A molecular dynamics study of branched α-cyclodextrin

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A branched α-cyclodextrin is a derivative of an α-cyclodextrin with a branch consisting of an extra glucose unit. Its water solubility is considerably higher than that of the unbranched one. We have studied the high solubility of the molecule in aqueous solution by molecular dynamics simulations. Trajectories of the molecule at 293 K were calculated using GROMOS programs in three different environments, i.e., in vacuo, *in the crystalline state, and in aqueous solution. A simulation* in vacuo *was carried out to explore stable conformations of the molecule in the isolated system. The quality of the simulations were examined by comparing the X-ray and the simulated crystal structures.*

The results of the simulations show three remarkable structural features of the molecule: self-inclusion with its branched portion, twist-boat conformation of a glucose ring, and wobbling of its macrocycle. Among these, the last feature is closely related to the water solubility of the molecule. The solubility of cyclodextrin appears to be mainly governed by its intramolecular interglucose hydrogen bonds, which inhibit hydration by solvent water molecules. The results of our simulations indicate that the capability to form hydrogen bonds in branched α-cyclodextrin decreased as the macrocycle of the molecule lost its regular circular shape. Such wobbling of the macrocycle was observed on a relatively short time scale (several picoseconds). An extra glucose unit introduced to α-cyclodextrin may cause the improved water solubility of the molecule through the greater wobbling motion of its macrocycle.

Keywords: Cyclodextrin, molecular dynamics, GROMOS, self-inclusion, hydrogen bond, solubility

INTRODUCTION

Cyclodextrins are naturally occurring cyclic oligosaccharides built up from 6, 7, or 8 glucose units (for α-, β- or γ-cyclodextrin, respectively), which are covalently linked to each other by α-(1-4) glycosidic bonds.[1] The doughnut-shaped molecule provides a hydrophobic cavity with a diameter of about 0.57–0.95 nm at the center, and a hydrophilic outer surface. Cyclodextrins can form inclusion complexes with many substances,[2] especially with hydrophobic drug molecules, which fit into their cavities. Due to their complexing capabilities, they have been used extensively as solubilizing and stabilizing agents in pharmaceutical and other industries. Cyclodextrins themselves, however, have relatively low water solubility, which appears to be related to their greater tendency to form intramolecular hydrogen bonds between adjacent hydroxyl groups, thus preventing hydration of the cyclodextrins by solvent water molecules. Recently, cyclodextrins with chemically modified hydroxyl groups have been developed, and their greater complexing capabilities and improved water solubilities were reported.[3–6]

Cyclodextrins with one or more branches of an α-D-glucopyranosyl unit or a (1-4)-α-D-glucan are called branched cyclodextrins. Branched cyclodextrins have been enzymatically synthesized, and have received considerable attention because of their physicochemical characteristics:[7,8] high water solubility, hemolytic activities decreasing with elongation of the branched chain, complexing capabilities comparable to their parent molecules, and greater solubilizing capabilities compared with their parent molecules. The reason for the improved water solubility is not so obvious as in the case of the chemically modified cyclodextrins, since the formation of intramolecular hydrogen bonds does not easily seem to be prevented by introducing the branches.

Previously we reported the X-ray structure of 6-O-α-D-glucosyl-α-cyclodextrin (G-α-CyD),[9] which is a derivative of α-cyclodextrin (α-CyD) with a branch of an α-D-glucopyranosyl unit at a C6 atom of a glucose unit of α-CyD (Figure 1). This molecule is approximately 5 times as water soluble as unmodified α-CyD.[7] In the reported structure, the G-α-CyD molecule forms an intermolecular inclusion complex with the branched glucose unit of the adjacent G-α-CyD molecule, as shown in Color Plate 1. The two molecules are crystallographically equivalent, and thus adopt the same conformation. Owing to the inclusion, the branched portion stays entirely out of the cavity of the molecule, to which the branched portion itself is attached. Although such an exposed form is stabilized by packing-energy in crystalline state, the form seems to be energetically unfavorable when isolated. We could not clarify the relationship between its structure and its solubility only from

Color Plates for this article are on page 294.

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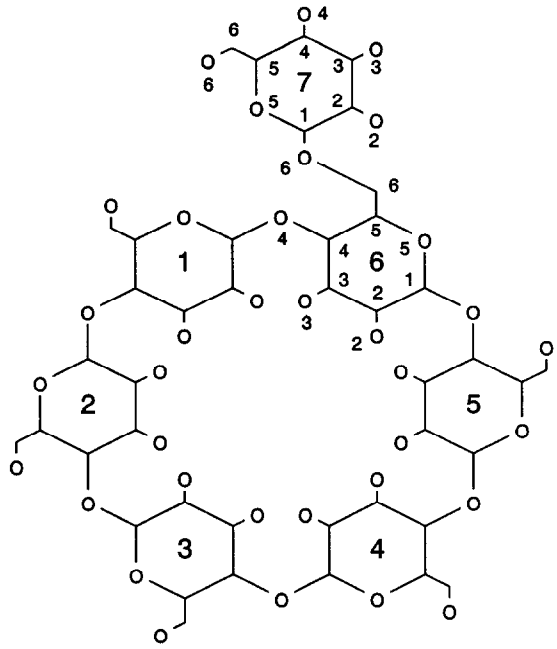

Figure 1. Chemical structure of G-α-CyD. Numberings for all glucose units (at the centers of hexagons of glucose rings) and the names of atoms in glucose units 6 and 7 are shown.

the X-ray structure of the G-α-CyD molecule. We have, thus, carried out molecular dynamics (MD) simulations of the G-α-CyD molecule so as to investigate the relationship between the improved solubility and the structure of the molecule. In the simulation, the G-α-CyD molecule revealed three remarkable structural features in aqueous solution: self-inclusion with its branched portion, twist-boat conformation of a glucose ring, and wobbling of its macrocycle. Especially, we found that the last feature is related to the molecule's intramolecular hydrogen bonds and water solubility. In this paper, we report the results of the MD simulations.

METHODS

Molecular modeling

The extended GROMOS force field parameters[10] were employed in our simulations of G-α-CyD. The extension was made by Koehler et al. to accurately reproduce the experimental properties of α-CyD and β-CyD.[11,12] For the modeling, hydroxyl hydrogen atoms were treated explicitly, while hydrogen atoms attached to carbon atoms were incorporated into the latter. The water molecules were modeled with a simple 3-point charge, SPC model.[13] During the simulations, all lengths of the bonds involving hydrogen were constrained by applying the SHAKE method[14] with relative geometric accuracy of 1×10^{-4}.

Simulation *in vacuo*

Starting coordinates for the trajectory *in vacuo* were taken from the X-ray structure of G-α-CyD molecule. After introducing hydrogen atoms geometrically, the molecule was energy minimized with 154 steps of the conjugate gradient algorithm, until the difference in energy decreased to less than 0.10 kJ/mol. A 100-ps trajectory was then calculated with a time step of 2 fs. In the simulation, we considered the interactions without introducing any cut-off.

Simulation in the crystalline state

As previously reported,[9] G-α-CyD crystallized in the space group $P2_12_12_1$ with lattice constant $a = 2.182$ nm, $b = 1.931$ nm, and $c = 1.351$ nm, containing a G-α-CyD and 8 water molecules in the crystallographic asymmetric unit. The crystal was mimicked by setting the computational box to be rectangular, with dimensions $a \times b \times 2c$ containing 8 G-α-CyD and 64 water molecules, and applying the periodic boundary condition corresponding to the unit cell translational symmetry $(a,b,2c)$. The dimension of c-axis was doubled because a cut-off radius of 0.8 nm was used for both Lennard–Jones and electrostatic interactions. The radius might be slightly smaller than those used in recent MD simulations. We, however, chose the value which Koehler et al. employed in the accurate simulation of α-CyD, so that our results could easily be compared with theirs.

Starting coordinates for the trajectory in the crystalline state were obtained from the X-ray structure. After hydrogen atoms were geometrically introduced, the molecular system was energy minimized with 107 steps of the conjugate gradient algorithm, until the difference in energy decreased to less than 0.1 kJ/mol. A 100-ps trajectory was then calculated with a time step of 2 fs. The system had been coupled to an external thermal bath at 293 K with a temperature relaxation time of 0.01 ps or 0.1 ps for the first 10 ps or the subsequent time period, respectively.[15]

Simulation in aqueous solution

A G-α-CyD and 8 water molecules in the crystallographic asymmetric unit were put on the center of the rectangular computational box (2.563 nm × 2.603 nm × 3.208 nm), which was 1 nm longer in length than the G-α-CyD molecule in any direction. Additional water molecules were then inserted into the box by immersing it in an equilibrium configuration of bulk SPC water,[13] and subsequently removing all water molecules of whose oxygen atom lay within 0.23 nm of a non-hydrogen G-α-CyD atom. Consequently, one G-α-CyD and 664 water molecules had been contained in the computational box. The positions of water atoms were first energy minimized with the conjugate gradient algorithm constraining the position of the G-α-CyD atoms. The entire system was then energy minimized until the difference decreased to less than 0.1 kJ/mol.

MD simulation was carried out, taking periodicity into account, at constant pressure (1 atm) and temperature (293 K). To avoid slow drifts, relaxation times of 0.05 ps and 0.01 ps were used for pressure and temperature, respectively, in the first 10 ps. In the subsequent period, relaxation times of 0.5 and 0.1 ps were used for the pressure and temperature, respectively. The compressibility of the system was set to 0.7624×10^{-3} kJ/mol/nm^3.[15] Other computational conditions were the same as those in the simulation for

the crystalline state. In this way, a 200-ps trajectory was calculated.

RESULTS AND DISCUSSION

Dynamics of G-α-CyD

The total potential energy had been sufficiently stabilized within the first few picoseconds of every simulation in the three different states, i.e., *in vacuo,* in the crystalline state and in aqueous solution. For every simulation, we chose an equilibration period of 20 ps to fully cover the first time period where the system seemed to be unequilibrated. In aqueous solution, considerable conformational transitions had been detected until 100 ps, as will be discussed later. We therefore performed an additional 100-ps simulation in aqueous solution. *In vacuo* or in the crystalline state, such transitions had not been detected after equilibration period, so that we ceased these simulations at 100 ps.

We carried out a simulation in the crystalline state to validate the employment of the GROMOS force field to the G-α-CyD molecule. The crystal structure of the G-α-CyD molecule as derived from the simulation was obtained by averaging over the 8 molecules in the computational box during the period from 20 ps to 100 ps. The root-mean-squared positional difference between the simulated and X-ray structures was 0.037 nm, except for hydrogen atoms. The agreement was comparable to that reported for the simulations of α- and β-CyDs.[11,12] All bond angles were also well reproduced by the simulation. The mean absolute difference of bond angles between the simulated and X-ray structures was 2.24 degrees, except for those concerned with hydrogen atoms. Dihedral angles were, as well, reproduced excellently. The mean absolute difference of dihedral angles between the simulated and X-ray structures was 4.35 degrees, except for those for hydrogen atoms and the O6 atom of glucose unit 7. It seems that the location of this terminal atom is not restricted so tightly as other atoms. These results suggest that our modeling of G-α-CyD using GROMOS force field is appropriate for investigating the structural feature of the molecule.

Self-inclusion behavior

G-α-CyD of the self-included form is one of the most interesting points in the results of our simulations. Color Plate 2 is a snapshot of a G-α-CyD molecule in aqueous solution at 75 ps. A part of the branched portion stays inside the cavity of the molecule to which the branched unit itself is attached. Figure 2 shows the time variation of the distance of the O2 atom of the branched unit from the least-squares plan for the six O4 atoms in cyclodextrin macrocycle. This distance is a good measure of self-inclusion behavior, because the O2 atom tended to be included into the cavity more deeply than other atoms. The G-α-CyD molecule is in self-included form during time periods when the distance is less than or comparable to that of the instance (at 75 ps) shown in Color Plate 2. With this measure, self-inclusion behavior was detected in the simulation *in vacuo* as well as for that in aqueous solution. On the other hand, the molecule in the crystalline state always adopts the exposed form. In its crystal structure, as mentioned previously, a branched portion of the G-α-CyD molecule forms an inclusion complex with another G-α-CyD molecule. Owing to the inclusion, the branched portion of a molecule is entirely located out of the cavity of this molecule (Color Plate 1). Although such an exposed form is stabilized by the packing-energy in the crystalline state, the form seems to be energetically unstable when the molecule is isolated. This is supported by the result that the G-α-CyD molecule remains in self-included form *in vacuo* after an equilibration period of 20 ps.

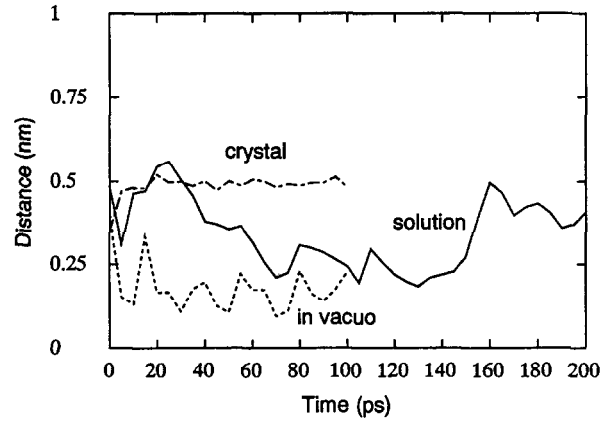

Figure 2. Time evolution of the distance of the O2 atom in the branched units from the least-squares plane for O4 atoms in the cyclodextrin macrocycle.

In aqueous solution, the G-α-CyD molecule adopted either the self-included form, which should be energetically favorable in an isolated situation, or the exposed form. Although our simulation might be too short to deduce what happens on a longer time scale, it may be that the G-α-CyD molecule makes a transition from the exposed form to the self-included form, or vice versa, in aqueous solution, repeatedly. The most obvious distinction between the gas phase (*in vacuo*) and solution is that water molecules are present only in the latter case. Solvent water molecules, therefore, play some important role in the self-inclusion behavior of the G-α-CyD molecule in aqueous solution.

Glucose ring conformation

As shown in Figure 3, remarkable conformational change on glucose unit 3 was observed. Figure 3 is a plot of the time variation of C1-C2-C3-C4 glucose ring torsion angles in aqueous solution. Glucose ring conformation of unit 3 changed from chair to twist-boat at 60 ps as the torsion angle of that unit changed from about −50 degrees to about 50 degrees; and it finally returned to chair at 100 ps. Such a conformational transition of glucose ring was observed on the molecule only in aqueous solution. To our knowledge, no glucose unit of cyclodextrins in crystal structure adopts any conformation other than the chair-type. The reason why the twist-boat conformation was observed only in aqueous solution could not been clarified through the results presented in this paper. However, solvent water molecules might be involved in the transition, as was the case for the self-inclusion behavior.

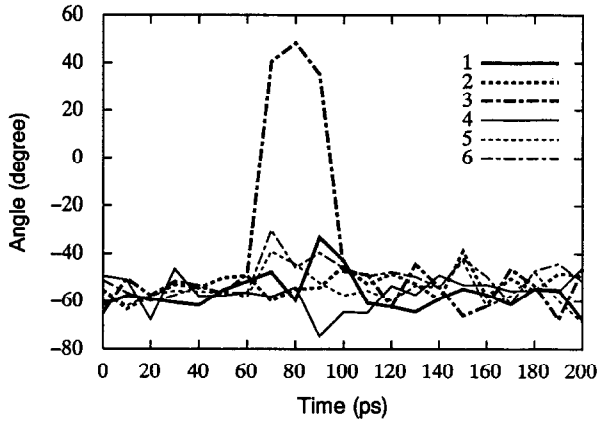

Figure 3. Time evolution from 0 ps to 200 ps of the six glucose ring torsion angles C1-C2-C3-C4 in aqueous solution. The torsion angles of the six glucose units, except the branched one, are plotted.

Conformation of macrocycle

Another interesting behavior of the G-α-CyD molecule is the wobbling of its macrocycle. We previously remarked the motion of a branched portion (self-inclusion behavior). Furthermore, the shape of the body of the G-α-CyD molecule also varies on a relatively short time scale. Color Plate 3 is a view of G-α-CyD molecule at 110 ps together with the least-squares elliptic ring. The elliptic ring was obtained by the least-square method considering the position of six O4 atoms in the cyclodextrin macrocycle. The instance shown in Color Plate 3 shows one of the most distorted shape of macrocycle. The larger semiaxis of the elliptic ring tended to be located along the line through glucose units 3 and 6, approximately. The location of the branched portion seems to affect the orientation of the axis.

Time variations of the ratio of the larger and smaller semiaxes of the ellipse is plotted in Figure 4. For the simulation of the crystalline state, a curve averaged over 8 molecules in a computational box is plotted for simplicity. The greatest ratio without averaging is 1.35 in this case. This value is considerably less than that in aqueous solution, and is greater than that *in vacuo*. Therefore, the wobbling motion of the G-α-CyD molecule in aqueous solution is more remarkable than other situations.

On the formation of host–guest complexes, the guest molecules may be hydrophobic as well as hydrophilic, and the dominant factor is whether the size and shape of the guest molecules permit it to fit spatially into the cavity of cyclodextrin. Greater wobbling of G-α-CyD implies the improved capability to form a host–guest complex with a flatter molecule. A drastic improvement in the solublity of 1-monoolein by G-α-CyD[7] may be due to this reason.

Solubility of G-α-CyD

Cyclodextrins can form intramolecular interglucose hydrogen bonds between the O2 and O3 atoms of glucose units adjoining each other. These hydrogen bonds are involved in the water solubility of cyclodextrins. When these hydrogen bonds have formed, solvent water molecules cannot hydrate the O2 and O3 atoms of a cyclodextrin molecule. The relatively low solubilities of some cyclodextrins are thus believed to be due to the intramolecular hydrogen bonds. Higher water solubility of G-α-CyD than α-CyD could be explained with the hydrogen bonds as the following. For both the α-CyD and G-α-CyD molecules, the number of possible O2—O3 hydrogen bonds is six. Figure 5 shows the time varation of these O2—O3 distances for the G-α-CyD molecule. In the simulation of α-CyD reported by Koehler et al.,[11] these six distances fluctuated within the range 0.25–0.40 nm, except for extreme instances in the equilibration period. Also, the distance averaged over the six glucose units was in the range 0.30–0.34 nm at all times.[11] In the case of G-α-CyD, however, instances with distances greater than 0.40 nm can be frequently seen, and the value averaged over the six glucose units is in the range 0.29–0.37 nm. The average value lies in almost the same range even if the extreme case before 100 ps is excluded. It is assumed that between two oxygen atoms O—O hydrogen bonds are never

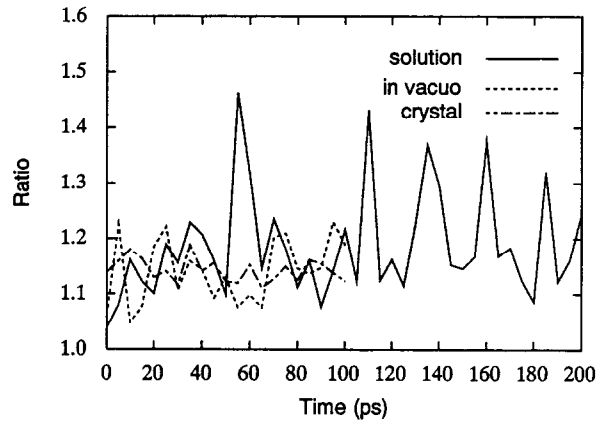

Figure 4. Time evolution of the ratio of larger and smaller semiaxes of the least-squares ellipse for the six O4 atoms in the cyclodextrin macrocycle.

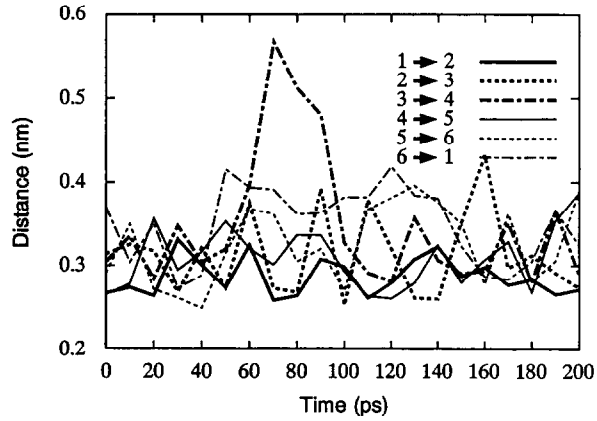

Figure 5. Time evolution from 0 ps to 200 ps of the distance between the O2 and O3 atoms of adjacent glucose units. The distance is a measure of the possibility of intramolecular and interglucose hydrogen bond formation.

formed with a distance greater than 0.35 nm. Therefore, fewer intramolecule interglucose hydrogen bonds than in α-CyD are formed in G-α-CyD. These results suggests that greater wobbling motion of G-α-CyD than that of α-CyD increases the solubility of the G-α-CyD molecule in water. Similar effects are expected for β-CyD, which is the most abundant and shows the lowest solubility in water among unmodified cyclodextrins.

In this paper, we have described the remarkable structural features of G-α-CyD, i.e., self-inclusion with its branched portion, twist-boat conformation of a glucose ring, and wobbling of the macrocycle. The last feature, especially, affects the formation of intramolecule interglucose hydrogen bonds which appear to be a dominant factor in the water solubility of the G-α-CyD molecule. Improved water solubility of G-α-CyD thus seems to be attributed to the wobbling of the macrocycle. However, we could not ascertain how the branch raises the extent of wobbling motion. And we have not investigated the quantitative relation between the structure and the solubility. Not only enthalpic factors, but also entropic ones, govern the solubility in a complicated manner. We would investigate the relation more deeply by examining the population of hydrogen bonds, the distribution of water molecules, and the hydration free energies.

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