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Competitive and Reversible Binding of a Guest Molecule to Its Host in Aqueous Solution through Molecular Dynamics Simulation: Benzyl Alcohol/β-Cyclodextrin System

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Understanding the fundamental principles that govern the binding of a guest molecule to its host and accurate prediction of the binding mode of the guest/host complex are important goals in guest−host chemistry and have implications in structure-based drug design. In this paper, we report our computational investigation of benzyl alcohol (the guest) binding to β-cyclodextrin (the host) in the presence of explicit water molecules using both the self-guided molecular dynamics (SGMD) simulation method and conventional MD simulation method. The simulation system was constructed in such a way that 6 guest and 1 host molecules were solvated in a cubic water box of 35 Å in dimension. Two SGMD simulations were performed for 1.5 ns and 2.5 ns at 300 K, respectively, starting from uncomplexed, two totally different initial configurations of the guest and host molecules. In both SGMD simulations, competitive and reversible binding of the guest molecules to the host is observed. Analysis of the simulation trajectories showed that one major complexed conformational cluster is in good agreement with the complex structure determined using the X-ray diffraction. In addition, several other major binding modes were also identified in aqueous solution. Investigation of the binding forces showed that the burial of the phenyl group in the cavity of β-cyclodextrin, but not the hydrogen bonding interaction between the guest and the host, is the major change for binding, suggesting that that hydrophobic interaction may be responsible for the formation of the complex. To verify the predictions made by the SGMD method, we performed two 12.5 ns conventional MD simulations with the same initial setup and same conditions as for the two SGMD simulation runs. Additionally, we have performed a 10 ns long conventional MD simulation starting from the crystal structure of the complex. The MD simulations predicted major solution binding modes similar to those identified through the SGMD simulations, including the conformational cluster that is essentially the same as that found in the X-ray structure. Our studies showed that the SGMD method is an efficient way to study competitive and reversible binding of guest molecules to their hosts in aqueous solution. The SGMD method may also be useful to study the binding of drug molecules to their marcomolecular targets.

Introduction

Molecular complexation is central for many chemical and biological processes. In chemistry, guest−host chemistry has been a very active field in the last three decades. Guest−host molecule systems have been used as model systems with which to study the fundamental principles that govern the complexation of a guest molecule to its host. Indeed, practical applications of guest−host chemistry are very popular in basic laboratory research, and in pharmaceutical and food industries. In biology, the binding or association between molecules plays a fundamental role in molecular and cellular functions. Understanding the basic mechanisms that govern the binding process between molecules is also essential for structure-based drug design.

Cyclodextrins (CDs) are cyclic oligomers of 1,4-linked, α-D-glucose monomers. They are often used as host molecules by scientists interested in applications of guest−host complexation, as well as in fundamental issues of molecule recognition. The structural, thermodynamics, and kinetic properties of cyclodextrins alone or in complex with small guest molecules have been explored extensively using a wide range of experimental methods including X-ray diffraction, ^{1}H NMR, UV spectroscopy, and circular dichroism, as well as computational and theoretical methods.[1−3] For example, combining molecular modeling and ^{1}H NMR techniques, the solution structure of β-cyclodextrin in complex with indomethacin sodium, a nonsteroidal antiinflammatory agent, as well as the selectivity of β-cyclodextrin to Z- and E-isomers of indomethacin was successfully characterized.[4]

In more recent years, computational methods have played an increasingly important role for the study of guest−host systems. Lipkowitz and colleagues successfully employed Monte Carlo (MC) and stochastic dynamics simulations to investigate where and how enantioselective binding takes place on permethylated β-cyclodextrin, a chiral stationary phase commonly used in gas chromatography.[5] Free-energy perturbation calculations were performed to the Rebek's "tennis ball" dimer complexation with CH_4, $CHCl_3$, and CF_4.[6] In another study, the free-energy difference between cryptophane-C (the host) and the (*R*)- and (*S*)-diastereomers of bromochlorofluoromethane (CHFClBr) was calculated. On the basis of the calculated free-energy difference, it was concluded that the absolute configuration of CHFClBr must be (*R*)−(−) or (*S*)−(+), in agreement with a recent independent assignment based upon Raman Optical Activity studies.[7] More recently, the dynamic behavior of

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cryptophane host−guest systems was investigated through MD simulations.[8] It was found that despite the similarity between two guest molecules, significant differences were observed in terms of their ability in modifying the conformational sampling of the host molecule, their binding orientations inside the host molecule, and their tumbling rates to the host, highlighting many challenges in describing molecular recognition.

For a given guest−host molecular system, a fundamental aspect is the precise manner in which the guest binds to its host. The solid state complex structure may be obtained through X-ray diffraction and the solution structure can be studied using NMR and other experimental techniques. However, an accurate prediction of the binding mode of a guest to its host through computational methods is an important goal for the study of the mechanism of the binding and especially for the design of new guest−host systems with desired properties. In the past, molecular mechanics, conformational searching, and energy minimization techniques were often used to assess the energetically favorable binding modes of a guest−host molecule system in the gas phase. However, prediction of the binding mode of a guest−host system in solution is a much more challenging problem. Because solvent molecules play an important role in determining the thermodynamics of the guest−host system and the manner the guest binds to its host, it is necessary to include the solvent effects in the calculation. To reduce the computational demand, implicit solvation models have been used and were often found to provide an inadequate representation of the solvent effects.[9−11] An explicit, atomic-detailed solvent model in theory should provide a much more accurate representation. But inclusion of many solvent molecules in the guest−host for binding-mode prediction presents a considerable challenge because of many degrees of freedom of the system.

Molecular dynamics (MD) simulation is a promising approach for the prediction of the binding mode of a guest−host system in solution with full-atom models for the guest, the host, and the solvent molecule, because the degrees of freedom of all the molecules in the system can be easily included in the simulation. One major challenge for such an approach is that lengthy MD simulations need to be performed in order to sample the conformational space adequately. The predicted binding modes from inadequate conformational sampling likely depend on the initial configurations of the system. To overcome the problem that the predicted binding mode is dependent upon the initial configuration, the simulation must be sufficiently long so that the guest molecule can bind to the host (association), then unbind (dissociation), and rebind during the simulation. This reversible binding process may need to be repeatedly observed if the purpose is to accurately predict the binding mode. In other words, "reversible binding" is essential during the MD simulations in order to achieve an accurate prediction.

Recently, we developed the self-guided molecular dynamics (SGMD) simulation method to improve the conformational searching efficiency of MD simulation.[14,15] Using model systems, we have demonstrated that the SGMD can achieve a much-improved conformational searching efficiency as compared to the conventional MD method, while it does not significantly alter the conformational distribution of the system or other thermodynamic properties with proper parameters.[14,15,26,27] Therefore, this new MD method with efficient conformational searching ability offers us the opportunity to investigate the binding of guest−host systems in solution.

A benzyl alcohol/β-cyclodextrin system, whose chemical structures are shown in Scheme 1, was chosen as our guest−host system in this investigation for several reasons. First, the experimental structure of benzyl alcohol in complex with β-cyclodextrin has been determined through X-ray diffraction.[12] Although the experimental structure is in solid state, it does provide us an experimental verification to our predicted binding mode derived from our simulations. Second, β-cyclodextrin is a widely used and studied host molecule. On the basis of the X-ray structure, the interaction between β-cyclodextrin and benzyl alcohol is primarily hydrophobic in nature. Thus, this guest−host system serves as an excellent model system for understanding the complexation between small guest molecules and host molecules whose interactions are predominantly hydrophobic.

Methods and Simulation Setup

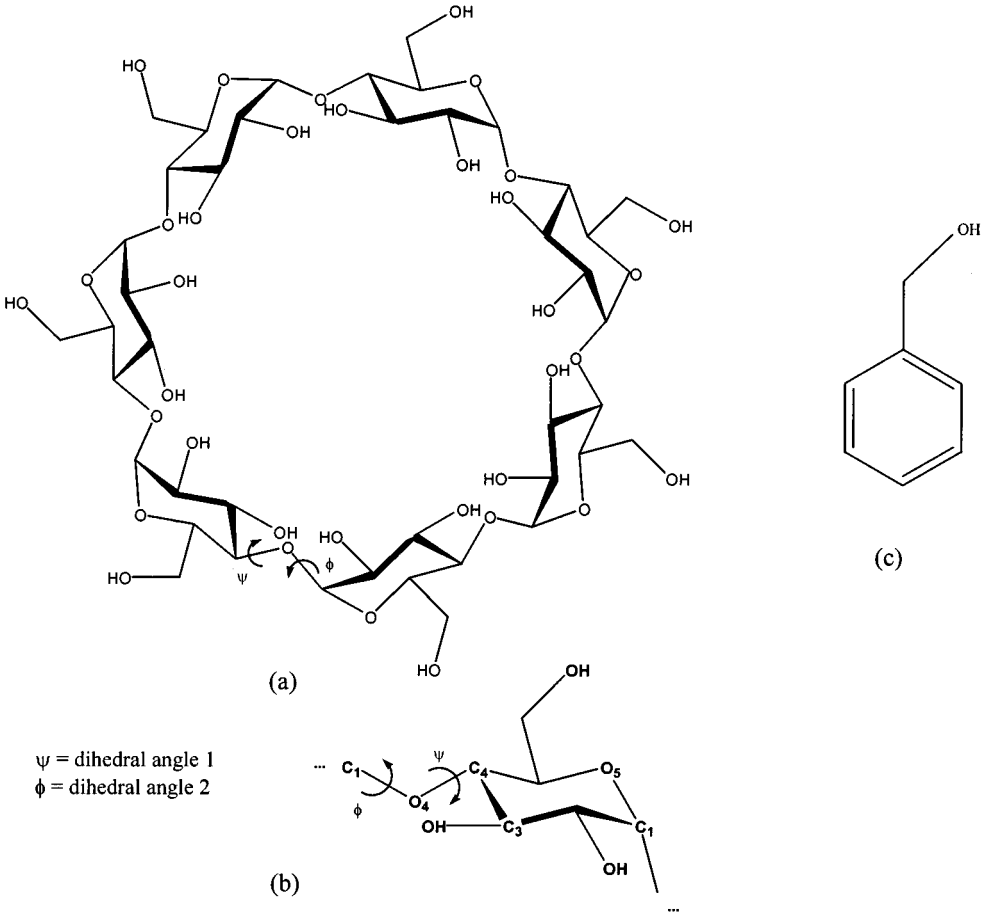
Conventional MD simulations were performed using the CHARMM program (version 26).[13] The SGMD algorithm[14,15] was implemented in the CHARMM program and used to perform the SGMD simulations in this study. Parameters, atom types, and charges for cyclodextrin were taken from the test files in the CHARMM program (version c24test), while those of benzyl alcohol were assigned using the program package Quanta 98. These parameters are provided as Supporting Information. TIP3P water model was used to represent the water molecules.[17] In all simulations, the MD time step was chosen to be 0.002 ps. NPT simulations were performed using the Berendsen method[18] ($T = 300$ K). The cutoff distance for the Lennard-Jones potential was set to 14.0 Å while electrostatic interactions were truncated at 12.0 Å. A smoothing function was applied for the electrostatic interactions between 8.0 and 12.0 Å (force switch method). The dielectric constant was set to 1. The nonbonding pair list was updated every 20 steps. The SHAKE algorithm was used to fix bonds for all atoms.[19] Cubic periodic conditions were used in all the simulations. The trajectory was recorded every picosecond (ps) for analysis.

To study the solution structure of β-cyclodextrin in complex with benzyl alcohol, a conventional MD simulation was performed for 10 ns starting with the structure determined by X-ray crystallography for this complex.[12] The solution conformation of β-cyclodextrin in the absence of benzyl alcohol was also investigated using a 10 ns conventional MD simulation. The system of β-cyclodextrin in the absence of benzyl alcohol was set up by immersing one β-cyclodextrin molecule in a cubic box of water molecules with dimension of 25 Å.

Since it is important to select proper guiding parameters in SGMD simulations to ensure that no significant conformational distortion of the β-cyclodextrin structure occurred,[14,15] a series of SGMD simulations with different guiding parameters were first tested and compared to MD. It was found that the using 0.1 for the guiding parameter (λ) and 0.2 for the averaging time (t_l) causes no significant conformational distortions while achieving a much improved conformational searching efficiency as compared to conventional MD simulation. This set of parameters was thus used in all the SGMD simulations in this study.

The system was set up by immersing six benzyl alcohol molecules and one β-cyclodextrin molecule into a cubic box of waters with dimension of 35 Å. The whole system centered around the center mass of the β-cyclodextrin molecule. The major advantage of using more than one benzyl alcohol (guest) molecule in the setup is that it would allow us to study competitive binding between different guest molecules to the host. Two initial setups were used in the SGMD simulations (Scheme 2). In the first setup, four guest molecules were placed

SCHEME 1: Chemical Structures of the Host and the Guest Molecules. (a) β-Cyclodextrin System; (b) One Unit of the β-Cyclodextrin System; (c) Benzyl Alcohol



around the rim of the cyclodextrin's macro-ring, the fifth guest molecule above the macro-ring and the sixth below the macro-ring. In the second setup, all six benzyl alcohols were placed around the rim, approximately in the plane of the glycosidic oxygens. The nearest distance between the center of the guest and host molecule is at least 10 Å to avoid potential bias to a particular binding mode. To validate the predictions made through the SGMD method, we have performed two parallel MD simulations. The protocol used for the SGMD and MD simulations were exactly the same except that the SGMD simulations were performed for 1.5 and 2.5 ns, while both MD runs were for 12.5 ns each.

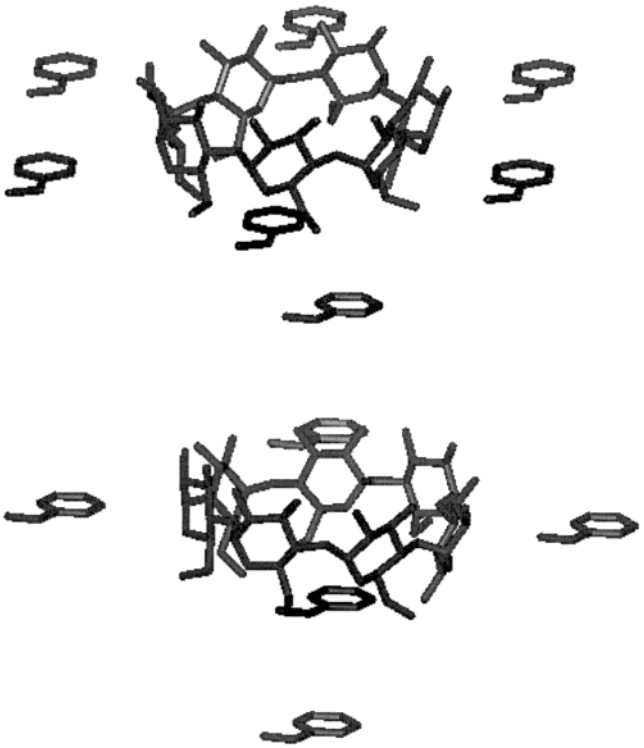
Since many conformations were generated from each simulation, it was necessary to perform cluster analysis for the purpose of identifying the favorable binding modes. The cluster analysis was done using the following criteria: (1) The distance between centers of mass of the host and the guest molecules. Only heavy atoms were included in the calculations of the center of mass for both guest and host molecules; (2) The number of hydrogen bonds between the benzyl alcohol guest and β-cyclodextrin's secondary hydroxyls; (3) The distance between the center of mass of the 14 oxygens in the secondary hydroxyls of β-cyclodextrin and the center of mass of the oxygen and the adjacent carbon in benzyl alcohol; (4) The angle between the plane of the glycosidic oxygens in β-cyclodextrin and that of the phenyl ring in benzyl alcohol. The first two criteria had a weighting factor of 2 while the other two had a weighting factor of 1 because we considered the first two more important for the position of the guest with respect to the host. A clustering algorithm based on a self-organizing neural net implemented into the CHARMM program package (version 26.0) was applied.[23−25]

Results and Discussion

Solution Conformations of β-Cyclodextrin/Benzyl Alcohol System. The solution conformations of the β-cyclodextrin and benzyl alcohol complex were investigated through a 10 ns long conventional MD simulation (MD #1) started from the crystal structure of the complex.[12] In Figure 1, we plotted the distance between the centers of mass of the guest and of the host molecules against the interaction energy between the guest and the host during the simulation. As can be seen, the benzyl alcohol molecule exhibits considerable mobility inside the host. During the 10 ns MD simulation, the distance between the centers of mass of benzyl alcohol and of β-cyclodextrin fluctuates between 0 and 6.5 Å, while the interaction energy fluctuates between −3.6 and −29.7 kcal/mol.

To determine major conformers obtained during the simulation, the structures were clustered as described under in the Methods section. Relative distribution of the obtained clusters is shown in Figure 2a. The most significant differences between several most favored conformers are compared using the following parameters: the depth of the penetration of the guest into the cavity of β-cyclodextrin, and the orientation angle of the aromatic ring of the guest and the host and some other properties (Table 1). Our analysis showed that cluster 3 in Figure 2a is close to the X-ray structure. In cluster 3, the average host−guest center of mass distance is 0.91 ± 0.34 Å, as compared to

SCHEME 2: Starting Conformations for SGMD (SGMD #1 and #2) and Corresponding MD Simulations (MD #3 and #4)[a]



[a] For clarity, only heavy atoms are shown. The six benzyl alcohol molecules are either all around the "rim" of the host (top) or four around the rim, one "below" and one "above" β-cyclodextrin (bottom).

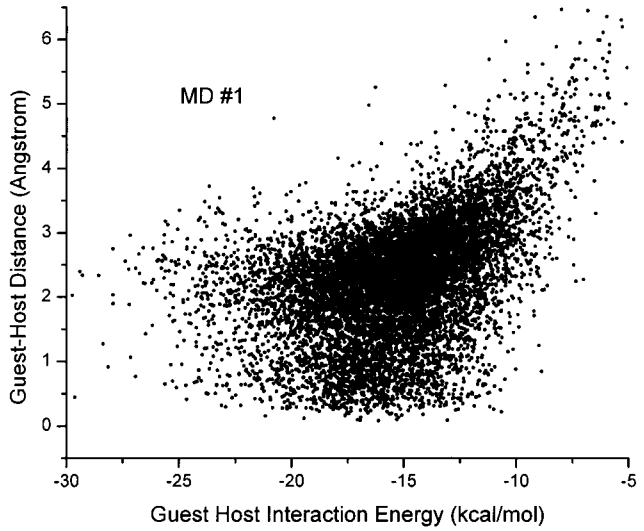


Figure 1. Relationship between the distance of the centers of the mass of guest and of the host molecules and their interaction energy obtained from a 10 ns MD simulation (MD #1).

0.53 Å in the X-ray structure; The phenyl ring is oriented at an angle of 77.04° ± 9.00° with respect to the plane formed by the seven glycosidic oxygen atoms in β-cyclodextrin, as compared to 69.6° in the X-ray structure. The center of mass distance between the "polar end" of the guest (the oxygen along with the carbon atom bonded to it) and the oxygens of the host's secondary hydroxyls is 1.42 ± 0.46 Å, a value comparable to 1.87 Å found in the crystal structure. It is of note that the binding mode as determined in the X-ray structure may be affected by crystal packing (herringbone-like pattern).

Two more populated binding modes (clusters 1 and 2) than cluster 3 were identified. Characteristics of the binding modes of benzyl alcohol in the two most populated clusters 1 and 2 differ somewhat from that of the crystal structure corresponding cluster (cluster 3), as shown in Table 1. The major difference is that the benzyl alcohol molecule does not penetrate inside the host's cavity as deeply (2.59 Å, 1.89 Å for clusters 1, 2 on average, respectively) compared to cluster 3 (0.91 Å). As a consequence, the average polar end distance is also larger for clusters 1 and 2 (2.90 and 2.45 Å) than for cluster 3 (1.42 Å). On the other hand, the orientations of the guest are essentially identical in all these three clusters. The angles between the phenyl ring of benzyl alcohol and the plane of the glycosidic oxygens of β-cyclodextrin are 76.4°, 78.1°, 77.0°, respectively, in clusters 1, 2, and 3. It should be noted that the most populated conformational clusters may be influenced by the simulation time and the force field used.

The host−guest distance and interaction energy relationship observed during 10 ns MD simulation starting with the crystal structure of the complex (MD #1) suggests that in a "tightly" bound benzyl alcohol−β-cyclodextrin complex, host−guest distances are overwhelmingly less than 3 Å (Figure 1). Only conformers on the higher end of the potential interaction energy range tend to exceed the 3 Å host−guest distance threshold, while the upper limit is 6.5 Å. Therefore in this work we refer to "tightly" bound guest if the host−guest distance is less than 3 Å, "loosely" bound if it is less than 6.5 Å (and larger than 3.0 Å). Guests are considered "unbound" if the host−guest distance becomes larger than 6.5 Å.

We then investigated whether binding of the guest leads to distortions of the β-cyclodextrin molecule. For this purpose, we performed another MD simulation (MD #2). The system setup is analogous to the previous simulation run but there is no guest molecule included and the length of the simulation is also 10 ns. Conformations adopted by the β-cyclodextrin macro-ring in the presence/absence of the bound benzyl alcohol were compared by calculating the dihedral angles involving the glycosidic link between the glucose units of β-cyclodextrin. The distribution is plotted in terms of number of conformers versus dihedral angles in Figure 3a and 3b. Dihedral angle 1 is defined by C3−C4−O4−C1, while dihedral angle 2 is defined by O5−C1−O4−C4, where O4 is the glycosidic oxygen, while O5 is the oxygen in the glucose ring. All seven sets of dihedral angles were included in the data set used for plotting. Another characteristic property, the tilt angle, is defined as the angle between the plane of the seven glycosidic oxygens and the plane formed by the O4, C1, C4 atoms and the O4 atom of the next unit; we considered the smallest angle between the two planes (angle "deviation" of one of the planes with respect to the other). This angle is a measure of the distortions of the β-cyclodextrin's ring that might be caused by the presence of a guest, and it was analogously plotted for both MD simulation trajectories (Figure 3c).

As can be seen from Figure 3, the dihedral and tilt angle distributions are approximately identical in the simulations with (MD #1) or without the guest (MD #2). Thus, the structure of β-cyclodextrin in solution is not significantly distorted by the binding of a benzyl alcohol. A possible reason for this relatively minor conformational distortion of β-cyclodextrin upon the binding of a benzyl alcohol is that the benzyl alcohol is relatively small as compared to the size of the binding cavity in β-cyclodextrin. Consequently, its averaged effect on the host is small. The presence of several major conformation clusters obtained from the simulation trajectory for the complex also

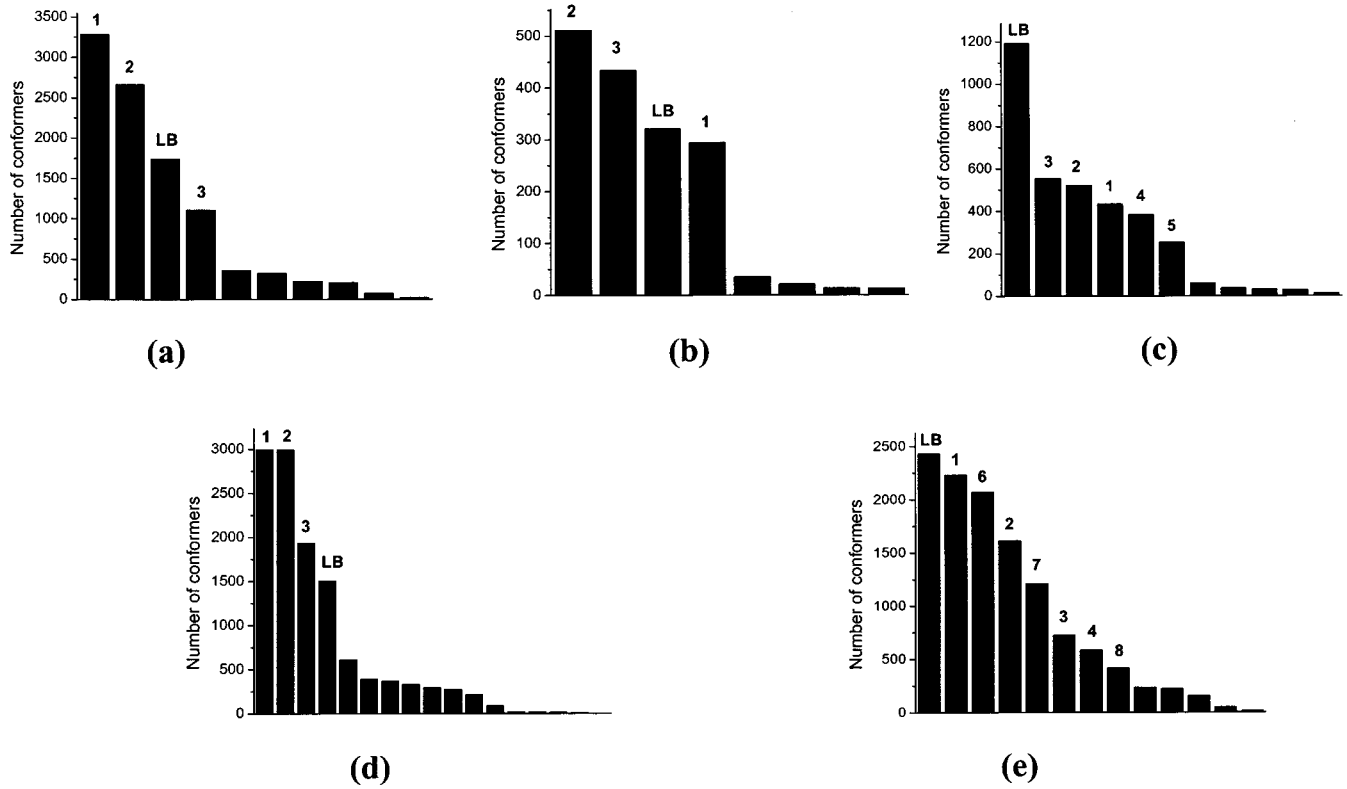


Figure 2. Conformational clusters obtained during MD and SGMD simulations. Major conformational clusters and loosely-bound (LB) conformational clusters are marked and discussed in the text. (a). MD #1. (b) SGMD #1. (c) SGMD #2. (d) MD #3. (e) MD #4.

TABLE 1: Properties of Conformations Averaged over All Conformations within a Cluster; Standard Deviations are Given in Parentheses[a]

MD/SGMD run #	host−guest distance (Å)	phenyl ring−O4 angle (degrees)	polar end distance (Å)	$E_{interaction}$ (kcal/mol)	H bond (%)
		Properties of Conformational Cluster 1			
MD #1	2.59 (±0.22)	76.40 (±8.74)	2.90 (±0.35)	−15.59 (±3.05)	0
MD #3	2.51 (±0.25)	75.27 (±9.35)	2.85 (±0.35)	−15.35 (±2.96)	0
MD #4	2.57 (±0.23)	70.58 (±6.47)	2.90 (±0.31)	−15.30 (±2.86)	0
SGMD #1	2.37 (±0.30)	69.24 (±11.31)	2.68 (±0.37)	−14.06 (±2.60)	0
SGMD #2	2.39 (±0.30)	63.66 (±11.76)	2.65 (±0.32)	−13.05 (±2.80)	0
		Properties of Conformational Cluster 2			
MD #1	1.89 (±0.25)	78.15 (±8.46)	2.45 (±0.33)	−16.51 (±2.96)	0
MD #3	1.76 (±0.25)	77.13 (±8.90)	2.27 (±0.32)	−16.47 (±2.97)	0
MD #4	1.86 (±0.26)	71.46 (±6.55)	2.35 (±0.30)	−16.65 (±2.78)	0
SGMD #1	1.51 (±0.27)	74.70 (±10.28)	2.01 (±0.31)	−16.69 (±3.36)	0
SGMD #2	1.60 (±0.27)	70.11 (±12.32)	2.19 (±0.48)	−15.01 (±3.60)	0
		Properties of Conformational Cluster 3			
MD #1	0.91 (±0.34)	77.04 (±9.00)	1.42 (±0.46)	−16.38 (±2.91)	0
MD #3	0.89 (±0.31)	76.55 (±9.31)	1.48 (±0.43)	−16.58 (±2.93)	0
MD #4	0.92 (±0.31)	76.39 (±8.63)	1.49 (±0.41)	−17.47 (±2.61)	0
SGMD #1	0.71 (±0.26)	75.32 (±9.87)	1.23 (±0.42)	−16.88 (±2.69)	0
SGMD #2	0.81 (±0.29)	65.66 (±11.56)	1.38 (±0.37)	−15.54 (±3.07)	0
crystal structure	0.53	69.6	1.87	0	

[a] Host−guest distance is the center of mass distance between the host and guest (considering heavy atoms only). Phenyl ring−O4 is the angle the phenyl ring of benzyl alcohol guest makes with the plane of the glycosidic oxygens. Polar end distance is between the center of mass of the "polar end" of the guest (the oxygen in benzyl alcohol along with the carbon atom bonded to it) and the center of the oxygens of the secondary hydroxyls in β-cyclodextrin. The interaction energy between β-cyclodextrin and the benzyl alcohol guest considered is listed as $E_{interaction}$. H bond is the percentage of conformers collected during the simulation trajectories in which the benzyl alcohol guest forms at least one hydrogen bond with any of the host's secondary hydroxyls. Distances are given in angstroms, angles in degrees, energies in kcal/mol.

suggests that the benzyl alcohol is mobile inside the cavity of β-cyclodextrin.

Thus, our MD simulation results complement experimental results and suggest that benzyl alcohol binds to β-cyclodextrin in solution with several major conformational clusters. The binding mode as determined by X-ray crystallography is one of these major conformational clusters. MD simulations also suggest that two of these binding modes are even more populated than the binding mode as determined by X-ray crystallography. Binding of the benzyl alcohol guest is found to cause no significant distortion to the structure of the β-cyclodextrin in solution.

Binding Simulations Using Conventional MD and SGMD Methods. The SGMD method was shown to have a much-

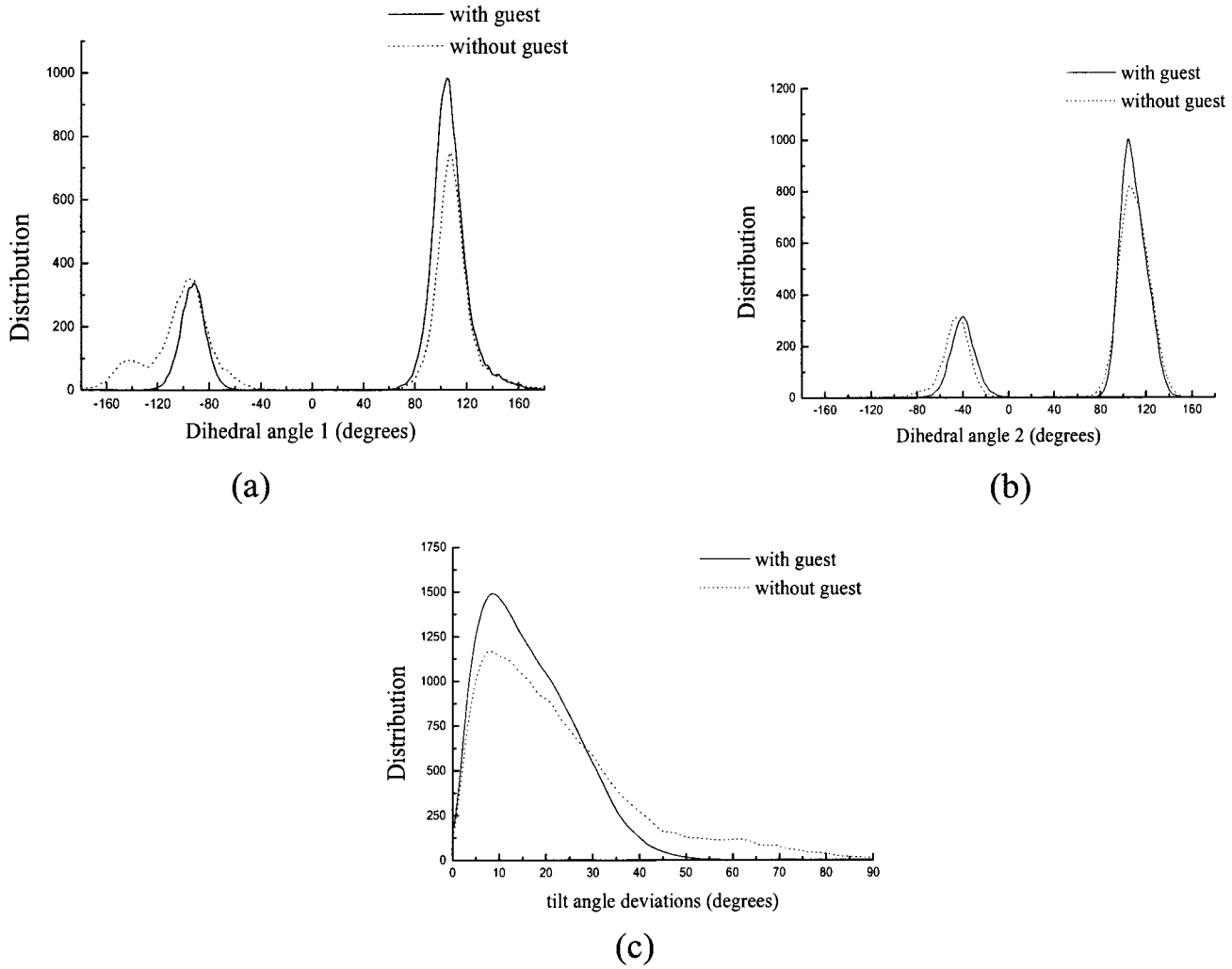


Figure 3. Comparison of the conformations of cyclodextrin during MD #1 run with a guest and a 10 ns long MD simulation of cyclodextrin without a guest (MD #2). All seven glucose units are considered. (a). Dihedral angle 1 (C3−C4−O4−C1) distribution. (b). Dihedral angle 2 (O5−C1−O4−C4) distribution. (c). Tilt angle deviation distribution.

improved efficiency in conformational searching as compared to conventional MD method.[14,15,26,27] We have therefore employed the SGMD method to study the binding of benzyl alcohol and β-cyclodextrin. In addition, we have also performed lengthy simulations using the conventional MD method to study the binding of benzyl alcohol and β-cyclodextrin. We investigated if it is possible to study reversible (binding, un-binding, and re-binding) and competitive (one unbound guest molecule displacing the bound guest molecule during the simulation) with this system using relatively short SGMD simulations and long MD simulations. The reversible and competitive binding then would allow us to investigate additional aspects on host−guest binding, such as the role of hydrophobic interactions and hydrogen bonding in binding. Most importantly, the reversible and competitive binding simulations make it possible for us to predict the binding mode of the guest to the host molecule starting from unbiased unbound configurations for the system.

Two SGMD simulations were performed for 1.5 and 2.5 ns, respectively, on a system containing one β-cyclodextrin and six benzyl alcohol molecules in solution. The setup of the two SGMD simulations (SGMD #1 and SGMD #2) were essentially the same but with two totally different starting configurations, as shown in Scheme 2. The distances between the center of mass of β-cyclodextrin and the center of mass of each of the six benzyl alcohol molecules in both starting conformations are between 10 and 12 Å. As mentioned above, including several guest molecules in the system allows the investigation of competitive guest molecule binding to the host.

We performed two 12.5 ns long conventional MD simulation runs (MD #3 and MD #4) using the same initial conformations and simulation conditions as for the two SGMD runs in order to investigate the following additional aspects. Does guest to host binding occur within the time frame of the MD simulations? If so, how are the predicted binding models compared to those obtained through the SGMD method and those obtained from the MD simulation started from the X-ray complex structure?

First, we investigated whether the guiding force introduced by the SGMD method with the current parameter set causes any significant distortion in our system. For this purpose, we repeated the 10 ns simulation on β-cyclodextrin in the absence of benzyl alcohol using the SGMD method and compared to the results obtained using the conventional MD simulation. The results are shown in Figure 4a−c. As can be seen, the dihedral angle and tilt angle distributions show that there are no considerably more distortions in the SGMD simulation as compared to that in the MD simulation.

Examination of the SGMD and MD simulation trajectories show that in each of these four simulations, binding of one or more guest molecule to the host does occur. The results thus suggest that the binding of benzyl alcohol to β-cyclodextrin takes places within the 12.5 ns of MD simulations and within 1.5 and 2.5 ns of SGMD simulations. The favored solution

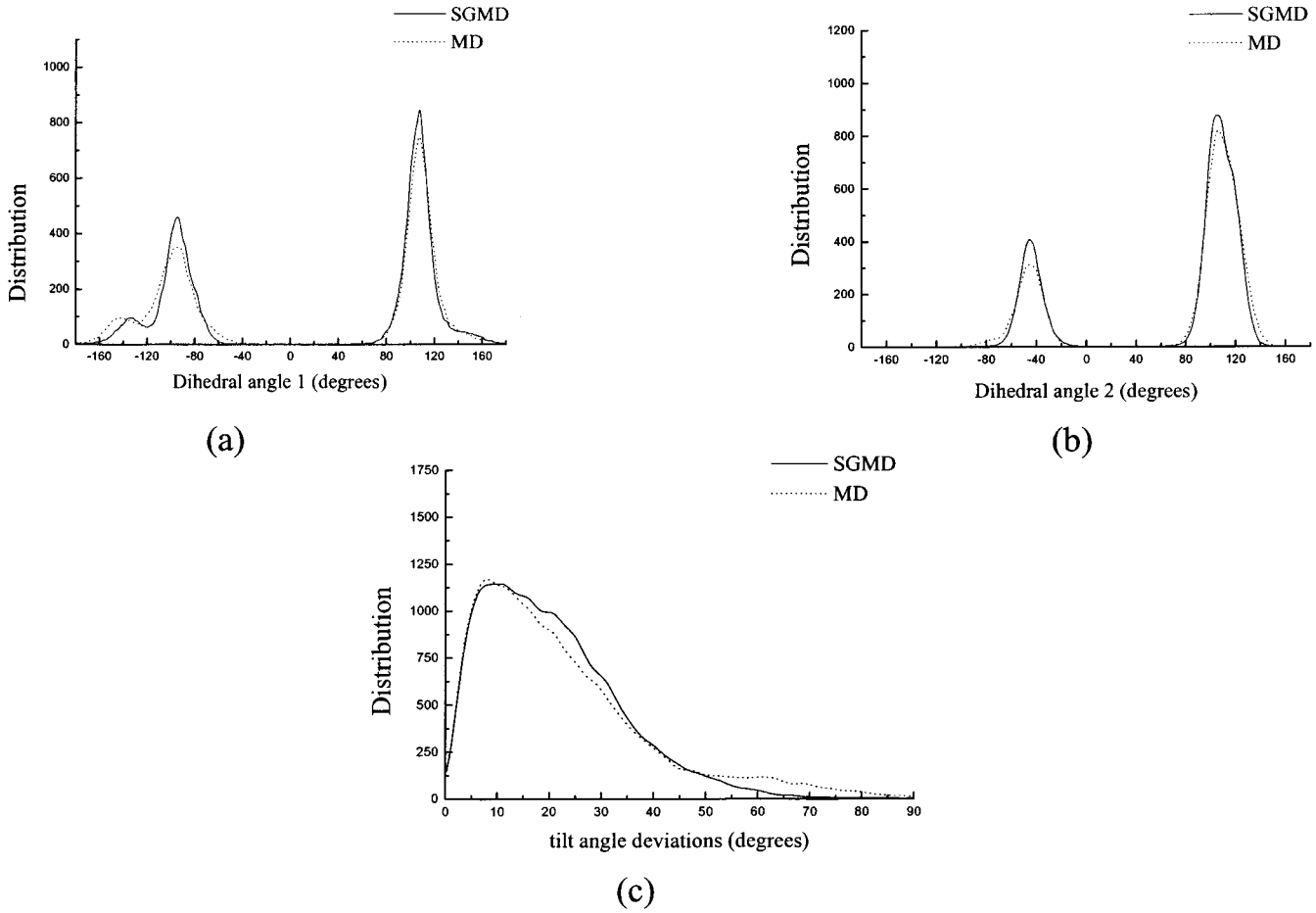


Figure 4. Comparison of the conformations of cyclodextrin during MD and SGMD simulations. (a). Dihedral angle 1 (C3−C4−O4−C1) distribution. (b). Dihedral angle 2 (O5−C1−O4−C4) distribution. (c). Tilt angle deviation distribution.

conformations predicted by the MD and SGMD simulations were determined by clustering the saved structures obtained from each simulation run. The same clustering parameters and criteria were used in all MD/SGMD simulation runs, as described in detail in the Methods section. Characteristic properties of the most populated conformational clusters in the SGMD simulations were compared with those obtained from the conventional MD simulation (Table 1). Those found essentially identical are labeled with the same cluster numbers throughout Figure 2. All benzyl alcohol molecules that become "tightly" bound to the host any time during the simulation run were included in conformation clustering.

Except for loosely bound conformational cluster (LB), clusters 1, 2, and 3 were identified as the most populated conformational clusters in SGMD #1 and SGMD #2 (Figures 2b and 2c) started from totally different initial conformations. This is in good agreement with the MD simulation started from the X-ray determined complex structure (Figure 2a). Thus, cluster 3, which closely resembles the X-ray structure, was identified as a significant conformational cluster in all the three simulations, although not the most populated one in MD #1 and SGMD #1. In SGMD #2, two other clusters 4 and 5, emerge as two other major clusters in addition to clusters 1, 2, and 3.

The major conformational clusters obtained from MD #3 and #4 are provided in Figure 2d and 2e. As can be seen, both MD simulations identify clusters 1, 2, and 3 as major conformational clusters. In addition, several other clusters emerge as major conformational clusters from MD #4. Taken together, the two lengthy MD simulations confirmed that clusters 1, 2, and 3 identified from SGMD simulations as three major conformational clusters. All the MD and SGMD simulations started from unbound configurations identify cluster 3, which closely resembles the X-ray complex structure as one of the major conformational clusters, although not the most populated one in most of the simulations. It is of note that the rank of order of these three major conformational clusters (clusters 1, 2,and 3) differs somewhat, suggesting that current simulations are probably still not sufficiently long to allow for accurate quantitative prediction of the population for each conformational cluster.

To investigate whether reversible and/or competitive binding takes place during the simulations, we plotted the distance between the centers of the mass of the host and guest for the two SGMD simulations and two corresponding MD simulations (Figure 5a−d). For clarity, benzyl alcohol molecules that do not become bound to the host during the entire simulation time are not shown in Figure 5. Examination of the distance plots for all the 6 guest molecules in MD#3 showed that only one guest molecule was found to bind to the host during this simulation (Figure 5a). Once the guest molecule becomes bound to the host, it stays bound during the entire remaining period of the simulation. Thus, in MD #3, no reversible binding or competitive binding takes place within the 12.5 ns simulation time.

In MD #4, two guest molecules were found to bind to the host during the simulation (Figure 5b). The first guest molecule begins to bind to the host at approximately 1.3 ns and remains bound until 3.5 ns, when the second guest molecule starts to bind to the host molecule, by displacing the first guest molecule (Figure 5b). The second molecule remains bound until 5.0 ns,

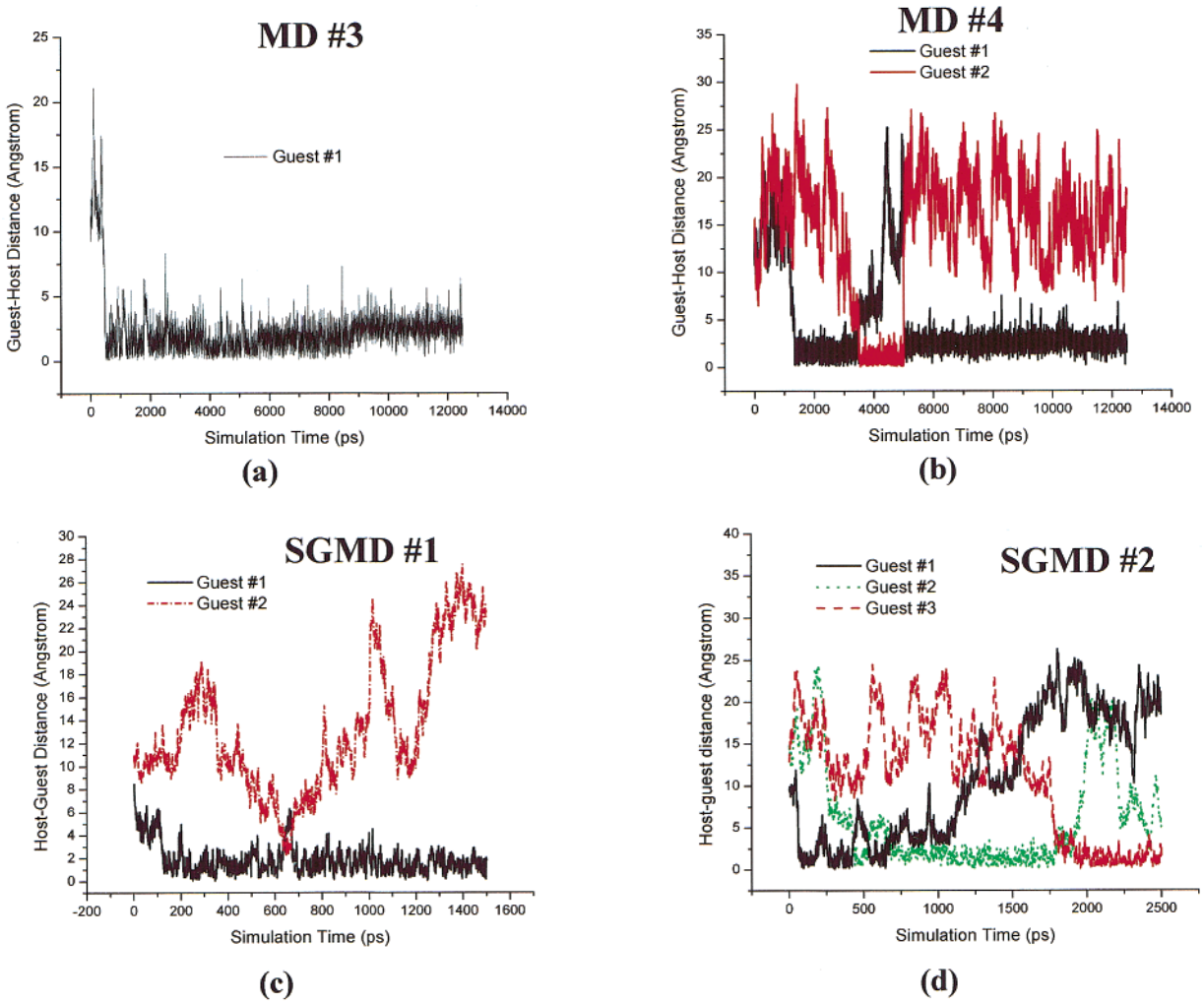


Figure 5. Distance between the centers of the mass of guest and host molecules during MD and SGMD simulations. For clarity, we plotted only the distances for guest molecules, which become tightly bound (within 3 Å) to the host molecule for longer than 30 ps during simulations.

when the first guest molecule begins to form a complex with the host, by displacing the second guest molecule. The first guest molecule remains bound to the host for the entire remaining period of the simulation. It is also clear that from Figure 5b, the binding is competitive since the binding of a "new" guest molecule to the host always results in displacement of the "old" guest molecule. Although two guest molecules can "loosely" bind to the host for a certain time period, they never simultaneously "tightly" bind to the host. Therefore, during MD simulation #4, reversible and competitive binding takes places.

Analysis of the SGMD simulation #1 showed that reversible and competitive binding also takes place during this simulation (Figure 5c). The first guest molecule quickly forms a complex with the host at 100 ps and remains bound until 635 ps, when the second guest molecule starts to form a new complex and stays tightly bound until 667 ps. However, the first guest molecule stays loosely bound during this period. At 667 ps, the first guest molecule becomes "tightly" bound to the host, by displacing the second guest molecule and remains tightly bound for the remaining period of the simulation.

Analysis of the second SGMD simulation (SGMD#2) showed that three guest molecules form a "tight" complex during the simulation period (Figure 5d). The first guest molecule starts to form a complex at 62 ps and stays "tightly" bound until 200 ps, when it becomes loosely bound. It remains loosely bound until 250 ps, when it forms a "tight" complex until 430 ps. At this point, the first guest molecule is displaced by the second guest molecule. The second guest molecule forms a tight complex for 100 ps. At approximately 530 ps, the first guest molecule returns to the host and forms a "tight" complex with the host, by displacing the second guest molecule. But the second guest molecule still remains loosely bound to the host. The first guest molecule remains tightly bound to the host until 670 ps, when it is displaced by the second guest molecule. The first guest molecule gradually becomes totally unbound from the host. The second guest molecule remains tightly bound to the host until approximately 1800 ps, when the third guest molecule becomes bound to the host. Between 1800 and 1900 ps, both the second and third guest molecules are loosely bound to the host. At 1900 ps, the third guest molecule becomes tightly bound to the host by displacing the second guest molecule. The third guest molecule remains tightly bound during the remaining period except for a brief period between 2413 and 2430 ps, when both the second and the third guest molecules are loosely bound to the host. Therefore, during the 2.5 ns SGMD simulation, we observe several reversible and competitive binding events.

In summary, during a total of 25 ns MD binding simulations, a total of 4 binding "events" take place. In comparison, during a total of 4 ns SGMD binding simulations, a total of 8 binding "events" take place. Therefore, the SGMD method is approximately 12-times more efficient in simulating the binding of benzyl alcohol to β-cyclodextrin than the conventional MD simulation. Importantly, in both relatively short SGMD simula-

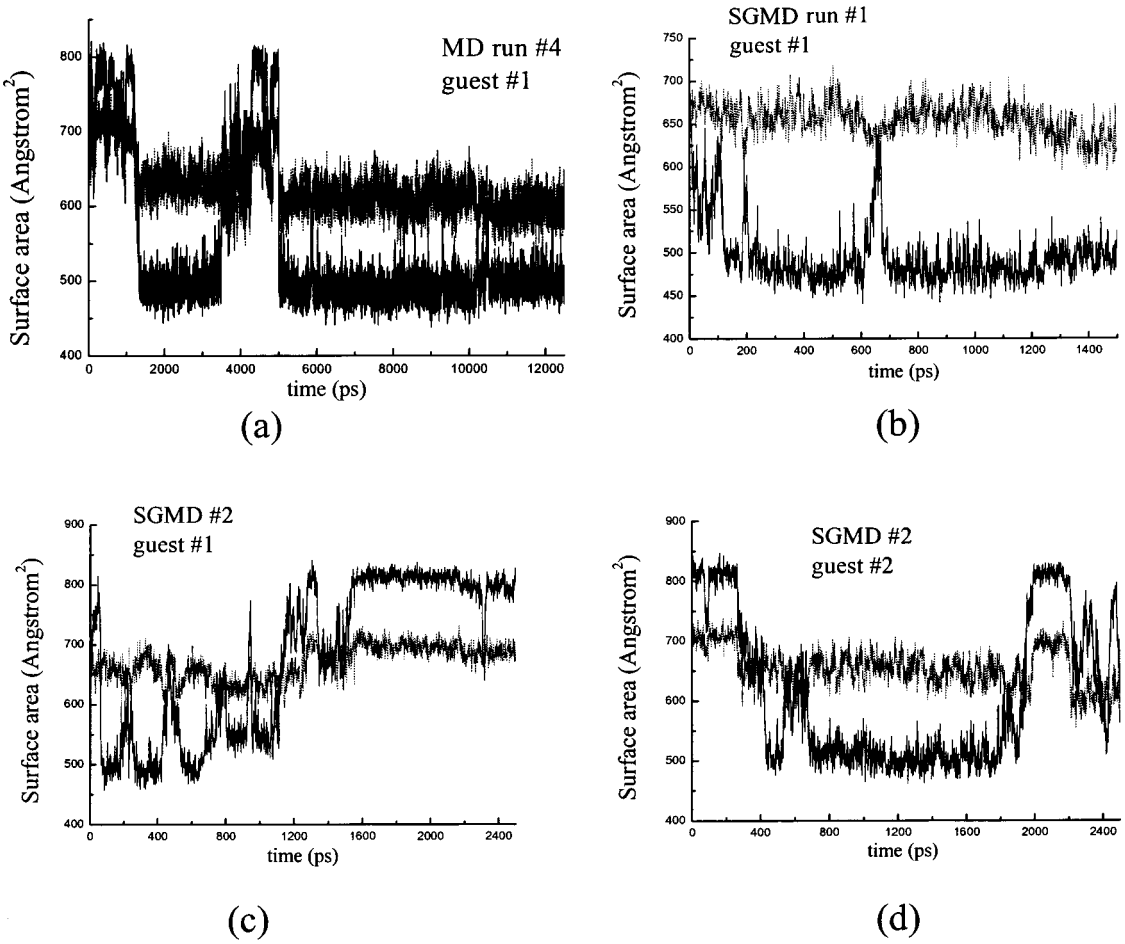


Figure 6. Combined host−guest surface area changes during MD and SGMD simulation runs. The hydrophobic surface area is in solid lines, the hydrophilic in dashed lines.

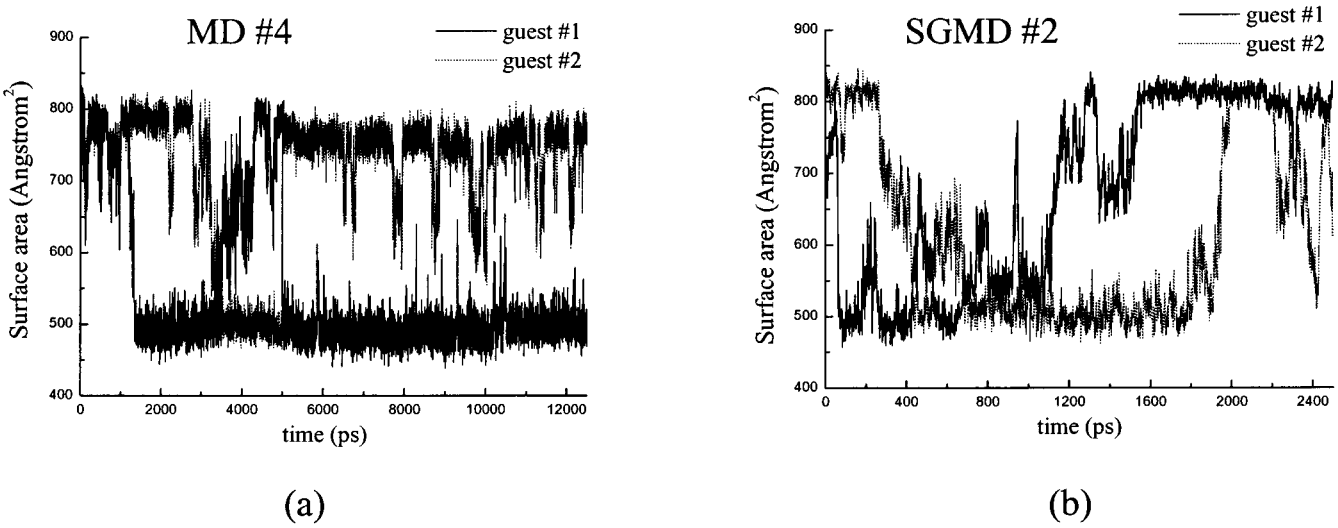


Figure 7. Hydrophobic surface area changes during competitive binding. (a) Guest #1 and #2 in MD #4. (b) Guest #1 and #2 in SGMD #2.

tions, reversible and competitive binding is observed. In contrast, in the first 12.5 ns MD binding simulation, reversible and competitive binding is not observed. It is of note that each SGMD step takes essentially the same amount of computer time as each MD step.[14,15]

To investigate what type of forces may be responsible for benzyl alcohol binding to β-cyclodextrin, we examined the change in hydrophobic and hydrophilic surface during the simulations during both SGMD and MD simulations. The results are shown in Figures 6 and 7. In all the cases, no significant change is found for the combined host−guest hydrophilic surface exposed to solvent before and after the guest becomes bound to the host (Figure 6). In contrast, there is always a significant reduction in the combined hydrophobic surface of the guest and the host exposed to solvent upon binding. Furthermore, in simulation periods when competitive binding occurs, there is also a significant reduction in the combined solvent-exposed hydrophobic surface of the incoming guest and

the host molecule (Figure 7) during both MD and SGMD simulations. Simultaneously, the combined solvent exposed hydrophobic surface area of the host and the leaving guest increases (Figure 7).

Interestingly, none of the major conformational clusters show hydrogen bonding between the bound guest molecule and the host (Table 1). Our results suggest that the burial of the hydrophobic moiety of the benzyl alcohol into the host but not the hydrogen bonding is responsible for the benzyl alcohol−β-cyclodextrin binding, though it is possible that hydrogen bonding plays a role in "guiding" the guest during complexation into the "right" position. Our prediction that hydrophobic forces dominate benzyl alcohol−β-cyclodextrin binding is consistent with other studies.[1,20−21]

Summary

In this study, we have used SGMD and conventional MD simulation methods to study the binding of benzyl alcohol and β-cyclodextrin in aqueous solution. Through lengthy MD simulations, we showed that the X-ray determined binding mode represents one major conformational cluster in solution but there are several other major conformational clusters identified. During two relatively short SGMD and one lengthy MD simulations starting from unbound configurations for the system, we observed reversible and competitive binding of the guest molecule to the host. On the basis of the analysis of conformations saved during the MD and SGMD simulations we have predicted the binding modes for the guest and host system. The X-ray determined binding mode is one of the major conformational clusters. Furthermore, the reversible and competitive binding simulations suggest that the major host−guest interactions during binding are associated with the burial of the hydrophobic surface area of the guest molecule to the host but not the hydrophilic/hydrogen bonding interactions. Our study paves the way for the study of other host−guest systems in aqueous solution.

Supporting Information Available: Topology and parameter files for cyclodextrin and benzyl alcohol. This material is available free of charge via the Internet at http://pubs.acs.org.

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