition of B$_{12}$Cl$_{12}$~2~ or B$_{12}$Br$_{12}$~2~; only the largest guest, B$_{12}$I$_{12}$~2~, showed sizable $^1$H NMR shifts even for the two largest investigated homologues (Fig. 4). In general, we concluded that when $\Delta \delta_{145} > \Delta \delta_{115}$, a partial inclusion of the cluster inside the cavity applies, while full inclusion is signaled by $\Delta \delta_{145} > \Delta \delta_{115}$. The $^1$H NMR results are in accordance with expectations from the size complementarity principle. 

ITC was used to determine the association constants of the halogenated clusters to LRCDs. The resulting binding affinities are shown in Table 3. Very strong binding affinities to δ-CD were observed, with the highest affinities measured for B$_{12}$Br$_{12}$~2~ (2.6 × 10$^6$ M$^{-1}$) and B$_{12}$Cl$_{12}$~2~ (2.5 × 10$^6$ M$^{-1}$), followed by B$_{12}$I$_{12}$~2~ (6.8 × 10$^5$ M$^{-1}$). These values even exceed the values previously measured for these clusters to γ-CD, and set record benchmarks for LRCDs (compare Table 3 with Table 1). The increase in affinity from γ-CD to δ-CD occurs at the expense of a decreased selectivity, that is, the binding constants for B$_{12}$Cl$_{12}$~2~, B$_{12}$Br$_{12}$~2~, and B$_{12}$I$_{12}$~2~ vary by almost a factor of 70 for γ-CD but by less than a factor of 4 for δ-CD. Presumably, the higher flexibility of δ-CD allows for a better induced fit. For example, it is likely that the smaller B$_{12}$Cl$_{12}$~2~ cluster is accommodated through an elliptical distortion of the LRCD cavity, which has been experimentally observed in the crystal structure of δ-CD$^{6a}$ and which has also been found in molecular dynamics simulations of LRCDs.$^{7e,df}$ The resulting clamp-like binding site (Fig. 5) offers a tighter cavity with more dispersive contact points, which accounts for the absolute (from 0.014 to 2.5 × 10$^8$ M$^{-1}$) and relative (to B$_{12}$Br$_{12}$~2~) enhancement in binding of B$_{12}$Cl$_{12}$~2~ with the larger macrocycle; this offsets simple size complementarity arguments, which would have led to the expectation of a reduced affinity of the smaller guest as the cavity becomes larger. These simple arguments are again sufficient to account for the variation in affinities as the LRCD series expands from δ-CD to ζ-CD. Particularly noteworthy is the fact that B$_{12}$I$_{12}$~2~ becomes the strongest binder for ε-CD where also a micromolar affinity is achieved. And even for ζ-CD a sizable binding constant of 8000 M$^{-1}$ is obtained.

Thermodynamic parameters for complexation are shown in Table 4. In general, the binding is an enthalpically driven process with an entropic penalty (ε-CD was an exception), in agreement with our previous report on the binding of same clusters with γ-CD.$^{13}$ In our previous study with γ-CD, a correlation between enthalpy and guest size was observed: the enthalpy of complexation (ΔH) increased with increasing the dianion size. This trend discontinues for the LRCDs, presumably due to the different binding modes (Fig. 5). However, large enthalpy values are accompanied by an increased entropic penalty for the LRCDs as well, that is, enthalpy-entropy compensation applies, as is common for CDs.$^{2c,13}$

Besides the studies in aqueous solution, we have tested the stability of the formed complexes in the gas phase using mass spectrometry experiments. Fig. 6 shows the mass spectra of δ-CD with B$_{12}$Br$_{12}$~2~ and B$_{12}$I$_{12}$~2~. For both clusters, doubly charged ions were observed at m/z 545 and 825, corresponding to the naked anions, B$_{12}$Br$_{12}$~2~ and B$_{12}$I$_{12}$~2~, respectively. The 1 : 1 complexes were also observed as doubly negatively charged species (δ-CD-B$_{12}$Br$_{12}$~2~ at m/z 1276 and δ-CD-B$_{12}$Br$_{12}$~2~ at

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**Table 3** Association constants (K$^a$) of dodecaborate cluster dianions$^b$ with LRCDs

| Host | B$_{12}$Cl$_{12}$~2~ | B$_{12}$Br$_{12}$~2~ | B$_{12}$I$_{12}$~2~ |
|------|-------------------|-------------------|-------------------|
| γ-CD | 14 | 960 | 67 |
| δ-CD | 2500 | 2600 | 680 |
| ε-CD | 29 | 140 | 2100 |
| ζ-CD | 2 ± 1 | 6 ± 1 | 8 ± 1 |

$^a$ Measured by ITC in H$_2$O at 25 °C and analyzed for a 1 : 1 complexation model; 10% error unless explicitly stated. $^b$ Measured as sodium salts. $^c$ From ref. 13. 

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**Table 4** Thermodynamic parameters (in kcal mol$^{-1}$) for the binding of dodecaborate cluster dianions$^b$ with LRCDs

| Host | B$_{12}$Cl$_{12}$~2~ | B$_{12}$Br$_{12}$~2~ | B$_{12}$I$_{12}$~2~ |
|------|-------------------|-------------------|-------------------|
| γ-CD | ΔH$^c$ | −14.4 | −21.4 | −25.0 |
| TΔS$^o$ | −8.6 | −13.3 | −18.4 |
| δ-CD | ΔH$^c$ | −11.2 | −11.9 | −8.7 |
| TΔS$^o$ | −2.5 | −3.1 | −0.6 |
| ε-CD | ΔH$^c$ | −3.6 | −4.6 | −7.7 |
| TΔS$^o$ | 2.5 | 2.4 | 0.9 |
| ζ-CD | ΔH$^c$ | −16.9 | −6.4 | −10.4 |
| TΔS$^o$ | −12.5 | −1.3 | −5.1 |

$^a$ Measured by ITC in H$_2$O at 25 °C and analyzed for a 1 : 1 complexation model; errors in ΔH and TΔS are 10% or ±0.8 kcal mol$^{-1}$, whichever is larger. $^b$ Measured as sodium salts. $^c$ From ref. 13.
B12Br12, Na2B12Br12, and Na2B12I12, were synthesized according to published procedures, while K3B12F12 was purchased from Sigma-Aldrich (Germany) and used without further purification. Nuclear Magnetic Resonance (NMR) spectra were recorded on a JEOL ECX 400 MHz NMR spectrometer in D2O. Isothermal titration calorimetry experiments were carried out in water (unbuffered) on a VP-ITC from Microcal, Inc., at 25 °C, pH 6.5–7. The binding equilibria were studied using a cellular host concentration of 50 μM, to which a 10–30 times more concentrated guest solution was titrated. Typically, 27 consecutive injections of 10 μL were used. All solutions were degassed prior to titration. Heats of dilution were determined by titration of the guest solution into water. The first data point was removed from the data set prior to curve fitting (Origin 7.0 software) according to a one-set-of-sites model. The knowledge of the complex stability constant (K) and molar reaction enthalpy (ΔH°) enabled the calculation of the standard free energy (ΔG°) and entropy changes (ΔS°) according to ΔG° = −RT ln K = ΔH° − TΔS°. Mass spectrometry experiments were performed with a Bruker Micro-TOF MS.

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