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Complexation mechanism of cucurbit[6]uril with hexamethylene diammonium cations in saline solution[†]

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Binding of cucurbit[6]uril (CB[6]) with hexamethylene diammonium cation (HD²⁺) in the presence of sodium ions is elucidated at the atomic level. The most probable complex of CB[6] in saline solution is found to be CB[6]:Na⁺. A two-stage binding process of CB[6]:Na⁺ with HD²⁺ is proposed.

Cucurbit[*n*]uril (CB[*n*])^{1,2} form a family of macrocyclic compounds containing *n* glycoluril units. The increasing interest in this area can be attributed to the potential of CB[*n*]s to act as hosts for many small molecules, especially alkyl-ammonium-based compounds, with extremely high association constants. High affinity towards the guests is critical in many applications of CB[*n*]s as part of sophisticated nanodevices, notably as valves of drug carriers^{3,4}, as drug stabilizers⁵ or in gene-delivery supramolecular assemblies.^{6,7} The high affinity in these complexes triggered much research interest to characterize its origins.

As a member of CB[*n*]s, CB[6] possesses structural features shared by all CB[*n*]s. A hydrophobic cavity, approximately 9-Å deep, is surrounded by two portals formed by a rim of carbonyl groups. This hydrophobic cavity accommodates the nonpolar moieties of the guests. Carbonyl-oxygen atoms attract cationic groups through long-range electrostatic interactions.⁸ However, these hydrophilic functional groups are insufficient to make CB[6] soluble in water. Addition of alkali metal salts^{9,10} is required to enhance the solubility of CB[6] in an aqueous environment, which is a prerequisite to bind guest molecules. How the CB[6]-guest complexes form, especially in the presence of alkali metal ions, therefore, constitutes an open question.

In the past twenty years, much effort has been devoted in this field. CB[6]:alkali metal ion complexes have been investigated experimentally^{11,12} and theoretically¹³ in detail. Binding of CB[6] with ammonium derivatives has been studied systematically.¹⁴ Meanwhile, a variety of mechanisms have been proposed to interpret the association of CB[6] with monoammonium^{15,16} and diammonium

cations.¹⁷ Further evidences show that alkali metal salts have a remarkable effect on the stability of supramolecular complexes formed by CB[6] and guest molecules. Nau et al.¹⁵ and Kim and Bohne et al.¹⁸ have reported, respectively, the detailed binding mechanisms of CB[6] and CB[7] with monoammonium guests in saline solution. To the best of our knowledge, however, investigation of the binding process of CB[*n*] with diammonium guests in the presence of alkali metal ions at the atomic level has hitherto never been undertaken.

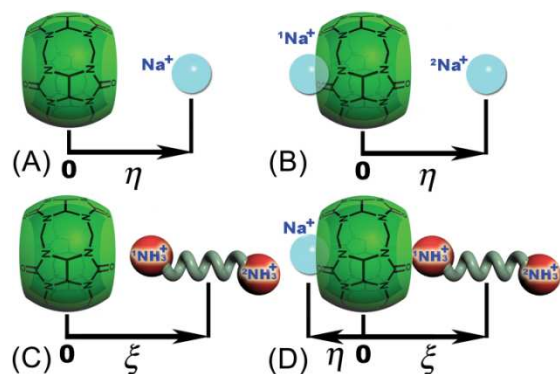
In the present work, the binding process of CB[6] with the hexamethylene diammonium cation (HD²⁺) in the presence of sodium ions was investigated by unconstrained molecular dynamics (MD) simulations combined with free-energy calculations. The potentials of mean force (PMFs) characterizing successive binding processes of CB[6] with two Na⁺ and HD²⁺ were determined. Combining with the analysis of the structural features obtained from additional molecular dynamics trajectories, the stable complexes CB[6]:(Na⁺)_{*n*}, where *n* = 1 or 2, are revealed. Based on the PMFs reported herein, the standard binding free energy of CB[6] with HD²⁺ in saline solution was determined with an accuracy comparable to that of experiment. Moreover, the reaction pathway for the binding of CB[6]:(Na⁺)_{*n*} complexes with HD²⁺ was investigated by mapping the underlying free-energy landscape in two dimensions. Characterizing the thermodynamics of the binding process constitutes the primary thrust of the present study, from whence novel insight will be gained into the recognition and association phenomena of CBs with guests in an aqueous solution.

The MD simulations described here rely upon the CHARMM general force field.^{19,20} Four supramolecular assemblies, namely CB[6] with Na⁺, CB[6]:Na⁺ with Na⁺, CB[6] with HD²⁺, and CB[6]:Na⁺ with HD²⁺, have been constructed. These assemblies were subsequently immersed in a periodic box of TIP3P water.²¹ All MD simulations were carried out with the NAMD 2.9 program.²²

The adaptive biasing force algorithm²³ implemented within NAMD^{24,25} combined with unconstrained MD simulations was

employed to investigate the binding processes. The transition coordinate, either one- or two-dimensional, was formed by collective variables²⁶ η and ζ , chosen, respectively, as the projection along the z direction of the vector connecting the center of mass of CB[6] to Na^+ , and to HD^{2+} (see Scheme 1). The variation of the free energy, ΔG , was determined by integrating the average force acting on η and/or ζ . Instantaneous values of the force were accrued in bins 0.1-Å wide for each collective variable. The barycenter of the CB[6] carbon atoms was maintained arbitrarily at the origin of the coordinate system with the longitudinal axis of the central cavity aligned with the z -axis. To achieve this goal, the backbone of CB[6] was restrained by means of weak harmonic potentials having a force constant of 2.0 kcal/mol/Å², and shown to have no influence on the final PMFs. Block-average regression was utilized to estimate the standard error associated with the free-energy differences.^{23,27} Detailed information about the molecular assemblies can be found in Table S1 of the Supporting Information.

The PMFs characterizing the successive binding of CB[6] with two Na^+ are depicted in Figure 1. As can be seen in panel A thereof, the profile for the binding of CB[6] with the first Na^+ possesses a deep minimum. A closer look at this figure reveals that the free energy decreases as the alkali cation approaches CB[6], leading to the global minimum found around 2.9 Å, with a free energy of about -6.6 kcal/mol. The structure of this stationary point is shown in Figure 1B and C. In this CB[6]: Na^+ complex, Na^+ is coordinated by three carbonyl oxygen atoms located in one portal of CB[6] (see Figure 1B), and the oxygen atom of three water molecules (see Figure 1C). The total coordination number for Na^+ is six, in agreement with experimental observations²⁸ and numerical simulations.²⁹



Scheme 1. Definition of the collective variables, η and ζ , in the binding processes of (A) CB[6] with Na^+ , (B) CB[6]: Na^+ with Na^+ . The bound Na^+ within the CB[6]: Na^+ complex and the incoming Na^+ are denoted $^1\text{Na}^+$ and $^2\text{Na}^+$, respectively. (C) CB[6] with HD^{2+} , and (D) CB[6]: Na^+ with HD^{2+} .

Figure 1D shows the free-energy profile delineating the binding of CB[6]: Na^+ with the second alkali cation. The free-energy landscape consists of two shallow minima emerging at 4.2 and 3.0 Å, corresponding to free energies of -1.2 and -1.3 kcal/mol. The structure of the stationary point around 4.2 Å is shown in Figure 1E. In this structure, the hydration shell of each sodium ion contains six oxygen atoms. CB[6] and water molecules contribute three oxygen atoms to the hydration shell of $^1\text{Na}^+$. Meanwhile, two and four oxygen atoms in the shell of $^2\text{Na}^+$ originate from CB[6] and water

molecules, respectively. Further observation reveals that the clash of water molecules lying in the cavity of CB[6] and coordinating the two Na^+ rules out symmetrical coordination of the cations. The structure of the complex near 3.0 Å is depicted in Figure 1F. Compared with the structure near 4.2 Å, the composition of the oxygen atoms pertaining to the hydration shell of $^1\text{Na}^+$ and $^2\text{Na}^+$ is permuted owing to the structural symmetry of CB[6]. These structural observations tend to indicate that binding of CB[6]: Na^+ with a second sodium ion is thermodynamically only slightly favoured. In other words, in the formation of a CB[6]:(Na^+)₂ complex, binding of CB[6] with the first sodium ion is strong, whereas binding with the second sodium ion is relatively weak, which is in agreement with experimental measurements.^{15,18}

To gain further insight into the stability of these complexes, additional MD simulations were performed over 10 ns, using the configurations corresponding to the free-energy minima as a starting point. The results reveal that the CB[6]:(Na^+)₂ complex is fragile. After three nanoseconds, it dissociates into CB[6]: Na^+ and Na^+ in solution. Conversely, CB[6]: Na^+ remains intact over the entire length of the simulation. It can, therefore, be concluded that CB[6]: Na^+ is the most probable complex of CB[6] formed in saline solution. The relevant geometrical analysis of the complexes can be found in the Supporting Information.

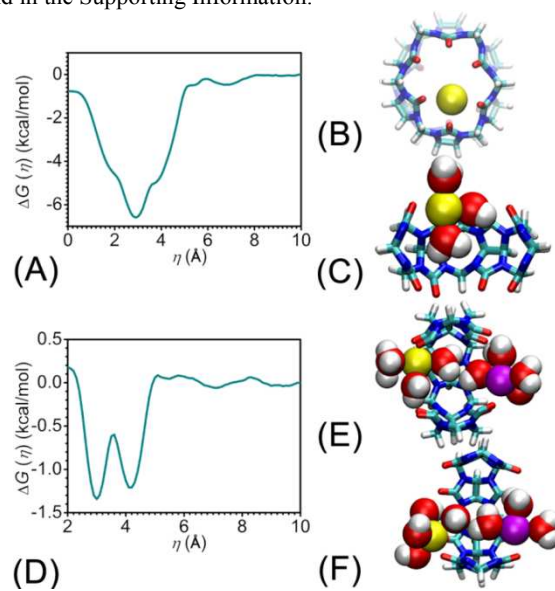


Figure 1. (A) Free-energy profile for the binding of CB[6] with Na^+ . (B) Top view of CB[6]: Na^+ . (C) Side view of the complex in its stable state, i.e., $\eta = 2.9$ Å, coordinated by three water molecules. (D) Free-energy profile for the binding of CB[6]: Na^+ with Na^+ . Snapshot of the CB[6]:(Na^+)₂ complex (E) in one stable state, i.e., near $\eta = 4.2$ Å and (F) in another stable state, i.e., near $\eta = 3.0$ Å. CB[6] and water molecules coordinating the sodium ions are shown as opaque sticks and van der Waals spheres, respectively. $^1\text{Na}^+$ and $^2\text{Na}^+$ are shown as yellow and purple spheres, respectively. The image rendering was done with VMD.³⁰

Based on the above analysis, binding of CB[6] with HD^{2+} in saline solution should in principle be that of CB[6]: Na^+ with HD^{2+} . To understand this process, two-dimensional PMFs along ζ and η were determined (see Figure 2). Binding of HD^{2+} to CB[6]: Na^+ can be decomposed in two distinct stages. In the first stage (see Figure

2A), the free-energy landscape characterizing the formation of the metastable states consists of a global minimum, emerging below the crossroad formed by two perpendicular channels. The horizontal channel found in the region spanning ca. $-3.5 \leq \eta \leq -2.5$ Å corresponds to the approach of HD^{2+} towards $\text{CB}[6]:\text{Na}^+$, indicating that the latter complex (I) is intact during this recognition process. The vertical channel found in the $5.0 \leq \zeta \leq 8.0$ Å range, characterizes a metastable region. In this region, the $\text{CB}[6]:\text{Na}^+:\text{HD}^{2+}$ complex (II) is the most stable state. As shown in Figure 2, Na^+ is coordinated by two oxygen atoms of one rim of $\text{CB}[6]$ and four oxygen atoms of water. Concomitantly, one ammonium moiety binds to the other rim of $\text{CB}[6]$ and one water molecule inside the cavity. In light of the above observation, $\text{CB}[6]:\text{Na}^+:\text{HD}^{2+}$ appears to be the most stable structure in the first stage of binding, underlined by I–II as a possible pathway.

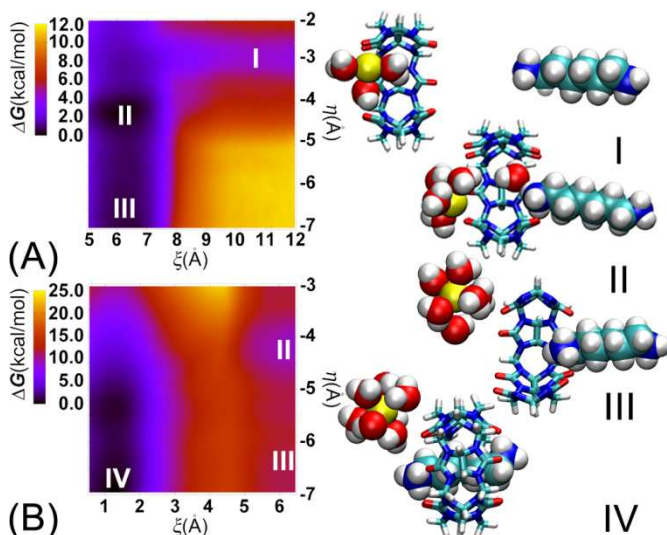


Figure 2. Free-energy landscapes characterizing (A) the first, and (B) the second stage of the binding of $\text{CB}[6]:\text{Na}^+$ complex with HD^{2+} . Configurations I–IV charted on the map correspond to four milestones in the course of the binding process. The corresponding structures are depicted on the right hand side.

In the second stage (see Figure 2B), the free-energy surface consists of two parallel channels separated by a ridge. The right channel spanning $5.0 \leq \zeta \leq 6.5$ Å is identical to the vertical channel in the first stage, as depicted in Figure 2A. The left channel, in the region of $0.5 \leq \zeta \leq 3.0$ Å, features the $\text{CB}[6]:\text{HD}^{2+}$ complex (IV) with a departing sodium ion. In configuration IV, the alkyl moiety of HD^{2+} is fully included in the cavity of $\text{CB}[6]$. The ammonium moieties of HD^{2+} lie at the carbonylated rims of $\text{CB}[6]$. This structural feature rationalizes the significant decrease of the free energy caused by the departing Na^+ . Beyond $\eta \leq -5.0$ Å, the decreasing trend of the free energy fades out. A flat landscape is then found in the region of $-7.0 \leq \eta \leq -6.0$ Å. The absence of stationary points along the ridge indicates that transition from the metastable states to the most stable state does not involve a specific pathway. The actual reaction pathway may consist of a linear combination of two independent pathways (II–III, i.e., dissociation of Na^+ from $\text{CB}[6]:\text{Na}^+:\text{HD}^{2+}$, and III–IV, threading of HD^{2+} into the cavity of $\text{CB}[6]$). The individual contribution of each pathway is, however,

difficult to ascertain and would require additional analysis,²³ which falls outside the scope of the present investigation.

Put together, binding of $\text{CB}[6]$ with HD^{2+} in saline solution can be viewed as a combination of the association of $\text{CB}[6]$ with HD^{2+} in a bulk aqueous medium and dissociation of $\text{CB}[6]:\text{Na}^+$. This hypothesis can be validated by comparison of the standard binding free energies determined at the experimental and theoretical levels. This comparison, however, requires as a preamble mapping of the free-energy landscape that underlies binding of $\text{CB}[6]$ with HD^{2+} .

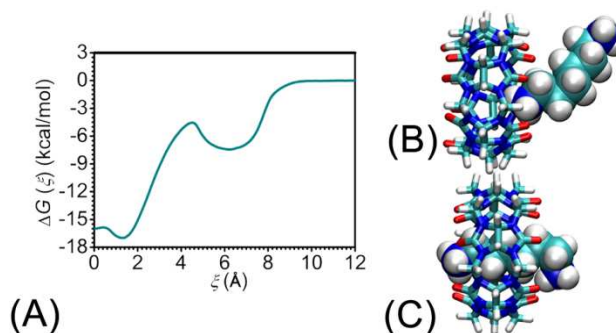


Figure 3. (A) Free-energy profile for the binding of $\text{CB}[6]$ with HD^{2+} . Representative configuration of $\text{CB}[6]:\text{HD}^{2+}$ (B) in a metastable state, near $\zeta = 6.2$ Å and (C) in its most stable state, near $\zeta = 1.3$ Å.

Figure 3 shows the free-energy profile for the binding of $\text{CB}[6]$ with HD^{2+} . In this PMF, the two minima are separated by an appreciable barrier, indicative that $\text{CB}[6]$ binds HD^{2+} at two locations, namely around 6.2 Å with a free energy of -7.4 kcal/mol and around 1.3 Å with a free energy of -17.0 kcal/mol. At the former location, the complex is in a metastable state, one ammonium moiety of HD^{2+} lying at the carbonylated rim of $\text{CB}[6]$ (see Figure 3B) – the alkyl group and another ammonium moiety being exposed to the aqueous phase. Conversely, at the latter location, the complex is in its most stable state, wherein the alkyl group of HD^{2+} is included in the cavity, while the two ammonium moieties lie at the rims of $\text{CB}[6]$ (see Figure 3C).

From the PMFs of Figure 1A and Figure 3A, the binding free energy of $\text{CB}[6]$ with HD^{2+} in saline solution may be recovered through,

$$\Delta G_{\text{bind}}^{\circ} = -\frac{1}{\beta} \ln \frac{\int d\xi \xi \exp[-\beta \Delta G_{\text{CB}[6]-\text{HD}^{2+}}(\xi)]}{\int d\xi \xi \exp[-\beta \Delta G_{\text{CB}[6]-\text{Na}^+}(\xi)]}$$

where, $\beta = (k_B T)^{-1}$, where k_B is the Boltzmann constant and T is the temperature. From this equation, the standard binding free energy of $\text{CB}[6]$ with HD^{2+} in saline solution was estimated to be equal to -11.7 ± 0.2 kcal/mol, in remarkable agreement with the experimental value of -11.69 kcal/mol.¹² Following the above discussion, it can be inferred that binding of $\text{CB}[6]$ with HD^{2+} in saline solution corresponds in fact to the binding of $\text{CB}[6]:\text{Na}^+$ with HD^{2+} .

Conclusions

In summary, the free-energy profiles delineating the successive binding of $\text{CB}[6]$ with two sodium ions have been determined. With the aid of additional MD simulations, the

CB[6]:Na⁺ complex was found as the most probable species of CB[6] in saline solution. Based on these findings and the free-energy profile characterizing the binding of CB[6] with HD²⁺, the standard binding free energy of CB[6] with HD²⁺ in saline solution has been estimated, and was found to agree well with experiment. This agreement validates by ricochet the conjecture of CB[6]:Na⁺ being the most probable complex. Furthermore, binding of CB[6]:Na⁺ with HD²⁺ was examined by mapping the underlying free-energy landscape, which unveiled possible reaction pathways. Put together, explicit consideration of sodium ions in the study of the association of CB[6] with guest molecules is a sine qua non condition to elucidate at atomic resolution the binding mechanisms, while rationalizing experimental binding constants. In this sense, the present work takes a further step towards understanding the ultrahigh binding affinity of CB[*n*] with guest molecules.

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† Electronic Supplementary Information (ESI) available: [Simulation details. Variations of the distance between the barycenter of CB[6] and Na⁺ obtained from additional 10-ns MD simulations for the CB[6]:Na⁺ and CB[6]:(Na⁺)₂ complexes. Time evolution of the RMSD over the gradients of free-energy surfaces characterizing the binding of CB[6]:Na⁺ complex with HD²⁺. The complete ref. 19.] See DOI: 10.1039/c000000x/

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