

Molecular dynamics simulations of inclusion complexation of glycyrrhizic acid and cyclodextrins (1:1) in water

Masayuki Oda¹ · Masataka Kuroda^{1,2}

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Abstract By means of molecular dynamics (MD) simulations, we analyzed the formation of inclusion complex consisting of cyclodextrins and the triterpene glycoside, glycyrrhizic acid, to obtain information about the transient binding pathway and the stable complex structures in equilibrium. For each of the two possible orientations of a glycyrrhizic acid molecule, β - and γ -cyclodextrins were initially positioned on 20 different sites of the molecule at intervals of 1 Å, and the MD run was performed for 0.8 nsec for the sampling conformations. The position-dependent energy contributions derived from van der Waals interactions and electrostatic interactions showed that there exist two distribution gaps responsible for the formation of β -cyclodextrin complexes, indicating that glycyrrhizic acid could not pass through the hydrophobic pocket of β -cyclodextrin, as opposed to γ -cyclodextrin. In the most stable complex structures for both β - and γ -cyclodextrins, the glucuronic acid of glycyrrhizic acid binds to the hydrophobic pocket of cyclodextrins. This is also consistent with the analysis of hydrogen bonding. These energy contributions are larger for the binding to γ -cyclodextrin than to β -cyclodextrin, which correlates well with the results of isothermal titration calorimetry experiments. We also analyzed configurational entropies based on the trajectory of the MD runs, which showed that there

would be little difference in configurational entropy on the binding entropy change between β - and γ - cyclodextrins.

Keywords Binding energy · Complex structure · Cyclodextrin · Glycyrrhizic acid · Molecular dynamics simulations

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of α -(1 \rightarrow 4)-linked α -D-glucosyl unit. The most common and best studied CDs are α -, β - and γ -CDs, consisting of 6-, 7-, and 8-glucosyl units, respectively [1]. The CD structures resemble toroidal, hollow, truncated cones with a hydrophilic surface and a hydrophobic pocket. Because of the unique features, they can form an inclusion complex with an organic compound, in a process known as a host–guest interaction of CDs [2, 3]. In the complex, the guest molecule with low water solubility is solubilized and stabilized, making it possible to use CDs in pharmacology and drug delivery systems [4, 5]. Upon the binding of a guest molecule in the hydrophobic pocket of CDs, the water molecules inside the cavity are released into the solvent. The host–guest interaction is mainly governed by van der Waals interactions and hydrogen bonding [6]. The hydrophobic pocket diameters of α -, β - and γ -CDs are 4.7–5.3, 6.0–6.5, and 7.5–8.3 Å, respectively [1]. The respective CDs can be used for the specific binding to compounds with different sizes and shapes [2, 7].

Glycyrrhizic acid (GlyA) is the major triterpene glycoside contained in licorice root (Fig. 1), and has a sweetish taste, with an approximately 170-fold higher degree of sweetness than sucrose [8]. GlyA also exhibits anti-inflammatory and anti-cancer activities [9, 10] Zhao

✉ Masayuki Oda
oda@kpu.ac.jp

¹ Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, 1-5 Hangi-cho, Shimogamo, Sakyo-ku, Kyoto, Kyoto 606-8522, Japan

² Discovery Technology Laboratories, Sohyaku Innovative Research Division, Mitsubishi Tanabe Pharma Corporation, 1000 Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa 227-0033, Japan

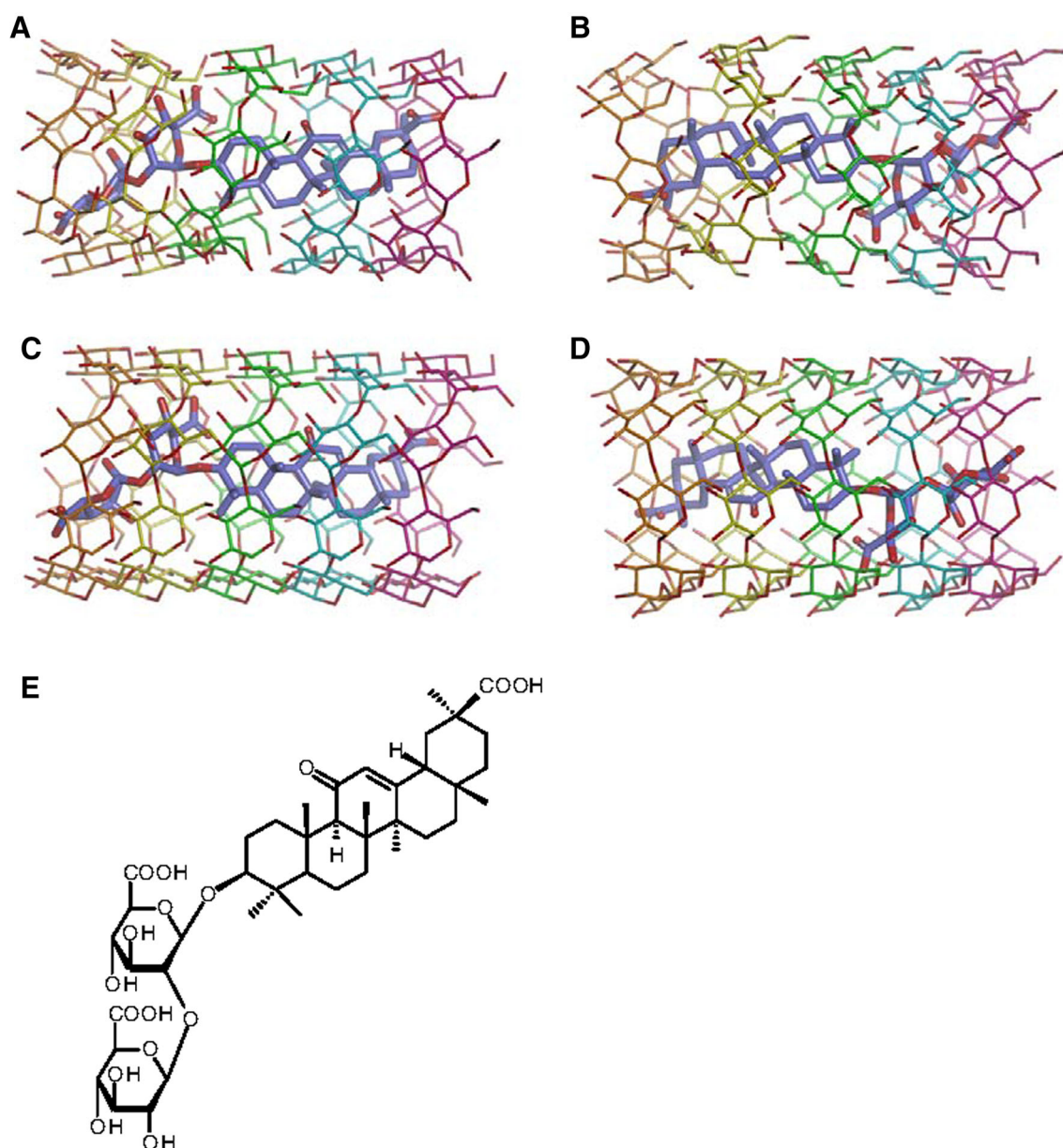


Fig. 1 The initial configurations of GlyA and CDs in “left-glu” of β -CD (a), “right-glu” of β -CD (b), “left-glu” of γ -CD (c), and “right-glu” of γ -CD (d). Among 20 initial configurations, 1st, 5th, 10th,

15th, and 20th configurations of CDs are drawn with superimposed GlyA. The chemical structure of GlyA is indicated as “left-glu” (e)

et al. reported that GlyA/ β -CD functionalised quantum dots could induce apoptosis in hepatocarcinoma cells [11]. We have analyzed the structural and physical properties of GlyA, and also analyzed its interactions with CDs, showing that the binding affinity of GlyA to γ -CD is about 300-times higher than that to β -CD [12]. Possibly due to the hydrogen bonding and van der Waals interactions associated with dehydration, the interactions of GlyA with β - and γ -CDs are accompanied with negative enthalpy changes and positive entropy changes [12]. The binding thermodynamics could be compared with the present

results of MD simulations. The isothermal titration calorimetry (ITC) experiments also showed that the binding stoichiometry of GlyA with β -CD and γ -CD was 1:1 [12].

In this study, the inclusion complexation of GlyA with β -CD and γ -CD was analyzed using molecular dynamics (MD) simulations. The first purpose of this study is to analyze the stable structures of GlyA and CD complexes, in consideration with the binding pathway. Because it is difficult to take the long-time MD run to simulate GlyA from entering into CD to going out at another side of the CD, the

inclusion complexation was analyzed from the complex structures at different stages starting from the beginning when the CDs were positioned on 20 different sites of the GlyA at intervals of 1 Å. The second purpose is to analyze the respective energy contributions of van der Waals interactions, electrostatic interactions, and hydrogen bonding in the inclusion complexes. The third purpose is to analyze the conformational entropy of the inclusion complexes. The results can be compared with the recently reported results of binding thermodynamics experiments [12]. The dynamic properties and energy contributions will help us to understand how GlyA binds to CDs, as the case study of host–guest interactions.

Materials and methods

Models

The structures of β - and γ -CDs were taken from PDB, 1Z0 N and 1D3C, respectively. The conformation of GlyA was built in DiscoveryStudio (Dassault Systemes Biovia K.K.) and the topology was roughly optimized. The two carboxyl groups of GlyA were ionized. The GlyA was automatically positioned along the center axis of the CD pocket from the opening end to the other end, with the CD molecules separated from one another by 1 Å interval. For β -CD, the complex models were manually adjusted to avoid atomic collision between GlyA and β -CD, owing a shorter inner diameter than that of γ -CD.

Twenty initial conformation models were generated for each form. Another binding mode should be considered due to the asymmetric natures of both types of molecules in the complex. Water molecules were added around the complex within the radius of 18 Å to generate a sphere system. The numbers of water molecules ranged from 674 to 688 for the β -CD complex and from 657 to 675 for γ -CD. Three sodium ion atoms were added to make the total charge of the system neutral.

MD

GAFF was applied for the force field, and TIP3P was used for the water molecule model [13]. The atomic charge of each molecule was calculated by the AM1-BCC method [14, 15]. The CAP restraint was used to keep the water molecules in the system and that for the solutes was especially set to be 1 Å shorter, to keep them from staying around the surface. The step was 1 fsec. The initial structures were minimized energetically.

The MD run started at 10 K and the temperature was gradually heated to 300 K finally in 10,000 steps, and equilibrated in 200,000 steps. The run was performed for

800,000 steps for the sampling conformations, which added up to 0.8 nsec. The coordinates of the system were sampled every 1000 steps. These runs were performed with the molecular simulation suite, PRESTO ver.3 [16].

Entropy calculation

The configuration entropy for each positional group was calculated based on the trajectory of the MD runs. The entropy was calculated using the method proposed by Schlitter [17], as follows,

$$S < S' = \frac{1}{2} k \ln \det \left[\mathbf{1} + \frac{kT e^2}{\hbar^2} \mathbf{M} \boldsymbol{\sigma} \right]$$

where \mathbf{M} is the mass matrix in which the diagonal elements are the mass of the atoms in the solute and others are zero, and $\boldsymbol{\sigma}$ is the covariance matrix. Each element is calculated by the equation,

$$\sigma_{ij} = \langle (x_i - \langle x_i \rangle)(x_j - \langle x_j \rangle) \rangle$$

Results and discussion

The inclusion complex of GlyA and β -CD or γ -CD can take two different forms. With the wider rim of CDs, i.e., the side of C3 of glucose, fixed to the left side, we designate the form in which the glucuronic acid of the GlyA is positioned to the left hand side as “left-glu”, and other form as “right-glu”. In the initial configurations, CDs were positioned on 20 different sites on GlyA at intervals of 1 Å. As shown in Fig. 1, the 1st initial configuration of β -CD “left-glu” corresponds to the relative position in which the left side of glucuronic acid sticks into the hydrophobic pocket of β -CD, and the 20th initial configuration of β -CD “left-glu” corresponds to the relative position in which the right side of aglycone sticks into the hydrophobic pocket of β -CD. The 20 initial structures generated for each of the system, namely “left-glu” of β -CD, “right-glu” of β -CD, “left-glu” of γ -CD, and “right-glu” of γ -CD, covered all the binding sites of GlyA and CDs.

Each MD run produced 800 configurations. Consequently, 1600 were sampled for each form. The configurations in each run were not distributed widely, and those in several runs were existed in the area around the initial structures. Considering the structural repetition of the glucose unit and the inner diameter of CD, initial structure would not affect the trajectory largely. To test the impact of initial structure against the trajectory, four initial structures were built manually from the 3rd initial configuration for ‘left-glu’ γ -CD, in which GlyA was rotated and tilted slightly around the center axis of the CD. Root-mean-

square deviation (RMSD) values between the four initial structures and that of the simulation 3 range from 1.0 to 1.5 Å, coming from the displacement of GlyA largely. Comparing each of the four trajectories with all from the 20 simulations, RMSD values of the closest structures are 1.5 Å or less. More or less, trajectories would not largely depend on the initial structures. For the convenience of analysis, a pseudo axis was introduced to present the position of GlyA relative to CDs. The axis vector of CD was defined using coordinates of the atoms of the six-membered rings present in the entire glucose molecule. The origin of the axis was set at the center of these atoms. The axis vector was defined as the average of the perpendicular unit vectors calculated using two vectors from the origin to the centers of the adjacent glucose rings. The absolute position of GlyA on the axis was the vertex of the perpendicular line drawn from a specific atom around the center of GlyA to the axis.

Eighty structures of inclusion complexes from the sampled trajectory of each run were selected, and their binding energies were plotted against the relative positions of GlyA and CDs (Fig. 2). The position-dependent energy contributions based on van der Waals interactions were similar to those from electrostatic interactions. We also analyzed the contribution from hydrogen bonding, which is included in electrostatic interactions. A hydrogen bond was defined as the distance of 2 Å or less between the hydrogen atom bound to a donor atom and an acceptor atom. The donor atoms are oxygen atoms of the hydroxyl group, and the acceptor atoms are the total number of oxygen atoms including that in the hydroxyl group. Figure 3 shows the number of hydrogen bonds in the simulated complex structures. The number seems to be correlated with the energy distribution in Fig. 2. There are maximum numbers of hydrogen bonds in the most stable structures of both β - and γ -CDs, in which the glucuronic acid interacts with the hydrophobic pocket of CDs. It should be also noted that the number of hydrogen bonds in the γ -CD stable complex is higher than that in the β -CD complex.

In the results of binding energies for β -CD complexes (Fig. 2), there exist two distribution gaps. In either the “left-glu” or the “right-glu” configuration, the distributions at the positions around -2 including the complex with lowest energy were isolated from those at both sides. It is likely that the putative initial structures at the gap regions are unstable, possibly due to the steric hindrance, resulting in alterations to form stable structures. Under such circumstances, the complex structures involved in the isolated distributions would not be considered, because GlyA can start interacting with β -CD from each side of the molecules. Therefore, in the case of inclusion complexation of β -CD, the complexes at the position around -8 for “left-glu” and at the position around 8 for “right-glu”

would be the most stable. In either case, the glucuronic acid of GlyA binds to the hydrophobic pocket of β -CD (Fig. 4a, b). The glucuronic acid can penetrate β -CD from the narrower rim, the C6 side of glucose, in the case of “left-glu”, and from the wider rim in the case of “right-glu”. Based on these energy level findings, the latter complexation would be more stable.

In the inclusion complexation of γ -CD, the results of binding energies shown in Fig. 2 indicate that the structures in which the glucuronic acid interacts with the hydrophobic pocket of γ -CD are most stable in both “left-glu” and “right-glu” configurations (Fig. 4c, d). For all the structures in which the glucuronic acid interacts with the hydrophobic pocket of CDs (Fig. 3), the hydrogen bond network could stabilize the complex configuration. Approximately 10 hydrogen bonds are found in the γ -CDs complexation (Fig. 3). The carboxyl groups of the glucuronic parts form hydrogen bonds to the hydrogen atoms at the CD rims opposite side of the aglycone parts (Fig. 4), possibly making the glucoses of GlyA stay inside of the CD stably. In the stable structures, van der Waals contacts between the atoms of glucuronic acid and CDs and electrostatic interaction including hydrogen-bonding would also contribute to the large binding energies. Additionally, the second stable state with local energy minimum was detected in the analysis of energy distribution, at the positions around 4 in “left-glu” and around -4 in “right-glu”, which corresponds to the structures where the aglycone part of GlyA interacts with the hydrophobic pocket of γ -CD. The binding and unbinding way of GlyA in the “right-glu” configuration could be just one, that is, GlyA dissociates from the same side to which it binds (Fig. 2l), similar to the case of β -CD (Fig. 2i, j). In contrast, in the case of “left-glu”, GlyA could go through the CD from the wide rim to the narrow and vice versa, and stay at other positions with local energy minima, such as 4 , 1 , and -1 , in short time, before reaching the most stable position around 6 (Fig. 2k). These binding mechanisms might result in the higher affinity toward γ -CD relative to β -CD. Although the complete equilibrium simulation was not performed, one possible pathway could be speculated from the complex structures with local energy minima acquired by the separate simulations.

We next analyzed the configurational entropies of GlyA and CDs, and their alternation upon the inclusion complexation. Figure 5 shows the entropies in bound state and the entropy changes between bound and unbound states of CDs plotted against the positions of simulated configurations. The configurational entropy of γ -CD in unbound state was calculated to be 6.11 kcal/mol K, which was higher than that of β -CD, 5.00 kcal/mol K. These entropy values were subtracted from those in the bound states, calculated from the typical complexed

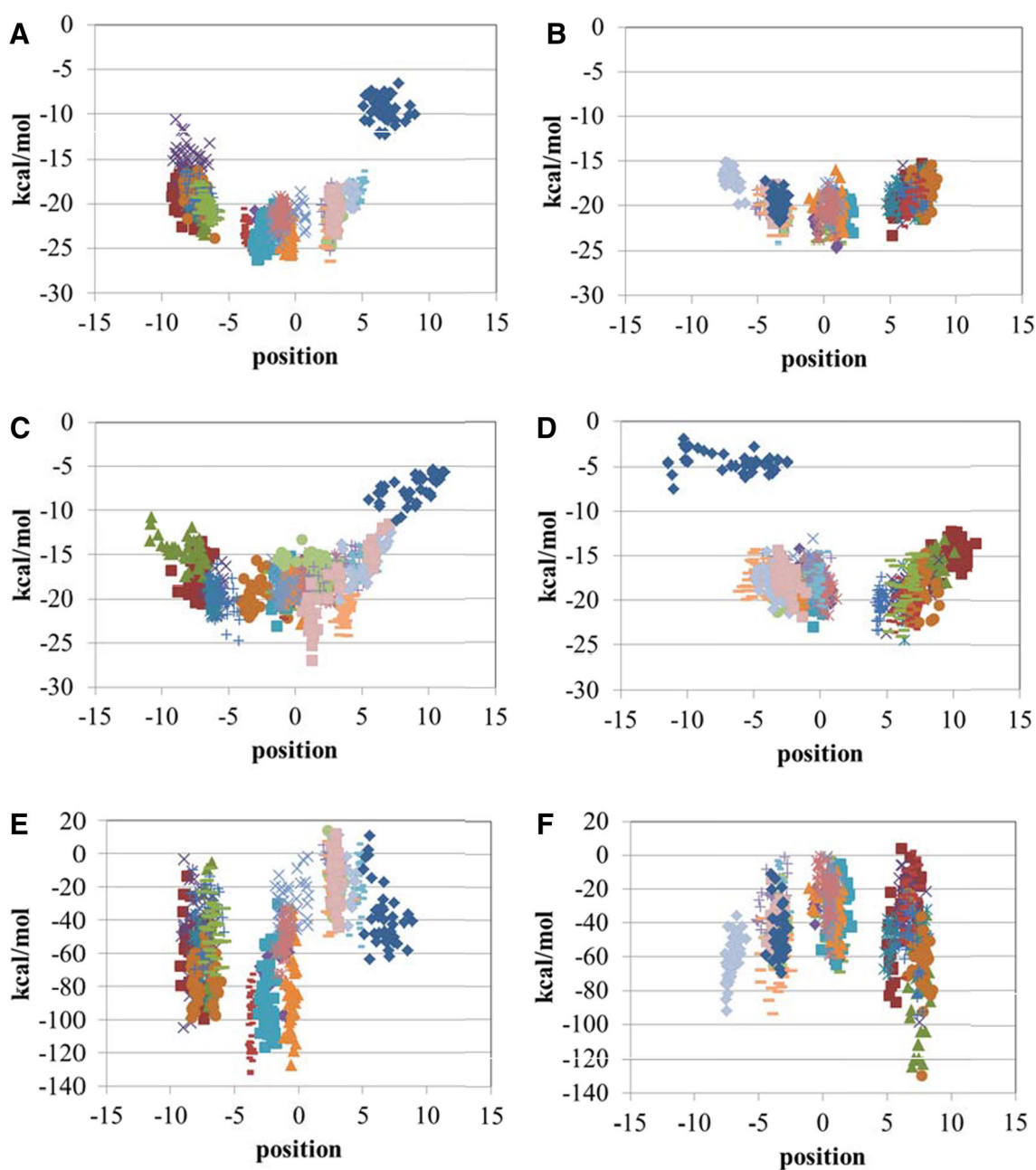


Fig. 2 Binding energy distribution of simulated configurations. Energies derived from van der Waals interactions (a–d), electrostatic interactions (e–h), and both (i–l) are plotted against the positions of simulated configurations. In the configuration “left-glu” of β -CD (a, e, i) and γ -CD (c, g, k), the position -10 corresponds to the 1st initial configuration in which the right side of glucuronic acid sticks into the hydrophobic pocket of CD, and the position 10 corresponds to the

20th initial configuration. In the configuration “right-glu” of β -CD (b, f, j) and γ -CD (d, h, l), the position -10 corresponds to the 1st initial configuration in which the left side of glucuronic acid sticks into the hydrophobic pocket of CD, and the position 10 corresponds to the 20th initial configuration. The respective *symbols* and *colors* indicate the energy distribution started from the respective initial structures. (Color figure online)

structures in the respective positions, to compare the change upon inclusion complexation between β - and γ -CDs, in consideration that the entropy of GlyA in unbound state is same for both CD complexations. As a result, the entropy change of γ -CD was similar to that of β -CD (Fig. 5c, d), indicating that there is little difference

in the configurational entropy of β - and γ -CDs in the binding entropy change.

In previous experiments on the binding of GlyA to CDs using ITC, the binding thermodynamics were determined as shown in Table 1 [12]. The present MD results indicate that the energy contributions of van der Waals interactions,

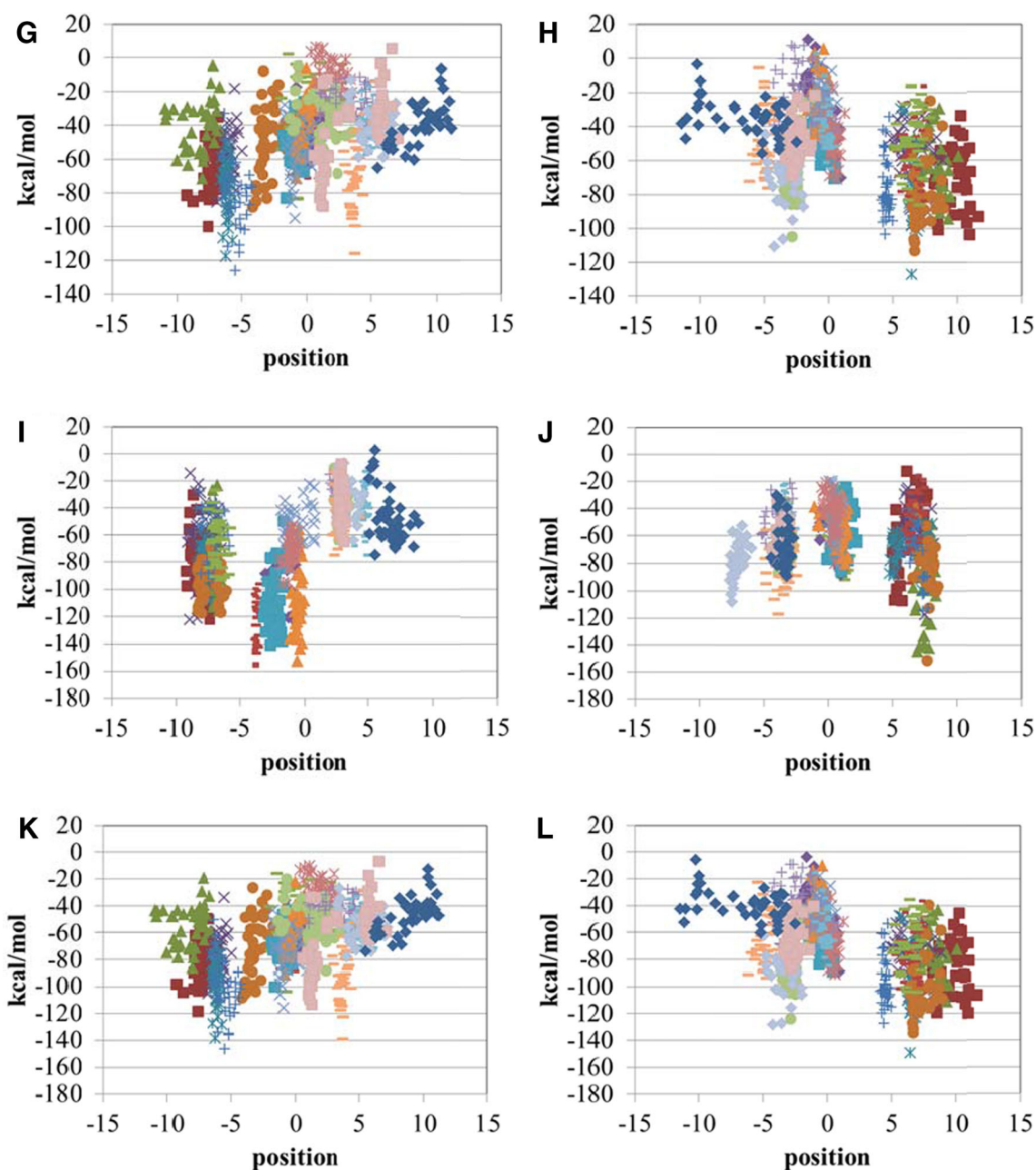


Fig. 2 continued

electrostatic interactions including hydrogen bonding for the binding of GlyA to γ -CD are greater than those for the binding to β -CD. This is highly consistent with the difference in enthalpy changes that was determined using ITC. The favorable enthalpy change is partially compensated by the unfavorable entropy change, which is a general phenomenon, also known as enthalpy-entropy compensation [18]. Actually, the ITC determined entropy change for the binding of GlyA to γ -CD is smaller than that for the binding to β -CD. Based on the MD results for the configurational entropy, the difference in structural

dynamics between β -CD and γ -CD complexations would be minimum, possibly resulting in minor effects on the difference in entropy change determined using ITC [12]. The dehydration effect of β -CD complexation on the entropy change might be larger than that of the γ -CD complexation.

ITC can effectively determine the correct and precise binding thermodynamics between unbound and bound states. Structural information regarding both the unbound and bound states could be obtained using biophysical methods such as NMR and X-ray crystallography. Despite

Fig. 3 Number of hydrogen bonds of simulated configurations. The numbers are plotted against the positions of simulated configurations in “left-glu” of β -CD (a), “right-glu” of β -CD (b), “left-glu” of γ -CD (c), and “right-glu” of γ -CD (d). The color codes on the right column indicate the structural possibilities appeared during the simulations. (Color figure online)

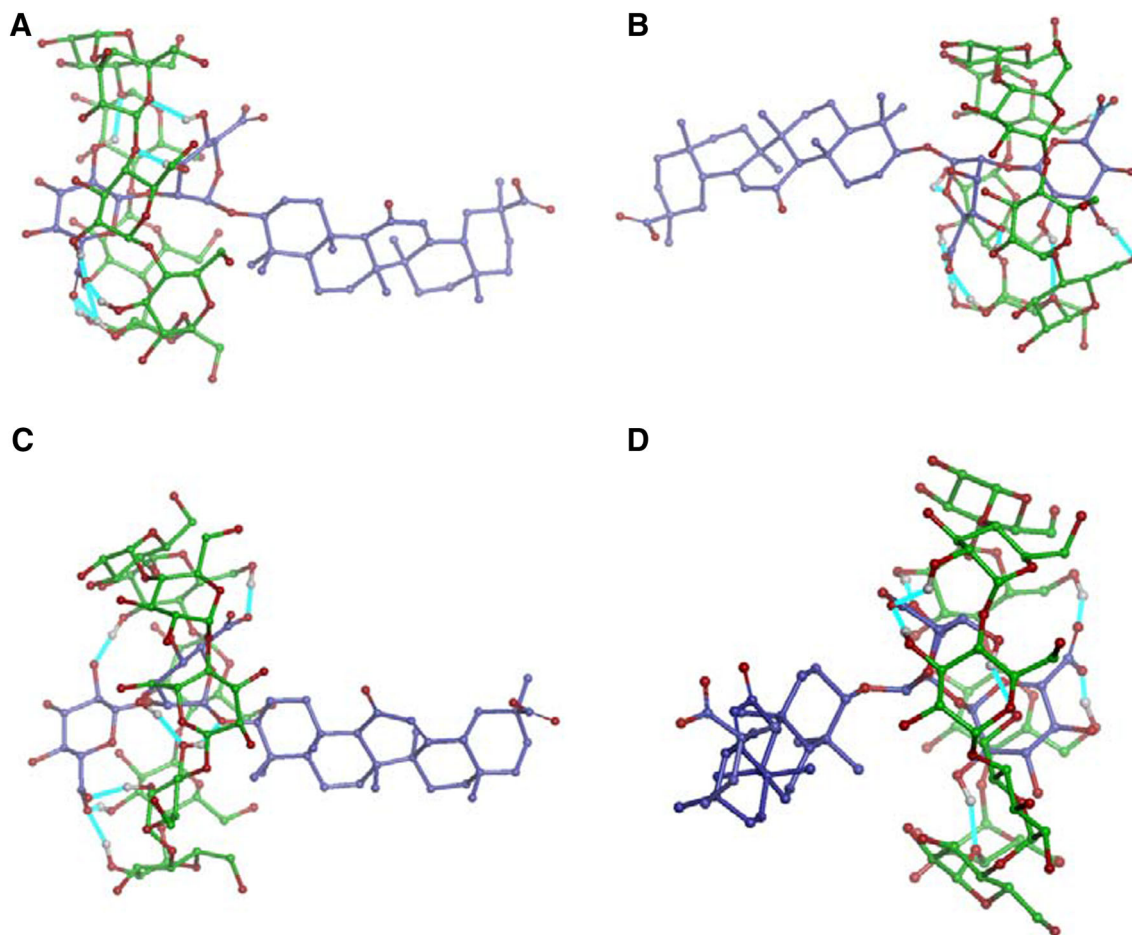
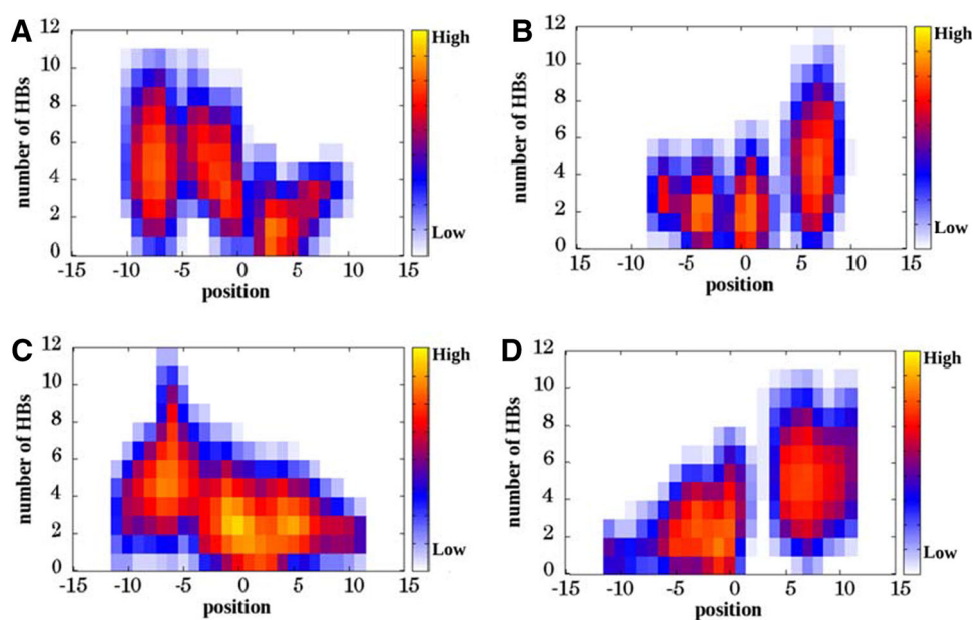


Fig. 4 Simulated configurations with low energies. Typical configurations with low energies were selected from the respective sets of “left-glu” of β -CD (a), “right-glu” of β -CD (b), “left-glu” of γ -CD (c), and “right-glu” of γ -CD (d). The carbon atoms in CDs and GlyA

are shown in *blue* and *green*, respectively, and the oxygen atoms are shown in *red*. The hydrogen bonds between CD and GlyA are indicated in cyan. (Color figure online)

Fig. 5 Difference in configurational entropy of the inclusion complexation. The configurational entropies of inclusion complexes with β -CD (open circle) and γ -CD (closed circle) (a, b), and the changes in the entropies of unbound states of β -CD (open circle) and γ -CD (closed circle) (c, d) are plotted against the positions of simulated configurations in “left-glu” (a, c) and “right-glu” (b, d)

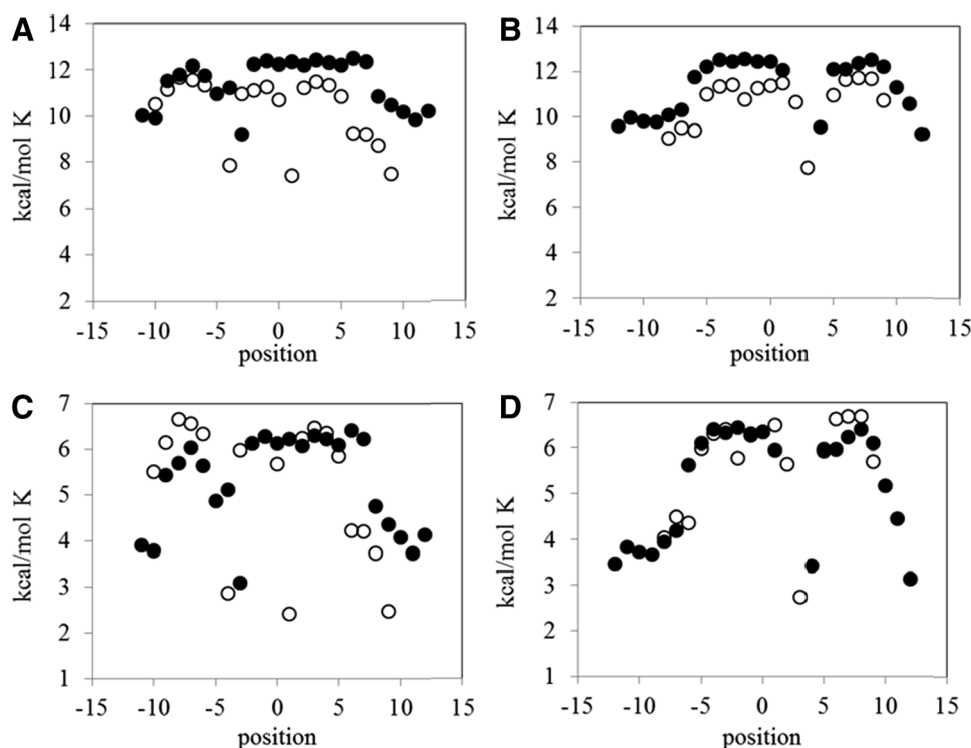


Table 1 Thermodynamic parameters of the interaction between GlyA and CDs at pH 7.4, 25 °C Data were taken from Izutani et al. [12]

CD	ΔG	ΔH	$T\Delta S$ (kcal mol ⁻¹)
β -CD	-4.8	-0.83	4.0
γ -CD	-8.1	-5.7	2.4

the advantage of these analyses, it is difficult to obtain information on the structural dynamics and transient binding pathway of the unbound and bound states. The present study showed the advantage of MD simulations in combination with other structural and thermodynamic analyses to understand the molecular recognition mechanism. We found that GlyA is unable to pass through the hydrophobic pocket of β -CD, while it is able to pass through that of γ -CD. The energy level of final attainable structure for the latter complex, including the contribution from hydrogen bonds, is lower than that for the former complex. This is in good correlation with the binding energy difference as determined using ITC. We could also show that the reasonable results were obtained by using the MD methods started from the initial different configurations of GlyA and CDs, without using the long-time MD run. While the experimental results using ITC showed the equilibrium binding thermodynamics, the present results

using MD simulations could indicate the respective complex structures of GlyA and CDs with different binding energies, which could help us to understand the dynamic properties of inclusion complexation of CDs [19, 20].

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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