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Molecular Modeling Studies of the β-Cyclodextrin in Monomer and Dimer Form as Hosts for the Complexation of Cholesterol

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Abstract

Molecular modeling studies on the inclusion complex formation of cholesterol with β-cyclodextrin in monomer and dimer form were performed. Monte Carlo docking simulations, molecular dynamics, and non-equilibrium molecular dynamics simulations were applied to assess the energetic driving force for the formation of these inclusion complexes. Both Monte Carlo docking and molecular dynamics simulations supported the more favorable inclusion complex formation of β-cyclodextrin dimer. Non-equilibrium molecular dynamics simulations provided a direct assessment of the binding force for the inclusion complexes, of which that of dimer form is much greater.

Introduction

Cyclodextrins (CDs) are toroidal-shaped cyclic oligosaccharides composed of six (α-CD), seven (β-CD) and eight (γ-CD) D-(+)-glucopyranoside units which are bonded through α-1,4 linkages. They have a relatively hydrophobic interior enabling the formation of inclusion complexes with a variety of organic molecules containing a hydrophobic moiety [1–5] and have attracted widespread interests in scientific and technological areas [6–9]. The hydrophobic cavity inside a CD has a diameter of approximately 5 to 8 Å, depending on the number of glucose moieties, and accommodates small, hydrophobic molecules to form stable, low energy inclusion complexes [10]. CDs have gained importance in the fundamental research and technological applications of inclusion phenomena such as chiral separation and drug solubilization [11–15].

Self-assembly of molecular systems or molecular recognition is a key process in biological systems, e.g., in the formation of enzyme complexes with substrates or drug-receptor interactions [10]. The inclusion complex formation of CDs is a valuable computational model system for studying the phenomena of molecular recognition because of abundant available experimental data and simple structural features. The theoretical prediction of the inclusion complex forming abilities of modified CDs would aid the usefulness of CDs. Various structural modifications were made to improve the usefulness of CDs as hosts for inclusion complex formation. In particular, a β-CD dimer with a very short linker between the two rings was recently reported to bind a cholesterol molecule cooperatively in the two rings with a several hundred times greater binding constant than β-CD [16]. We chose these molecules as our models for the computational analysis of inclusion complex formation to further investigate the difference of molecular recognition.

In this study, the inclusion complex formation of β-CD and its dimer with cholesterol was analyzed by Monte Carlo (MC) docking simulations, molecular dynamics (MD) calculations and by non-equilibrium molecular dynamics simulations.

Methods

Molecular mechanics and dynamics calculations were performed with the InsightII/Discover program (version 97.0, Molecular Simulations Inc., U.S.A.) using the consistent valence force field (CVFF) [17] on a SGI R4600 platform (Silicon Graphics Inc., U.S.A.).

Construction of molecular models

β-CD and β-CD dimer

The β-CD structure was obtained by energy minimization of a crystallographic geometry [5] and the initial model of the β-CD dimer was constructed referring to the experimental procedure [16]. A systematic grid search was performed for the dihedral angles of four single bonds connecting the two rings in the β-CD dimer. Each dihedral angle was rotated by 30° intervals from 0° to 360°, and the conformations with more than 10% overlap of van der Waals radii were discarded. Among the resulting conformations, the one with serial alignment of two β-CDs was used for further simulations because of the 1 : 1 molar stoichiometry [16].

Cholesterol

Conformational search of cholesterol was performed by the simulated annealing molecular dynamics-full minimization strategy [18]. In this procedure, the temperature was repeated between 300 K and 1000 K five times at intervals of 50 K. At each temperature, MD simulation was performed for 2.5 ps: 0.5 ps of equilibration phase and 2 ps of production phase. One structure was saved from the end of the production phase at each temperature. No cutoff was imposed on the calculation of non-bonded interactions. Constant NVT MD calculation was performed using the leap-frog algorithm with a 1fs time step. Temperature was controlled by velocity scaling in the equilibration phase and the Berendsen algorithm in the production phase with a coupling constant of 0.2 ps [19]. The dielectric constant was set to 1. After the MD simulation, all the 141 saved conformations were fully energy-minimized: 100 iterations of steepest descent minimization and conjugate gradient minimization until the maximum derivative reached below 0.001 kcal/mol Å (typically 4,000–10,000 iterations). From these conformations, the one with the lowest energy was selected for further simulation.

Monte Carlo docking simulations

The host and guest molecules were positioned in the neighborhood at a distance of ∼7 Å. From this initial configuration, Monte Carlo docking simulation started by minimizing the configuration for 100 iterations with the conjugate gradient algorithm and accepted it as the first frame. Several initial configurations were tried. In the course of a trial to a new configuration, cholesterol could take the maximum translational movement of 7 Å to x, y, and z axis and maximum rotation of 180° around the x, y, and z axis. Four dihedral angles of the hydrocarbon tail of cholesterol could rotate (maximum 180°) for flexible docking. A total of 10 degrees of freedom were present for this system (3 translational, 3 rotational, and 4 dihedral). Each cycle began with a random change of up to 5 degrees of freedom among them [20, 21]. If the energy of the resulting host–guest system was within 1000 kcal/mol from the previous accepted structure, the system was subjected to 100 iterations of conjugate gradient energy minimization. The energy tolerance of 1000 kcal/mol was imposed to avoid significant overlap of van der Waals radii in the random movement. The resulting structure was accepted based on two criteria. (a) an energy check which used Metropolis criteria at a temperature of 300 K [22], and (b) a root mean squared displacement (RMSD) check which compared the RMSD of a new structure against the structures accepted so far. If an accepted structure in the energy check was within 0.1 Å of RMSD with one of the previously accepted structures, it was regarded as the same one and discarded. The Monte Carlo simulations were performed until complete energy convergence. The non-bonded interactions were calculated by the cell multipole method [23] and the dielectric constant was set to 1.

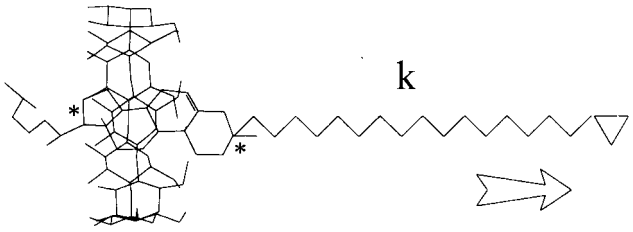

Figure 1. The schematic illustration of non-equilibrium MD simulations. By increasing the value of the force constant k, the force needed to dissociate the inclusion complex was measured. Direction 1 is shown. The atoms which were connected to the virtual spring are indicated by asterisks.

Molecular dynamics simulations

One of the low energy structures of the MC docking simulation of each host–guest complex was subjected to molecular dynamics simulation. MD simulations were performed *in vacuo*. The MD calculations were performed using the Velocity Verlet algorithm [24] at constant volume with the cell multipole method for the calculation of non-bonded interactions. The initial atomic velocities were assigned from a Gaussian distribution corresponding to a temperature of 300 K. The system was equilibrated for 50 ps and the production run was done for 500 ps. By constraining bond lengths (rattle algorithm) [25], a time step of 2 fs could be used. The temperature was controlled using weak coupling to a bath of constant temperature (T = 300 K, τ = 0.1443 ps). Intermediate structures were saved every 100 fs for analysis.

Non-equilibrium molecular dynamics simulations

We performed the non-equilibrium molecular dynamics simulations of inclusion complexes out of which the guest molecule was pulled by an external force to investigate the binding forces of guest molecules in inclusion complexes. We have attached a virtual harmonic spring to one of the sterol ring atoms to pull the guest molecule out of the host. By increasing the force constant of the spring gradually, the force needed to drag the guest molecule out of the host was recorded. Non-equilibrium MD simulations started after the 50 ps equilibration of the same molecular model system as the MD simulation above were equilibrated for 50 ps and then continued until the complete dissociation of host and guest molecule. For each inclusion complex, two simulations were performed in the direction of the pulling force: the direction of the polar head group of cholesterol (direction 1) and the other (direction 2). The spring was connected to the C3 atom of cholesterol in the former, and the C17 atom in the latter. A schematic illustration is shown in Figure 1. The force of the harmonic spring, F_{spring} could be described as:

$$F_{\text{spring}} = k(z_{\text{link}}(t) - z_{\text{spring}}(t)),$$

where k is the force constant, $z_{\text{link}}(t)$ is the position of the atom to which the spring is linked, and $z_{\text{spring}}(t)$ is the fixed position to which the other end of the spring was connected. The value of the force constant was increased gradually from zero to 2.0 kcal/mol Å^2 at a rate of 0.00125 kcal/mol Å^2 ps

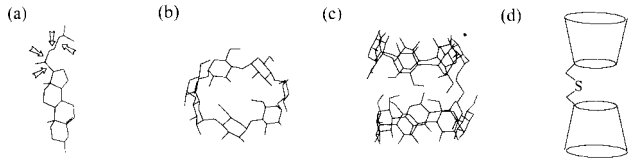

Figure 2. The molecular models for the MC and MD Simulations: (a) cholesterol; (b) β-CD; (c) β-CD dimer; (d) schematic illustration of β-CD dimer. The rotatable bonds of cholesterol in the MC runs are indicated by arrows.

to extract the guest out of the host. In order to prevent the whole inclusion complex from being pulled away, the center-of-mass of the host molecule was kept in the original place. For complete dissociation, a spring with a length of 20 Å was used. Other MD conditions were the same as described above.

Results and discussion

Molecular models

Figure 2 shows the molecular models of β-CD and β-CD dimer. Of the 97 conformations found in the systematic grid search of four dihedral angles connecting the two rings of β-CD dimer, only one conformation had two serially aligned rings. For the 1 : 1 molar stoichiometry for the formation of the inclusion complex of cholesterol and β-CD dimer [16], the two rings in the dimer should align in a serial position. So, this conformation was chosen for the further simulations.

Figure 3 shows the energy profile of the simulated annealing molecular dynamics – full minimization of cholesterol. This energy profile showed the existence of some closely spaced local energy minima, but the conformation of the sterol rings of these local minima were identical (Figure 3) indicating that the sterol ring was rigid. Therefore, the lowest energy one was selected as the initial conformation for the following MC docking simulations without considering the flexibility of the sterol ring.

Monte Carlo docking simulations

The pathways of Monte Carlo docking simulations showed a general tendency of inclusion complex formation and lowering interaction energy. The interaction energy was defined as the difference between the sum of the energy of individual host and guest molecule and the energy of the inclusion complex [26]. The interaction energies converged rapidly in the initial phase of the MC simulations. Figure 4 compares the interaction energies in Monte Carlo runs for the β-CD-cholesterol and the β-CD dimer-cholesterol system. The low energy conformations of the β-CD-cholesterol complex were found at -38.6 ± 1.3 kcal/mol and those of the β-CD dimer-cholesterol complex were found at -62.6 ± 1.3 kcal/mol, indicating that the inclusion complex formation of β-CD dimer with cholesterol was energetically more favorable than that of β-CD. Figure 5 shows representative snapshots during the MC docking simulations. In β-CD dimer, the guest molecule was fully embedded in the cavity,

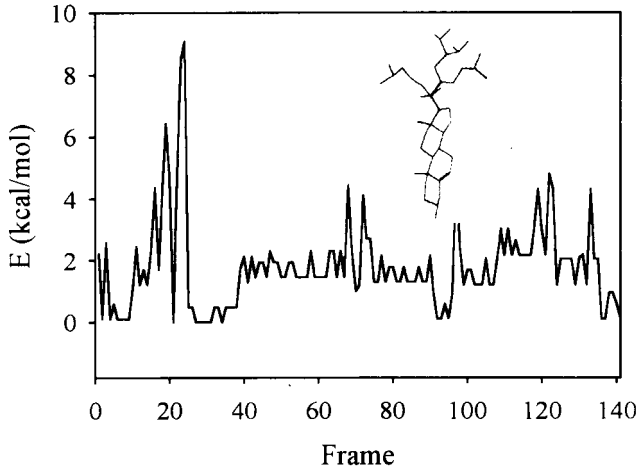

Figure 3. The energy profile of simulated annealing molecular dynamics simulation – full energy minimization of cholesterol. The y-axis is the energy difference of each frame (conformation number) to the energy of the lowest energy conformation. (Inset: The overlap of the energy minimized conformations of cholesterol. Five conformations were taken at the lowest temperature in the simulated annealing molecular dynamics. The conformations of the sterol rings were identical in these conformations although the hydrocarbon tails rotated freely.)

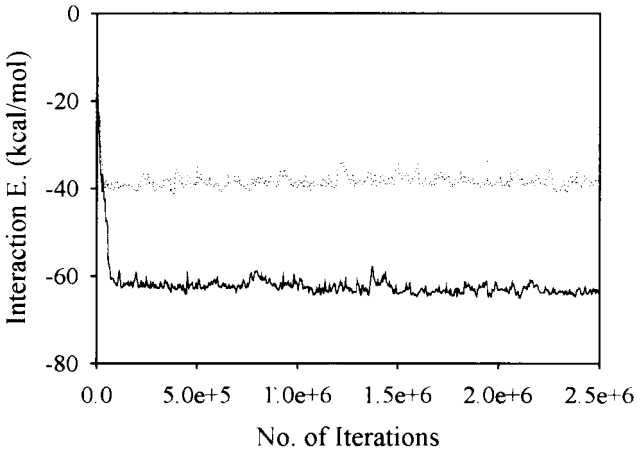

Figure 4. The energy profiles of MC docking simulations. The interaction energy was defined as the difference between the sum of the independently calculated energy of each host–guest molecule and the energy of each configuration in the process of MD docking simulation.

whereas the hydrocarbon tail of cholesterol was exposed out of the host in β-CD. In the case of β-CD, two types of configurations were found according to the orientation of cholesterol. The hydrocarbon tail of cholesterol is on the same side of O6 of β-CD in Type A and on the opposite side in Type B (see Figure 6). Both types appeared alternately in the course of MC simulation and had almost identical interaction energies. The MC simulations from different initial positions consistently provided similar or almost identical docking configurations with very close interaction energies to validate our results.

Molecular dynamics simulations

The general features from the MD trajectories were very similar to those from MC docking simulations. Figure 6 shows overlap of snapshots from MD trajectories. The in-

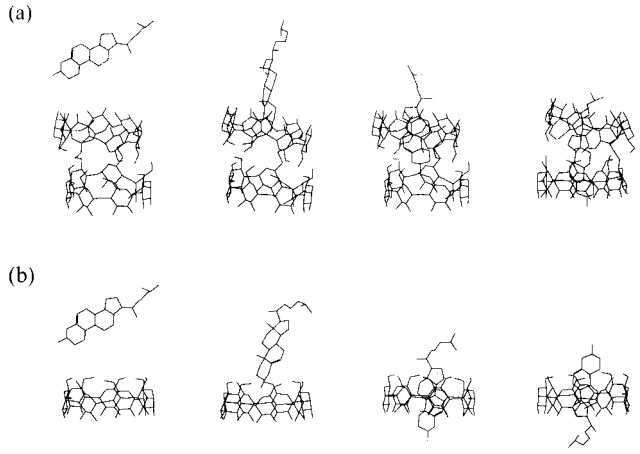

Figure 5. Snapshots of inclusion complexes during the MC docking simulations. (a) β-CD dimer-cholesterol complex formation. To maintain serial alignment of the two rings in the β-CD dimer, the dihedral angles of the bonds connecting the two rings were fixed in the initial phase of the MC simulation (until the interaction energy reached below -50 kcal/mol). (b) β-CD-cholesterol complex formation.

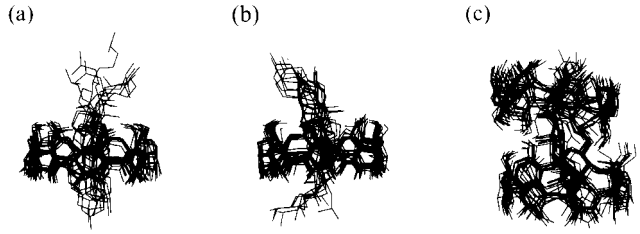

Figure 6. The overlap of snapshots during the MD simulations. A snapshot was taken at intervals of 50 ps and superimposed on the initial conformation in the production phase of the MD runs: (a) Type A of the β-CD-cholesterol complex; (b) Type B of the β-CD-cholesterol complex; and (c) the β-CD dimer-cholesterol complex. One can see from the figure that the β-CD dimer bound cholesterol more tightly than β-CD.

teraction energies after 100 iterations of conjugate gradient energy minimization showed good agreement with the results of MC docking simulations. The interaction energies were -57.4 ± 2.7 kcal/mol for the cholesterol-β-CD dimer, -37.3 ± 1.6 kcal/mol for cholesterol-β-CD Type A and -36.9 ± 1.7 kcal/mol for Type B. These results indicated the relative energetic stability of the β-CD dimer-cholesterol complex compared with β-CD-cholesterol as in the case of MC docking simulations. The center-of-mass distances between the host and guest molecule during the MD runs were 0.51 ± 0.19 Å for cholesterol-β-CD dimer, 1.83 ± 0.64 Å for cholesterol-β-CD Type A and 2.13 ± 0.93 Å for Type B. A wide range of host–guest distance fluctuations were observed in β-CD complexes compared with β-CD dimer. This result indicates that the β-CD bound the cholesterol more loosely than the β-CD dimer.

Non-equilibrium molecular dynamics simulations

Analyses of the computed forces and the concomitant configurational changes of the inclusion complex during the simulated extraction process showed a detailed picture of the dissociation process in an interesting perspective. Figure 7 shows the center-of-mass distance between the host

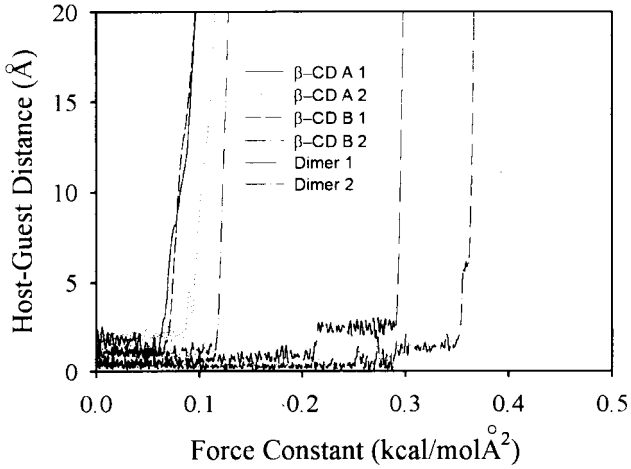

Figure 7. Center-of-mass distance between the host and guest molecule as a function of the force constant in the dissociation process by non-equilibrium molecular dynamics. At the dissociation point, the dissociation of host and guest molecule occurred abruptly. (A, B: Type A and B, 1,2: direction 1 and 2).

and guest molecules in the trajectories of non-equilibrium MD simulations. At a certain value of the force constant, the dissociation of host and guest molecule occurred abruptly. Figure 8 shows some representative snapshots from the non-equilibrium MD simulations. Non-bonded forces as well as the frictional forces would determine the dissociation point of the host and guest molecules. The pulling force gradually built up to its maximum value, after which it decreased again as the guest molecule left the host molecule (Figure 9). The height and position of the largest force maximum, the dissociation point, depends on the binding force. The values of force at this dissociation point corresponded to 1.2 ± 2.3 kcal/mol Å for the β-CD-cholesterol complexes and 5.3 ± 6.6 kcal/mol Å for the β-CD dimer-cholesterol complexes. The data could be represented in another useful way in the form of a binding energy, which was obtained by Hook's law (Figure 10). The dissociation energies tended to be lower than the docking energies especially in the case of the β-CD complex (Figure 4). This result appeared to be related to the natural dissociation tendency shown by the large center-of-mass fluctuations of cholesterol in the β-CD.

Conclusions

We studied the β-CD- and β-CD dimer-cholesterol inclusion complexes using computer simulations. The MC docking, MD simulations and non-equilibrium MD simulations all showed consistent results showing more favorable inclusion complex formation of cholesterol with β-CD dimer. In particular, the non-equilibrium MD simulations provided the direct assessment of the binding force of the inclusion complexes, which may be applicable to assessing and predicting the binding forces of various host–guest complexes. We believe that the present investigations provided interesting insights into the way the guest recognizes its host.

In many cases, it is difficult to simulate the binding process of molecular systems. MD simulations might take

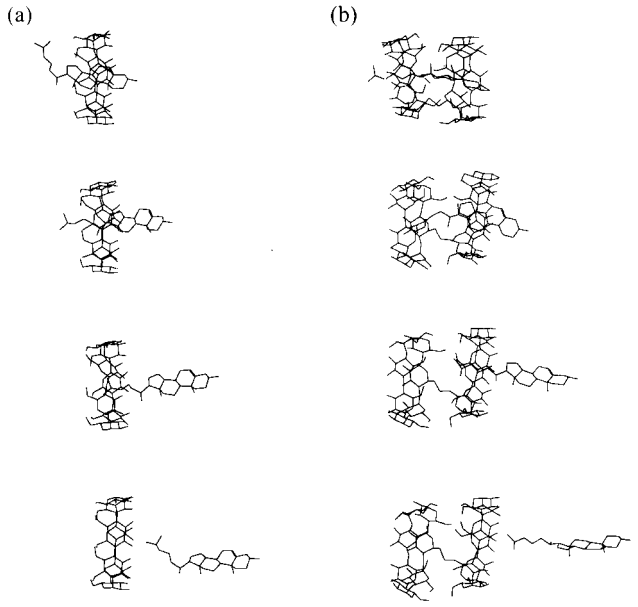

Figure 8. Snapshots from the non-equilibrium MD simulations; (a) β-CD-cholesterol complex type A in direction 1; and (b) β-CD dimer-cholesterol complex in direction 1.

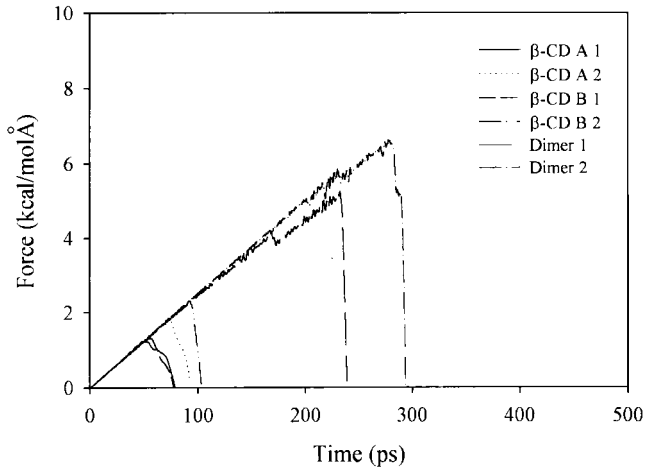

Figure 9. The force profile of non-equilibrium MD simulations. The highest point in each plot can be interpreted as the dissociation force of the inclusion complexes.

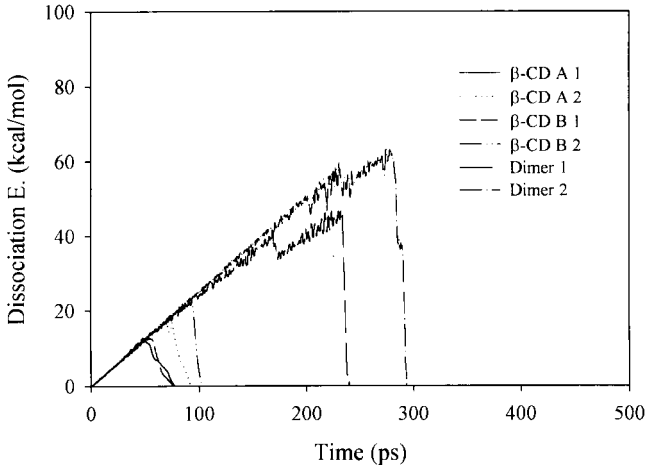

Figure 10. The dissociation energy profiles of non-equilibrium MD simulations. The energies were obtained by Hook's law. The highest point in each plot can be interpreted as the dissociation energy of the inclusion complexes.

too much time and MC simulations do not produce time-dependent properties. In such cases, simulating the reverse of the binding process would be useful. By applying an external force on the guest molecule, the dissociation process could be accelerated enabling us to monitor the process. In our simulated extraction method by non-equilibrium MD simulations, at a certain value of the external force, the dissociation of cholesterol and β-CD dimer occurred abruptly. This implies that the binding and dissociation process might be cooperative. The binding/dissociation of cholesterol to one β-CD may help the binding/dissociation to the other in the β-CD dimer. We believe that the simulated extraction process is especially useful for the understanding of the molecular recognition mechanism when applied to the experimentally determined structures of host-guest inclusion complexes.

We want to briefly discuss the limitations of our calculations. We did not consider solvent molecules in our simulations. Because it was practically impossible for us to perform Monte Carlo docking simulations in the presence of explicit water molecules, we decided to maintain consistency through the applied simulation methods of MC, normal MD and non-equilibrium MD to compare the results from different methods. In addition, the implicit treatment of solvent effect by a distance dependent dielectric constant would not be effective for closely contacted systems. And we calculated only energies not free energies. So, our simulation results fit the experimental results [16] qualitatively but not quantitatively. But we think that our results are useful for the assessment and prediction of the binding tendencies of inclusion complexes.

Finally, we want to discuss the new features of non-equilibrium molecular simulations. This method was new and provided some benefits to the calculation of binding energies. If one calculates binding energies from the difference of the energies of the inclusion complex and the individual host/guest molecule (as MC and normal MD simulations in our simulations), one should perform a conformational search to find the global energy minima for host and guest molecules. This might not be easy in many cases. On the other hand, if one considers only inter-molecular nonbonded energies for the binding energies, one could not help ignoring intra-molecular conformational changes in the course of inclusion complex formation and/or dissociation. This method, a simulated extraction process, can provide an alternative way for measuring the binding energy. It does not need the conformational search of host and guest molecules, and incorporates intra-molecular conformational changes as well as frictional forces and inter-molecular energies.

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