Netsu Sokutei **34** (5) 232-243

解　説

Thermodynamics of Cucurbituril Complexation in Aqueous Solutions

Mikhail V. Rekharsky and Yoshihisa Inoue

(Received August 17, 2007; Accepted October 6, 2007)

Recently cucurbituril macrocycles attract much attention in supramolecular chemistry and have widely been used to build a variety of self-assembled nanostructures, devices, and architectures. Appropriate design of self-assembling processes requires detailed knowledge of the thermodynamics of cucurbituril complexation with various guest molecules. Despite the significant efforts devoted in recent years to the thermodynamic investigation of cucurbituril complexation, there still remain serious discrepancies in the literature data. In this review, we wish to discuss the recent achievements as well as the remaining problems in the area of complexation thermodynamics of cucurbituril.

Keywords: cucurbituril; cucurbit[n]uril; thermodynamics; association constant

1. Introduction

Symmetrical hexameric macrocycle cucurbituril, i.e. cucurbit[6]uril (CB[6]), was synthesized for the first time in 1905 by acid-catalyzed condensation reaction of glycouril and formaldehyde.[1] However, its chemical nature and structure were unknown until 1981, when full characterization was reported by Mock and co-workers (**Fig.1**).[2] Cucurbituril, named after Cucurbitae (pumpkin) due to the similarity in shape, is a barrel-shaped molecular container with a central hydrophobic cavity and two identical portals rimmed with six carbonyl oxygens.

Until recently, application of CB[6] in chemistry has been rather limited since it is practically insoluble in water or organic solvents, and hence the first study on the guest-binding properties of CB[6] as a synthetic receptor was performed in a rather unusual solvent, i.e. 50 % aqueous formic acid, by Mock and co-workers.[3] The use of CB[6], however, has undergone an explosion in various fields of science[4,5] after the discovery of the fact that it becomes readily soluble in aqueous salt solutions containing alkali or other metal cations or organic ammonium ions.[6] It was subsequently found that alkali metal cations (M^+) coordinate to both portals of barrel-shaped CB[6] to give a dicationic complex $[CB[6] \cdot 2M]^{2+}$.[7]

In the condensation of glycoluril and formaldehyde (**Fig.1**), neither Behrend *et al.*[1] nor Mock *et al.*[2] detected any formation of lower or higher homologues of CB[6], composed of a different number of glycouril units in the macrocyclic ring (*e.g.*, CB[5], CB[7], and CB[8]). Two decades later, this reaction was conducted under milder, kinetically controlled conditions by the research groups of Kim and Day to give CB[5]-CB[8] and CB[10] (**Fig.2**).[8,9]

All CB homologues share characteristic features, possessing a hydrophobic cavity and polar carbonyl groups surrounding the portals. However, their varying cavity and portal sizes (**Table 1**) make the molecular recognition behavior very different from each other. As demonstrated for the first time by Mock *et al.*,[3] CB[6] forms stable complexes with protonated aminoalkanes and even more stable complexes with protonated diaminoalkanes (**Fig.3**). Upon complexation with CB, the hydrophobic aliphatic chain of (di)aminoalkanes is inserted into the cavity and the ammonium cation(s) electrostatically interact with the carbonyl oxygens at the portal(s).

Relatively small neutral guests, such as tetrahydrofuran or benzene, can also interact with CB[6] and be encapsulated inside the cavity. The smallest

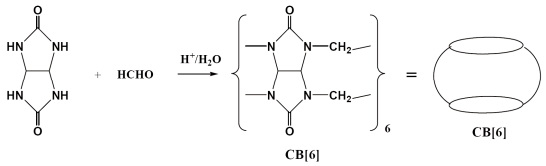


Fig.1 Synthesis and chemical structure of cucurbit[6]uril macrocycle.

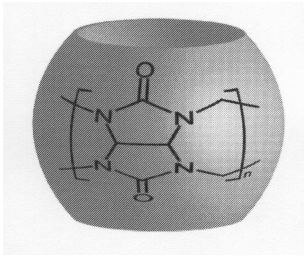
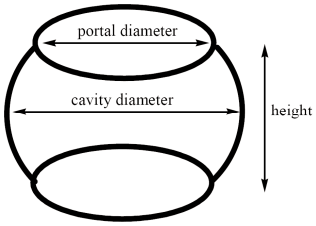


Fig.2 Chemical structure of cucurbituril homologues CB[*n*] (*n* = 5, 6, 7, 8, and 10).

Table 1 Geometrical dimensions of cucurbit[*n*]uril cavity.



Dimension	CB[5]	CB[6]	CB[7]	CB[8]
portal diameter (nm)	0.24	0.39	0.54	0.69
cavity diameter (nm)	0.44	0.58	0.73	0.88
Height (nm)	0.91	0.91	0.91	0.91
cavity volume (nm^3)	0.082	0.184	0.279	0.479

homologue CB[5] can encapsulate only very small neutral gases such as N_2, O_2, or Ar and strongly bind small cations, such as Li^+ or Na^+, at the portals. Larger CB[7] can comfortably accommodate in its cavity rather sizeable hydrophobic moieties such as naphthalene, ferrocene, and adamantane, while yet larger CB[8] is capable of forming not only 1:1 complexes with positively charged polycations, such as protonated cyclams, but also 2:1 complexes through simultaneous insertion of two aromatic

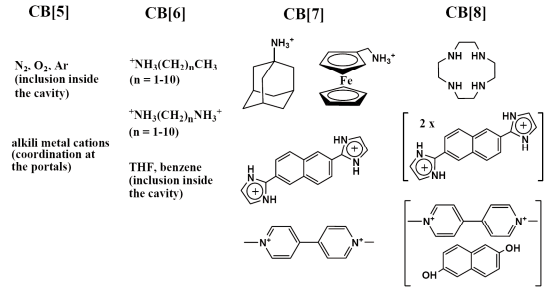
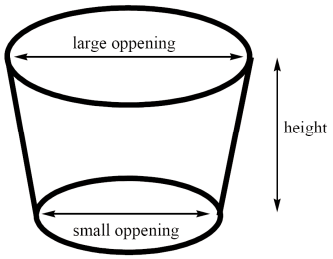


Fig.3 Chemical structure of guest molecules forming strong complexes with various cucurbituril macrocycles.

Table 2 Geometrical dimensions of cyclodextrin cavity.



	α-CD	β-CD	γ-CD
small opening (nm)	0.47	0.60	0.75
large opening (nm)	0.53	0.65	0.83
height (nm)	0.79	0.79	0.79
cavity volume (nm^3)	0.174	0.262	0.427

guests/moieties, such as naphthalene derivatives (**Fig.3**).

Geometrical dimensions of CB[6], CB[7], and CB[8] (**Table 1**) resemble those of more documented α-, β-, and γ-cyclodextrin (CD), respectively (**Table 2**). Both types of macrocyclic hosts have a central hydrophobic cavity suitable for accommodating hydrophobic guests of appropriate shape and size. Nevertheless, the binding properties of CBs are strikingly different from those of CDs. Complexation of benzylammonium with CB[7] versus β-CD can serve as an excellent illustration of this difference. In aqueous solution, benzylammonium forms very stable complex with CB[7] (**Fig.4**).[10] In contrast this guest exhibits practically no affinity toward β-CD.[11] Complexation behavior of cyclodextrins in aqueous solution was previously summarized and discussed in several reviews,[12,13] but the binding properties of

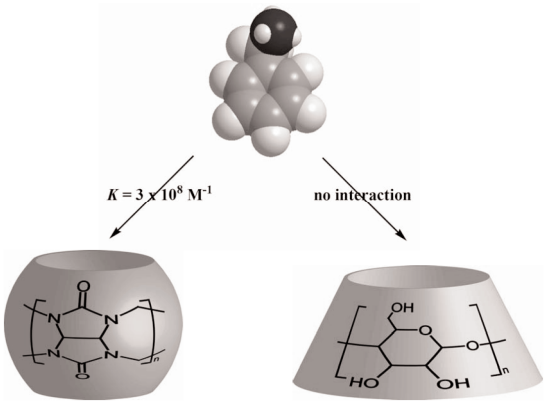


Fig.4 Complexation of benzylammonium toward CB[7] ($n=7$; left) and β-CD ($n=7$; right).

cucurbiturils is a relatively new topic in supramolecular chemistry and here we will discuss it in more detail.

2. Thermodynamics of Cucurbit[6]uril (CB[6]) Complexation in Aqueous Solutions

In their pioneering work, Mock *et al.*[3] examined a wide variety of aliphatic and aromatic amines as guests for CB[6]. Probably due to the extremely low solubilities in conventional solvents, the association constants (K_a) were determined in 50 % (v/v) aqueous formic acid by using the NMR and/or UV spectral methods. Since it was discovered[6] that CB[6] becomes readily soluble in aqueous solution that contains alkali or other metal ions or even organic ammonium ions, aqueous solutions of such salts were often employed as solvents (see for instance,[6,14,15]). Recently, Isaacs *et al.*[5] have carefully examined the complexation behavior of CB[6] and other CBs to find large differences in K_a determined by different groups. For example, Buschmann *et al.*[16] reported $K_a = 6.6 \times 10^5\ M^{-1}$ for complexation of tricationic spermidine with CB[6] in pure water, which is 20 times lower than that determined in 50 % formic acid by Mock *et al.*[3] Isaacs *et al.*[17] reported $K_a = 4.5 \times 10^8\ M^{-1}$ for complexation of 1,6-hexanediammonium with CB[6] in 50 mM CD_3CO_2Na buffered D_2O (pD 4.74), which is > 150 times larger than that determined in 50 % formic acid by Mock *et al.*[3] Obviously, varying solution condition is one of the major reasons for giving such different K_a values, and this apparently controversial and puzzling situation is discussed below.

2.1 CB[6] species existing in aqueous solution

To start any complexation thermodynamic study, one should know the molecular/ionic species existing in the solution. Surprisingly, there is no agreement in the literature on the identity of ionic form(s) of CB[6] solubilized in aqueous solution of metal salts. Buschmann *et al.*[18,19,20] reported that aqueous NaCl solution of CB[6] contains predominantly monocationic $[CB[6] \cdot Na]^+$ species. However, our recent ESI-MS study[21] has revealed that only dicationic $[CB[6] \cdot 2Na]^{2+}$ species exists even in the presence of a large excess of CB[6]. Recently we published[22] the systematic and comprehensive thermodynamic data for complexation of aliphatic amines and alcohols with CB[6] in a variety of aqueous solutions to reinforce our previous conclusion obtained by the ESI-MS study.[21]

Microcalorimetric titration of CB[6] with propylammonium was performed in aqueous 0.2 M LiCl, 0.05 M NaCl, and 0.05 M CsCl solutions (the higher LiCl concentration was needed due to the low solubility of CB[6] in aqueous LiCl solution).[22] Propylammonium as guest has two advantages in the present case. Firstly, it shows a relatively high affinity toward CB[6],[3] and hence one can determine the stability constant with high precision. Secondly, judging from the geometrical dimensions, the short alkyl chain of propylammonium can be comfortably accommodated inside the CB[6] cavity without touching the metal cation at the CB's second portal. Upon complexation with CB[6], the ammonium moiety of the guest coordinates to the carbonyl oxygens at one of CB[6] portals, while the hydrophobic part occupies the inner space of CB[6].[3,5] If CB[6] exists in a monocationic form ($[CB[6] \cdot M]^+$; M = Li, Na, or Cs) and only one portal is occupied by metal cation, alkylammonium guest can readily penetrate into the cavity from the open end of CB without competing with the metal cation at the opposite portal. In the propylammonium case, the insertion of short propyl chain does not significantly affect the original position/location of the metal cation at the opposite end. Consequently, the stability constants as well as the other thermodynamic parameters would resemble to each other in all three solutions, *i.e.* 0.2 M LiCl, 0.05 M NaCl, and 0.05 M CsCl. In contrast, both the affinity and the enthalpic gain gradually decrease by increasing the size of the metal ion in solution, *i.e.* on going from 0.2 M LiCl to 0.05

Table 3 Complex Stability Constant (K_a), Enthalpy Changes (ΔH°), and Entropy Changes ($T\Delta S^\circ$)for Complexation of Various Guests toward Cucurbit[6]uril (CB[6]) in aqueous solutions at $T = 298.15$ K (adapted from 22)).

Reaction	K_a / M^{-1}	ΔH° / kJ mol^{-1}	$T\Delta S^\circ$ / kJ mol^{-1}
$[CB[6] \cdot 2Na]^{2+}$ + Ethanol = $[CB[6] \cdot Ethanol \cdot 2Na]^{2+}$ (0.05 M NaCl)	90 ± 8	−11.2 ± 0.2	0.0 ± 0.4
$[CB[6] \cdot 2Cs]^{2+}$ + Ethanol = $[CB[6] \cdot Ethanol \cdot 2Cs]^{2+}$ (0.05 M CsCl)	26 ± 5	−9.1 ± 0.3	−1.0 ± 0.6
$[CB[6] \cdot 2Na]^{2+}$ + Propanol = $[CB[6] \cdot Propanol \cdot 2Na]^{2+}$ (0.05 M NaCl)	710 ± 30	-22.5 ± 0.2	−6.2 ± 0.2
$[CB[6] \cdot 2K]^{2+}$ + Propanol = $[CB[6] \cdot Propanol \cdot 2K]^{2+}$ (0.05 M KCl)	490 ± 20	-19.9 ± 0.2	−4.5 ± 0.3
$[CB[6] \cdot 2Rb]^{2+}$ + Propanol = $[CB[6] \cdot Propanol \cdot 2Rb]^{2+}$ (0.05 M RbCl)	120 ± 15	-16.8 ± 0.2	−4.9 ± 0.5
$[CB[6] \cdot 2Cs]^{2+}$ + Propanol = $[CB[6] \cdot Propanol \cdot 2Cs]^{2+}$ (0.05 M CsCl)	< 5		
$[CB[6] \cdot 2Na]^{2+}$ + Butanol = $[CB[6] \cdot Butanoll \cdot 2Na]^{2+}$ (0.05 M NaCl)	1220 ± 50	−30.3 ± 0.3	−12.7 ± 0.3
$[CB[6] \cdot 2Na]^{2+}$ + Pentanol = $[CB[6] \cdot Pentanol \cdot 2Na]^{2+}$ (0.05 M NaCl)	410 ± 20	-24.1 ± 0.3	−9.2 ± 0.3
$[CB[6] \cdot 2Na]^{2+}$ + 1-Propylammonium$^+$ = $[CB[6] \cdot 1\text{-}Propylammonium \cdot Na]^{2+}$ + Na^+ (0.05 M NaCl)	(1.55 ± 0.08)×10^5	−19.1 ± 0.3	10.6 ± 0.3
$[CB[6] \cdot 2Cs]^{2+}$ + 1-Propylammonium$^+$ = $[CB[6] \cdot 1\text{-}PropylammoniumoCs]^{2+}$ + Cs^+ (0.05 M CsCl)	8500 ± 500	−9.2 ± 0.4	13.2 ± 0.5
$[CB[6] \cdot 2Li]^{2+}$ + 1-Propylammonium$^+$ = $[CB[6] \cdot 1\text{-}Propylammonium \cdot Li]^{2+}$ + Li +(0.2 M LiCl)	(2.2 ± 0.1)×10^6	−41.7 ± 0.4	−5.5 ± 0.4
$[CB[6] \cdot 2Na]^{2+}$ + + 1-Propylammonium$^+$ = $[CB[6] \cdot 1\text{-}Propylammonium \cdot Na]^{2+}$ + Na^+ (0.1 M Na acetate buff.; pH 4.7)	(2.1 ± 0.7)×10^4	4.0 ± 0.5	28.7 ± 1.0
$[CB[6] \cdot 2Na]^{2+}$ + 1-Propylammonium+ = $[CB[6] \cdot 1\text{-}PropylammoniumoNa]^{2+}$ + Na^+ (0.05 M Na citrate buff.; pH 4.5)	(1.56 ± 0.09)×10^5	−18.9 ± 0.3	10.7 ± 0.3
$[CB[6] \cdot 2Na]^{2+}$ + 1-Propylammonium+ = $[CB[6] \cdot 1\text{-}Propylammonium \cdot Na]^{2+}$ + Na^+ (0.05 M Na citrate buff.; pH 3.1)	(1.55 ± 0.09)×10^5	−19.3 ± 0.3	10.3 ± 0.3
$[CB[6] \cdot 2Na]^{2+}$ + Acetic acid0 = $[CB[6] \cdot Acetic\ acid \cdot 2Na]^{2+}$ (0.05 M Na citrate buff.; pH 3.1)	150 ± 5	−24.0 ± 0.3	−11.6 ± 0.3

M NaCl and than to 0.05 M CsCl, as shown in **Table 3**. This thermodynamic behavior is compatible only with the dicationic form of CB[6] in the initial state, where the both portals are occupied by M^+. Indeed, the size matching of Cs^+ with CB[6] portal leads to the strongest ion-dipole interactions, making Cs^+ the hardest competitor for in-coming propylammonium to give the lowest affinity and enthalpic gain in CsCl solution. On the other hand, Li^+ is the weakest competitor and therefore the highest affinity and enthalpic gain were

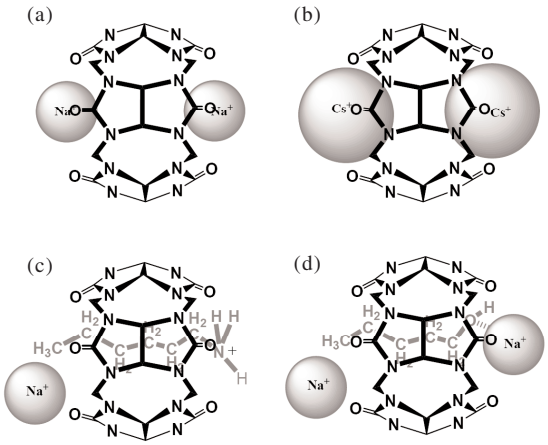


Fig.5 Coordination of (a) sodium and (b) cesium cations to the portals of CB[6] and dislocation of the sodium cation upon inclusion of (c) hexylammonium and (d) pentanol in the cavity.

obtained in LiCl solution. Coordination of Na^+ and Cs^+ ions to CB[6] portals is illustrated at **Fig.5(a)** and **(b)**.

A series of C_2-C_5 alkanols were also examined as guests for CB[6] in aqueous 0.05 M NaCl solution.[22] Complexation of neutral guests, such as aliphatic alcohols, ketones, and ethers, with CB[6] is facilitated by coordination of guest's oxygen to the metal cation at one of CB[6] portals as well as the insertion of the guest's hydrophobic part into the cavity.[6,14,15] If CB[6] exists in monocationic form (e.g. $[CB[6] \cdot Na]^+$) and the second portal is open, we may expect a continuously increasing trend in K_a with increasing alkyl chain length, as was the case with cyclodextrins [See for instance,[23]]. On the other hand, if both portals are capped by sodium cations, the maximum affinity should be observed for an alcohol with an optimum alkyl chain length that can be comfortably accommodated in the cavity. Further extension of the alkyl chain should cause steric clashes with the sodium ion (**Fig.5(d)**), leading to reduced affinities. The results in **Table 3** support the coordination of sodium ions to both portals. Indeed, the affinity becomes higher on going from ethanol to propanol and then to butanol, but is suddenly reduced upon further elongation of the alkyl chain.

Complexation of propanol with CB[6] was further investigated in aqueous NaCl, KCl, RbCl, and CsCl solutions (0.05 M).[22] By changing the cation size, the effective accessible volume of CB[6] cavity is expected to be manipulated (**Fig.5(a)** and **5(b)**). If both portals are occupied by metal cations, the inner volume of CB[6] cavity is gradually reduced by increasing cation size from Na^+ to Cs^+ with accompanying decrease in K_a. Indeed, the isothermal titration calorimetry (ITC) results show that the affinity of propanol decreases from 710 M^{-1} in NaCl solution to 490 M^{-1} in KCl solution and then to 120 M^{-1} in RbCl solution. In CsCl solution, no appreciable complexation of propanol with CB[6] was detected. Before giving a final conclusion, we should examine another interpretation of the observed tendency, in which the affinity decrease is related to the reducing coordination ability of cation to oxygen in the order: $Na^+ > K^+ > Rb^+ > Cs^+$. This explanation seems reasonable, since the most strongly coordinating Na^+ affords the largest affinity to alcohol, while the weakly coordinating Cs^+ fails to bind the alcohol guest. To check this possibility, ethanol was employed as a guest for CB[6] in 0.05 M CsCl to obtain an appreciable affinity of 26 M^{-1}, which is 3.5 times lower than that in 0.05 M NaCl (Table 3). Consequently, if CB[6] exists as a monocationic species in aqueous solution, a similar trend in affinity should be observed even in the case of propanol, and hence we can expect K_a of ca. 200 M^{-1} in CsCl solution, which however obviously contradicts with the experimental results presented in **Table 3**. Therefore, all newly obtained thermodynamic data[22] reinforce the previous conclusion[21] that CB[6] is solubilized in aqueous solution of various metal salts by forming exclusively dicationic species.

2.2 Affinity of alkylammonium versus 1, ω-alkanediammonium toward CB[6] in 50 % formic acid and in 0.05 M NaCl solution

Addition of formic acid (of up to 50 %) into water leads to total restructuring of the unique structure of water, and therefore it is difficult in general to expect similar complexation thermodynamic behavior in water and 1:1 water-formic acid mixture. Nevertheless, the profiles of affinity (log K_a) for both alkylammonium and alkanediammonium guests toward CB[6] (as a function of chain length) are similar in these two solvents, as illustrated in **Fig.6**. In both solvents, the affinity of C_2-C_8 alkylammonium guests gradually increases to reach

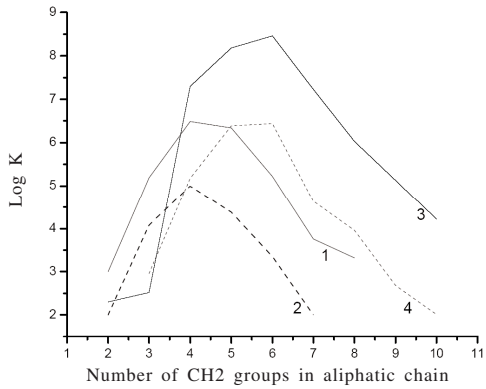


Fig.6 Affinity (log K_a) of CB[6] toward monocationic alkylammonium guests in 0.05 M NaCl (solid line 1) and in 50 % formic acid (dashed line 2) and toward dicationic 1, ω-alkanediammonium guests in 0.05 M NaCl (solid line 3) and in 50 % formic acid (dashed line 4).

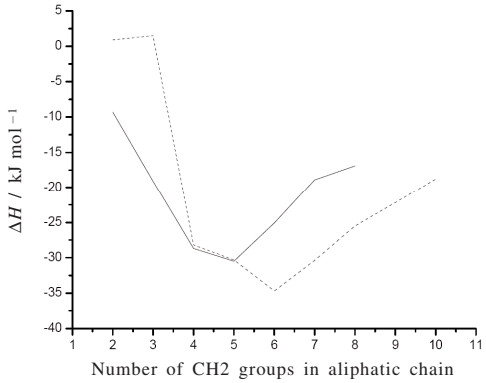


Fig.7 Reaction enthalpy (ΔH°) for complexation of alkylammonium (solid line) and alkanediammonium (dashed line) with CB[6].

a maximum at C_4 and then starts to decrease moderately to C_5 and rapidly thereafter. Similar affinity profiles are also seen for alkanediammonium guests with maxima at C_5-C_6 (**Fig.6**).

The reaction enthalpies (**Fig.7**) exhibit a trend similar to that observed for affinity (see above). The reaction enthalpy reaches the largest negative value at C_4-C_5 for alkylammonium and at C_6 for alkanediammonium (**Fig.7**). The reaction entropy is consistently positive with a profile somewhat similar to that for affinity (**Fig.8**). Thus, the complexation of amines

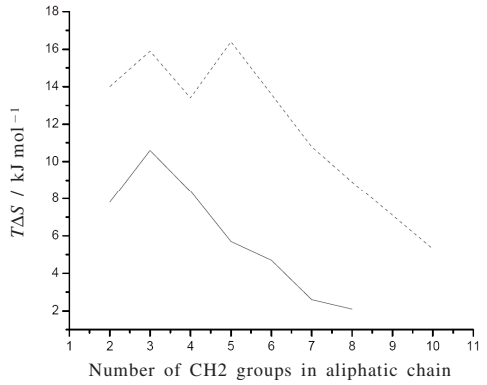


Fig.8 Reaction entropy ($T\Delta S^\circ$; $T = 298.15$ K) for complexation of alkylammonium (solid line) and alkanediammonium (dashed line) with CB[6].

and diamines with CB[6] is driven and controlled by both enthalpic and entropic terms.

Interestingly, despite the steady increase in enthalpy up to C_5, the reaction entropy starts to decrease at C_4 upon complexation of alkylammonium (**Fig.8**), probably indicating appreciable restriction of the alkyl chain in the cavity due to the steric clashes with Na^+ at the opposite portal. However, this entropic loss is over-compensated by the large per-methylene enthalpic gain of 9.6 kJ mol^{-1} most likely arising from the strong van der Waals interactions inside the cavity (due to the closer contacts with CB walls) to give the largest affinity of 3.1×10^6 M^{-1} for butyammonium. Conformational restrictions are more severe for pentylammonium and the small enthalpic gain (1.8 kJ mol-1) obtained by adding an extra methylene to butylammonium is completely cancelled out by a larger entropic loss (2.7 kJ mol^{-1}), eventually leading to a measurable reduction of affinity for pentylammonium. Volumes of longer alkylammonium guests exceed the available space of CB[6] cavity capped with a sodium ion. The only way to accommodate such a bulky guest is to displace the sodium ion from its optimal position/location at the opposite CB[6] portal (**Fig.5(c)**). Obviously, such displacement is enthalpically highly unfavorable and leads to a large decrease in affinity.

In contrast, alkanediammonium guests can replace two sodium cations at both portals of CB[6]. However, the alkyl chains of 1,2-ethylenediammonium and 1,3-propanediammonium guests are too short to allow

simultaneous coordination of the two ammonium groups to the both portals of CB[6]. This is the major reason why the particularly low affinities was observed for these short C_2 and C_3 diamines (**Fig.6**).[22] The dramatic affinity leap between C_3 and C_4 alkanediammonium guests leads us to a conclusion that at least four methylene units are needed for an alkanediammonium guest to allow the simultaneous replacement of the two sodium ions at the both openings of CB[6] (**Fig.6**). The affinity is enhanced by a factor of 60000 by simply adding a single methylene to propanediammonium. To the best of our knowledge, this is the largest per-methylene enhancement ever observed in supramolecular chemistry.

The more moderate affinity enhancement observed upon further extension of the alkyl chain from C_4 to C_5 is caused by more favorable enthalpy and entropy changes, which are attributable to the optimized van der Waals interactions (enthalpic gain) and the extensive cavity desolvation (entropic gain). The additional relatively small enhancement of affinity observed for hexanediammonium is exclusively enthalpy-driven, most probably due to very strong intra-cavity van der Waals interactions. However, as demonstrated in cyclodextrin complexation,[23] strong intra-cavity van der Waals interactions inevitably lead to restriction of guest conformation with accompanying decrease of entropy (**Fig.6-8**). Then, the inherent entropic gain from the dehydration of host cavity is constantly cancelled upon extension of the methylene chain from C_6 to C_{10} (**Fig.6-8**). The enthalpic gain reaches the highest value at C6 and then monotonically decreases up to C_{10}. Such a synchronized reduction of enthalpy and entropy is expected to occur when the alkyl chain length exceeds a certain limit. Thus, a very long alkyl chain is severely restricted in conformation inside the cavity (causing entropic losses) and at the same time two ammonium groups are poorly coordinated at the portals resulting in weak host-guest ion-dipole interactions (enthalpic losses).

As shown at Figure 6, complex stabilities in 50 % formic acid are much smaller than those obtained in aqueous 0.05 M NaCl solution. However, the reason for such large difference is not immediately clear. In a highly acidic solution of 50% formic acid, it is likely that concentration of hydronium ion (H_3O^+) is high enough to achieve effective coordination to CB[6] portals, forming $[CB[6] \cdot 2H_3O]^{2+}$ complex. This dicationic complex should behave in a way similar to $[CB[6] \cdot 2Na]^{2+}$and therefore the general affinity profiles would resemble to each other for the same guest series. However, there is a problem with such explanation. As we discussed above, the affinity of propylammonium increases with decreasing cation size of metal salt in solution, and therefore we would expect higher affinity for $[CB[6] \cdot 2H_3O]^{2+}$ than for $[CB[6] \cdot 2Na]^{2+}$. To explain the experimentally observed lower affinity in 50% formic acid versus 0.05 M NaCl solution we should take into account of the species residing in the CB[6] cavity. In 0.05 M NaCl, the cavity can contain several water molecules since there is no any other possible guest species in aqueous NaCl solution. On the other hand, it is likely that in 50 % formic acid, neutral HCOOH molecules are included inside the cavity. These HCOOH molecules may act as competitor upon guest inclusion to reduce the affinity for alkylammoium and alkanediammonium.

To explore the possible inclusion of organic acid into CB[6] cavity, the ITC experiments were performed with propylammonium in three different buffer solutions: 0.05 M sodium citrate buffer at pH 4.5, 0.05 M sodium citrate buffer at pH 3.1, and 0.1 M sodium acetate buffer at pH 4.7 (**Table 3**). The thermodynamic parameters for complexation of propylammonium with CB[6] in 0.05 M sodium citrate buffer (pH 4.5) and in 0.05 M sodium citrate buffer (pH 3.1) are the same as those obtained in 0.05 M NaCl solution (**Table 3**). This seems reasonable since bulky citric acid cannot be included in CB[6] cavity in any of these two solutions (**Fig.9(a)**). In contrast, the use of 0.1 M sodium acetate buffer greatly affects the complexation of propylammonium to give a 7.4-times smaller affinity and a positive enthalpy change (**Table 3**). The most likely explanation for the affinity drop in 0.1 M sodium acetate buffer is the inclusion of neutral acetic acid (CH_3COOH) into the cavity (**Fig.9(b)**). Hence, ITC experiments were performed to directly determine the thermodynamic parameters for complexation of neutral CH_3COOH with CB[6] in 0.05 M sodium citrate buffer (pH 3.1).[22] This complexation is exclusively enthalpy-driven and accompanied by a large negative entropy (**Table 3** and **Fig.9(b)**). The complexation enthalpy of CH_3COOH with CB[6] is more negative than that of propylammonium (**Table 3**). It is readily understood why

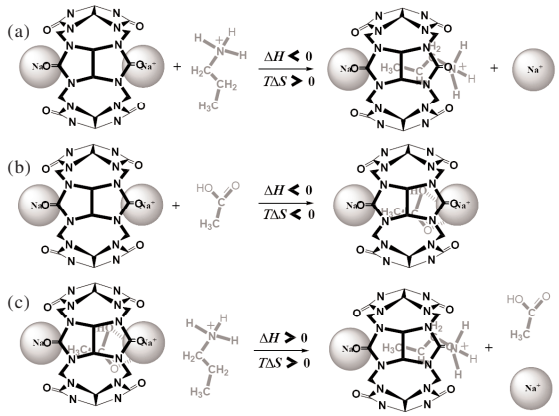


Fig.9 (a) Insertion of propylammonium into CB[6] cavity in 0.05 M NaCl, 0.05 M sodium citrate buffer (pH 4.5), and 0.05 M sodium citrate buffer (pH 3.1), where CB[6] cavity is occupied only by water molecule(s) (not shown); (b) Inclusion of neutral CH_3COOH molecule in CB[6] cavity; (c) Complexation of propylammonium with CB[6] in 0.1 M sodium acetate buffer (pH 4.7).

the complexation of propylammonium with CB[6] is associated with an unfavorable positive enthalpy in 0.1 M sodium acetate buffer. This is simply because the removal of neutral CH_3COOH from the cavity is not entirely compensated in enthalpy by the inclusion of propylammonium. This scenario is illustrated in **Fig.9(c)**. In the ethylammonium case, even a larger positive enthalpy was obtained in 0.1 M sodium acetate buffer. Again, the enthalpy difference in 0.05 M NaCl versus 0.1 M sodium acetate buffer is close to the enthalpy of insertion of neutral CH_3COOH into the cavity.

By using the ITC results obtained in 0.05 M NaCl and in 0.1 M sodium acetate buffer (**Table 3**), we may explain similar affinities determine for 1,6-hexanediammonium performed in 0.05 M NaCl (H_2O) solution[22] and in Isaacs' study[17] performed in 0.05 M CD_3CO_2Na-buffered (pD 4.74) D_2O solution. These two studies were performed in different solvents, *i.e.* H_2O versus D_2O, but we assume that the solvent isotope effect does not lead to $>15\sim20$ % difference in affinity, as was the case with cyclodextrin complexation.[24] As discussed above, the presence of neutral acetic acid molecules in 0.025 M CD_3CO_2Na-buffered D_2O (pD 4.74) should reduce the stability of 1,6-hexanediammonium-CB[6] complex. Taking into account the affinity of neutral acetic acid toward CB[6] (**Table 3**), we may expect that the stability of 1,6-hexanediammonium-CB[6] complex in 0.025 M CD_3CO_2Na-buffered D_2O is 3-4 times lower than that in neutral solution that contains the same concentration of sodium cations, e.g. 0.025 M NaCl. This concentration is two times lower than that employed in the ITC study (0.05 M NaCl).[22] Low concentration of Na^+ means weaker competition for the CB[6] portals and thus stronger inclusion of 1,6-hexanediammonium. These two opposite trends, *i.e.* the presence of neutral acetic acid molecules in the solution (leading to an affinity reduction) versus the lower Na^+ concentration (leading to an affinity enhancement), counterbalance to each other to eventually afford the very similar complex stabilities in both solutions.

The geometric dimensions of CB[6] cavity allow inclusion of up to two molecules of neutral formic acid probably forming a hydrogen-bonded dimer in the cavity. This idea is supported by comparing the affinities of alkylammonium and alkanediammonium guests toward CB[6] in 0.05 M NaCl[22] and in 50 % formic acid.[3] If there are two molecules of neutral formic acid in the cavity, then short alkylammonium guest, such as ethyl- or propylammonium, would replace only one of the two formic acids, while longer alkylammonium and alkanediammonium could replace both of the formic acid molecules. If this is the case, the difference in affinity (obtained in 0.05 M NaCl versus 50 % formic acid) should be smaller for short alkylammonium than for longer alkylammonium and alkanediammonium. Indeed, the affinity ratio, K_{NaCl}/K_{HCOOH}, is close to 10 for ethyl- and propylammonium, but well exceeds 100 for longer alkylammonium and alkanediammonium guests. We may conclude therefore that the earlier data obtained in 50 % formic acid[3] are well compatible with more recent ITC data obtained in aqueous metal salt solutions.[22]

2.3 Interaction of spermidine and spermine with CB[6]

As illustrated in **Fig.10** (solid and dashed lines), diamines consistently display much higher affinities toward CB[6] than the corresponding monoamines. This general tendency prompted us to further examine the complexation thermodynamic behavior of biologically important polyamines, such as spermidine and spermine.[22] These tri- and tetraammonium guests allow us to

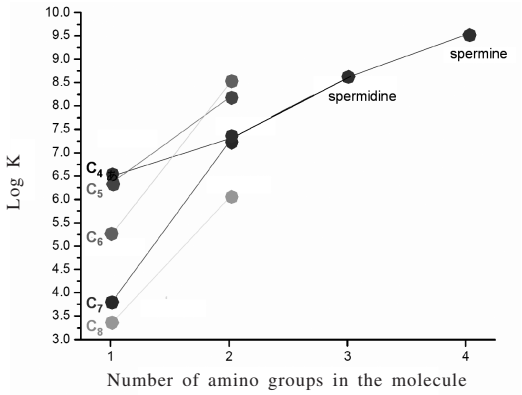


Fig.10 Dependence of complex stability upon the number of ammonium groups in guest molecule.

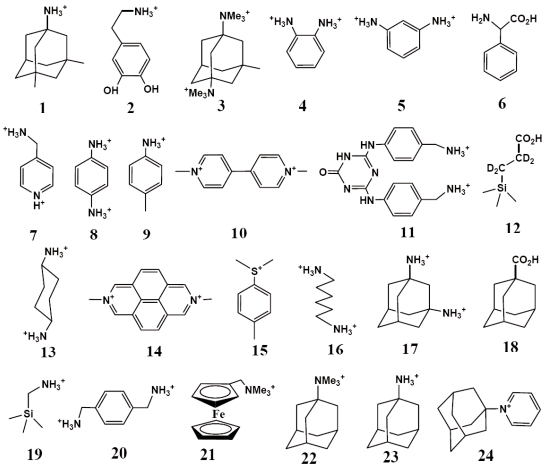


Fig.11 Structure of the guest molecules which form complexes with CB[7].

systematically investigate the effects of the number of ammonium groups in a guest. The affinities obtained for spermidine^{3+} and spermine^{4+} ($K_a = 4.1 \times 10^8\ M^{-1}$ and $K_a = 3.3 \times 10^9\ M^{-1}$, respectively[22]) are plotted against the number of ammonium groups in a guest (**Fig.10**), along with the data for butylammonium^{+} and butanediammonium^{2+}. Interestingly, the four points almost fall on a single straight line and each amino group enhances the affinity by ca. 10 times ($\Delta\log K_a \approx 1$). The affinities for spermidine and spermine in 0.05 M NaCl (this study) are 306 and 250 times higher than those in 50 % formic acid.[3] Such K_{NaCl}/K_{HCOOH} ratios are compatible with the above discussion.

Spermine shows the highest affinity of $3.3 \times 10^9\ M^{-1}$ in 0.05 M NaCl among the cationic and neutral guests examined in this study.[22] In order to further enhance the stability of spermine-CB[6] complex, it is reasonable to perform ITC measurements in the solution containing smaller cations, *e.g.* Li^+ which interacts weakly with CB[6] portal. Indeed, the stability of spermine-CB[6] complex was found 16 times higher in 0.2 M LiCl solution than in 0.05 M NaCl solution.[22] Up to now, the stability of spermine-CB[6] complex as large as $5.4 \times 10^{10}\ M^{-1}$ obtained in 0.2 M LiCl solution is the highest reported in the literature for any known complexes of CB[6] macrocycle under a variety of conditions.

3. Thermodynamics of Cucurbit[7]uril (CB[7]) Complexation in Aqueous Solutions

CB[7] was discovered only several years ago[8,9] and the first systematic study on its binding properties was published in 2005 by Isaacs *et al.*[17] In this study, binding constants for CB[7] were determined by indirect method using ^{1}H NMR spectroscopy introduced by Mock in his pioneering work on CB[6].[3] NMR measurements were performed in 50 mM CD_3CO_2Na-buffered D_2O (pD 4.74) to determine the affinities for the guests shown in **Fig.11**.

Association constants (K_a in M^{-1}) for the complexation of these guests with CB[7] are summarized in **Table 4**.[17] These data allow us to correlate the guest structure with the affinity (K_a) and further provide us with a clue to approximately estimate affinity for a potential guest which has not been studied. The lowest affinities for guest **1** and **2** (**Fig.11**) are most likely to be assigned to the over-sized hydrophobic moiety in **1** which cannot be comfortably accommodated inside the CB[7] cavity and to the hydrophilic nature of hydroxyl group in **2** which are heavily hydrated in bulk water and their dehydration upon insertion into the CB[7] cavity is energetically unfavorable. The two ammonium groups in guests **3**, **4** and **5** are not aligned in line in the molecule and do not well coordinate to the CB[7] portals. Consequently, steric clashes upon complex formation result in relatively low affinity.

In general, the relative position of ammonium cations in guest molecule and the size/shape of hydrophobic moiety are two most important factors to form a strong complex. Comparison of the affinity of **8** versus **20** provides us with insights into the profound effect of the distance of two charges. The distance between

two ammonium cations of **20** is sufficient for simultaneous coordination of each guest cation at the opposite CB[7] portal with the xylene moiety located inside the cavity.

Table 4 Association constants (K_a in M^{-1}) for the complexation of the guests shown in Figure 11 with CB[7] and CB[8] in 50 mM CD_3CO_2Na buffered D_2O (pD 4.74) at $T = 298$ K (adapted from 17)).

Guest	CB[7] (M^{-1})	CB[8] (M^{-1})
1	$(2.5 \pm 0.4)\times10^4$	$(4.3 \pm 1.1)\times10^{11}$
2	$(4.3 \pm 0.7)\times10^4$	–
3	$(6.4 \pm 1.0)\times10^4$	$(1.1 \pm 0.3)\times10^{11}$
4	$(8.0 \pm 1.3)\times10^4$	–
5	$(8.1 \pm 0.6)\times10^4$	–
6	$(1.5 \pm 0.2)\times10^5$	–
7	$(3.6 \pm 0.6)\times10^5$	–
8	$(2.1 \pm 0.3)\times10^6$	–
9	$(8.4 \pm 1.3)\times10^6$	–
10	$(1.3 \pm 0.2)\times10^7$	–
11	$(1.8 \pm 0.3)\times10^7$	$(5.8 \pm 1.4)\times10^{10}$
12	$(1.8 \pm 0.2)\times10^7$	–
13	$(2.3 \pm 0.4)\times10^7$	–
14	$(3.8 \pm 0.6)\times10^7$	$(6.4 \pm 1.2)\times10^8$
15	$(5.2 \pm 0.8)\times10^7$	–
16	$(9.0 \pm 1.4)\times10^7$	–
17	$(2.1 \pm 0.3)\times10^8$	–
18	$(3.2 \pm 0.6)\times10^8$	–
19	$(8.9 \pm 1.4)\times10^8$	–
20	$(1.8 \pm 0.3)\times10^9$	–
21	$(3.3 \pm 0.6)\times10^{11}$	$(3.1 \pm 0.8)\times10^9$
22	$(1.7 \pm 0.4)\times10^{12}$	$(9.7 \pm 2.3)\times10^{10}$
23	$(4.2 \pm 1.0)\times10^{12}$	$(8.2 \pm 1.8)\times10^8$
24	$(2.0 \pm 0.4)\times10^{12}$	$(2.0 \pm 0.5)\times10^9$

In contrast, the inter-charge distance is too short for 8 and hence the affinity of **20** with CB[7] exceeds that of **8** by a factor of 860.

It is expected that guest affinity in pure water is larger than that in 50mM sodium acetate buffer discussed above.[17] This is simply because sodium cations coordinate at CB[7] portals and thus act as competitor against incoming guest reducing complex stability. Comparison of complex stability in sodium acetate buffer and in pure water would give us a direct measure of the effect of sodium cations existing in the solution. In this context, it is interesting to examine the complexation thermodynamic parameters for three ferrocene guests toward CB[7] in H_2O (**Table 5**), since the complexation behavior of exactly the same guest **21** (**Fig.11**) has been examined in 50mM sodium acetate buffer by NMR technique. As can be seen from **Tables 4** and **5**, the affinity of guest **21** is about one order of magnitude higher in H_2O (**Table 5**) than in acetate buffer (**Table 4**). In this discussion, we may neglect the solvent isotope effect (H_2O in **Table 5** vs. D_2O in **Table 4**), since it is not expected to exceed 15～20 %.[24]

Data presented in **Table 5** allow us to draw one more important conclusion that positively charged guests exhibit much higher affinity as compare with neutral analogues and that this affinity enhancement is exclusively entropy driven (for more details see, [10]).

4. Thermodynamics of Cucurbit[8]uril (CB[8]) Complexation in Aqueous Solutions

Volume of CB[8] cavity exceeds that of CB[7] and CB[6] by a factor of 1.7 and 2.6, respectively (**Table 1**). Accordingly, we may expect comfortable accommodation of very bulky guests in CB[8] cavity.

Table 5 Complex Stability Constant (K_a), Enthalpy Changes (ΔH°), and Entropy Changes ($T\Delta S^\circ$) for Selected Ferrocenyl Guests toward Cucurbit[7]uril (CB[7]) in H_2O at $T = 298.15$ K (adapted from 10)).

Reaction	K_a / M^{-1}	ΔH° / kJ mol^{-1}	$T\Delta S^\circ$ / kJ mol^{-1}
CB[7]+hydroxymethylferrocene = [CB[7] · hydroxymethylferrocene]^0	$(3.0 \pm 0.5)\times10^9$	-88 ± 3	-34 ± 3
CB[7]+(dimethylamino)methyl-ferrocene^{1+}= [CB[7] · (dimethylamino)methyl-ferrocene]$^{1+}$	$(2 \pm 1)\times10^{12}$	-88 ± 3	-18 ± 3
CB[7]+(trimethylamino)methyl-ferrocene^{1+}= [CB[7] · (trimethylamino)methyl-ferrocene]$^{1+}$	$(4 \pm 1)\times10^{12}$	-89 ± 3	-17 ± 3

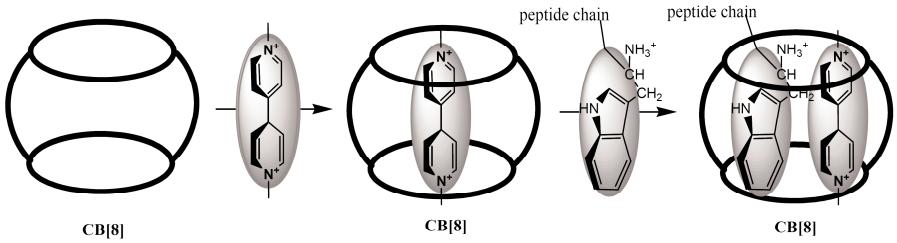


Fig.12 Consecutive complexation of CB[8] with methylviologen and then with tryptophan-containing peptide leading to formation of 1:1:1 complex.

For example, methylated aminoadmantanes **1** and **3** (**Fig.11**), which are only partially inserted in smaller CB[7] cavity, exhibit rather modest affinity of $3\sim6\times10^4\ M^{-1}$ toward CB[7] (**Table 4**). In contrast, larger-sized CB[8] allows full penetration of **1** and **3** into the cavity to give tremendous affinity enhancement by a factor of 17.2×10^6 and 1.7×10^6, respectively, as compare with CB[7] (**Table 4**). Similarly, bulky **11** and **14** also exhibit larger affinities toward CB[8] than CB[7]. Less bulky guests may have geometrical dimensions somewhat smaller than that of CB[8] cavity but almost perfectly fit into CB[7] cavity. In such a case, we may expect higher affinity toward CB[7] rather than CB[8]. This kind of situation can be illustrated by guests **21**-**24** (**Table 4**). Guest **22** shows rather modest CB[7]/CB[8] affinity ratio of 17 times, whilst the affinity of **23** toward CB[7] is more than 5000 times higher than that for CB[8].

CB[8] can form not only 1:1 but also 1:2 complexes where two guests molecules are simultaneously accommodated in the same host cavity as illustrated in Figure 3 and discussed in more details by Kim *et al.*[4]

Recently, Urbach *et al.*[25,26] performed ITC study on peptide sequence recognition using CB[8]. Initially, CB[8] macrocycle interacts with dicationic methylviologen forming 1:1 complex ($K_a=8.5\times10^5\ M^{-1}$)[25] as shown at **Fig.12**. Although large CB[8] cavity has enough space to comfortably accommodate a second molecule of methylviologen, the second complexation never occurs due to strong electrostatic guest-guest repulsion. Upon addition of tryptophan (Trp) derivatives or Trp-containing peptides to the solution of the 1:1 complex, they[25] observed large changes in UV-vis spectra corresponding to the formation of a charge-transfer complex between methylviologen and indol ring of Trp residue inside the CB[8] cavity. Based on the results of ITC experiments, they[25] reported binding constants in the range $10^3\sim10^5\ M^{-1}$ for the complexation of peptide with the 1:1 complex of methylviologen with CB[8]; the strongest complex ($K_a=1.3\times10^5\ M^{-1}$) is formed between CB[8], methylviologen and Trp-Gly-Gly.[25] Later, Urbach *at el.*[26] found that not only 1:1 CB[8]-methylviologen complex but also CB[8] itself can interact with Trp-containing peptides forming in a stepwise fashion 1:1 and then 1:2 host-guest complexes with affinities in the range $10^4\sim10^5\ M^{-1}$ at each step of complex formation. The same authors[26] have also reported the stepwise complexation of phenylalanine-containing peptides with CB[8].

5. Conclusion.

Investigation of the complex formation between cucurbiturils (CB[6], CB[7] and CB[8]) and various inorganic and organic guest molecules is a new and fast-developing area of supramolecular chemistry. An important message emerging from this review article is that the application of ITC is a powerful, indispensable method to characterize cucurbituril complexes. The strength of this method lies in its universal nature. We may conclude therefore that ITC has become a mainstream tool to study formation and to determine thermodynamic parameters of cucurbituril complexes.

References

1) R. Behrend, E. Meyer, and F. Rusche, *Liebigs Ann. Chem.* **339**, 1 (1905).
2) W. A. Freeman, W. L. Mock, and N.-Y. Shih, *J. Am. Chem. Soc.* **103**, 7367 (1981).
3) W. L. Mock and N.-Y. Shih, *J. Org. Chem.* **51**, 4440 (1986).

4) J. W. Lee, S. Samal, N. Selvapalam, H.-J. Kim, and K. Kim, *Acc. Chem. Res.* **36**, 621 (2003).
5) J. Lagona, P. Mukhopadhyay, S. Chakrabarti, and L. Isaaxs, *Angew. Chem. Int. Ed.* **44**, 4844 (2005).
6) Y.-M. Jeon, J. Kim, D. Whang, and K. Kim, *J. Am. Chem. Soc.* **118**, 9790 (1996).
7) I. Osaka, M. Kondou, N. Selvapalam, S. Samal, K. Kim, M.V. Rekharsky, Y. Inoue, and R. Arakawa, *J. Mass Spectr.* **41**, 202 (2006).
8) J. Kin, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi, and K. Kim, *J. Am. Chem. Soc.* **122**, 540 (2000).
9) A. I. Day, A. P. Arnold, R. J. Blanch, and B. Snushall, *J. Org. Chem.* **66**, 8094 (2001).
10) W. S. Jeon, K. Moon, S. H. Park, H. Chun, Y. H. Ko, J. Y. Lee, E. S. Lee, S. Samal, N. Selvapalam, M. V. Rekharsky, V. Sindelar, D. Sobransingh, Y. Inoue, A. E. Kaifer, and K. Kim, *J. Am. Chem. Soc*. **127**, 12984 (2005).
11) M. V. Rekharsky, M. P. Mayhew, R. N. Goldberg, P. D. Ross, Y. Yamashoji, and Y. Inoue, *J. Phys. Chem. A* **101**, 87 (1997).
12) Special Issue on Cyclodextrins. *Chem. Rev.* **98**, 1741-2076 (1998)
13) M. V. Rekharsky and Y. Inoue, in *Cyclodextrins and their Complexes*, H. Dodziuk, Ed. (Willey-VCH, Weinheim) pp.199-230 (2006).
14) D. Whang, J. Heo, J. H. Park, and K. Kim, *Angew. Chem. Int. Ed.* **37**, 78 (1998).
15) J. W. Lee, S, Samal, N. Selvapalam, H.-J. Kim, and K. Kim, *Acc. Chem. Res.* **36**, 621 (2003).
16) H.-J. Buschmann, L. Mutihac, and E. Schollmeyer, *J. Incl. Phenom. Macrocycl. Chem.* **53**, 85 (2005).
17) S. Liu, C. Ruspic, P. Mukhopadhyay, S. Chakrabatri, P. Y. Zavalij, and L, Isaacs, *J. Am. Chem. Soc.* **127**, 15959 (2005).
18) R. Hoffmann, W. Knoche, C. Fenn, and H.-J. Buschmann, *J. Chem. Soc., Faraday Trans.* **90**, 1507 (1994).
19) H.-J. Buschmann, K. Jansen, C. Meschke, and E. Schollmeyer, *J. Solut. Chem.* **27**, 135 (1998).
20) H.-J.; Buschmann, E. Cleve, K. Jansen, A. Wego, and E. Schollmeyer, *J. Inclus. Phenom. Macrocycl. Chem.* **40**, 117 (2001).
21) I. Osaka, M. Kondou, N. Selvapalam, S. Samal, K. Kim, M. V. Rekharsky, Y. Inoue, and R. Arakawa, *J. Mass Spectr.* **41**, 202 (2006).
22) M.V. Rekharsky, Y.H. Ko, N. Selvapalam, K. Kim, and Y. Inoue, *Supramol. Chem.* **19**, 39 (2007).
23) M. V. Rekharsky and Y. Inoue, *Chem. Rev.* **98**, 1875 (1998).
24) M. V. Rekharsky and Y. Inoue, *J. Am. Chem. Soc.* **124**, 12361 (2002).
25) M. E. Bush, N. D. Bouley, and A. R. Urbach, *J. Am. Chem. Soc.* **127**, 14511 (2005).
26) L. M. Heitmann, A. B. Taylor, P. J. Hart, and A. R. Urbach, *J. Am. Chem. Soc.* **128**, 12574 (2006).

Mikhail V. Rekharsky
Entropy Control Project, ICORP, JST and Department of Applied Chemistry, Osaka Univ., TEL. 06-6879-7920, FAX. 06-6879-7920, e-mail: mvr@chem.eng.osaka-u.ac.jp
Research area: Thermodynamics of host-guest and supramolecular reactions
Hobby: Fishing

Yoshihisa Inoue
Entropy Control Project, ICORP, JST and Department of Applied Chemistry, Osaka Univ., TEL. 06-6879-7920, FAX. 06-6879-7920, e-mail: inoue@chem.eng.osaka-u.ac.jp
Research area: Supramolecular chemistry, Photochemistry
Hobby: Trekking