



Hydration of β -cyclodextrin: A molecular dynamics simulation study

R.G. Winkler^a, S. Fioravanti^{a,b}, G. Ciccotti^b, C. Margheritis^c & M. Villa^c

^aAbteilung Theoretische Physik, Universität Ulm, D-89069 Ulm, Germany; ^bINFM-Dipartimento di Fisica, Università 'La Sapienza', Piazzale A. Moro 5, I-00185 Roma, Italy; ^cDipartimento di Chimica Fisica e Unità INFM di Pavia, Via Taramelli 16, I-27000 Pavia, Italy

Received 22 November 1999; Accepted 4 February 2000

Key words: β -cyclodextrin, hydration, hydrophobicity, molecular dynamics simulation

Summary

We study by molecular dynamics simulations the hydration of β -cyclodextrin. Our simulations show that within these barrel-shaped molecules hydrophobicity dominates, while at the top and bottom sides of the barrel interactions with water are mostly hydrophilic in nature. These results agree with crystallographic data at 120 K and, in particular, with the spontaneous hydration process of a cyclodextrin crystal in wet atmosphere. The predicted structure of the hydration shells is discussed and compared with previous molecular mechanics calculations which report an overall hydrophobic behavior. Moreover, the temperature dependence of the hydration process is discussed.

Introduction

Cyclodextrins (CDs) are a family of macrocyclic oligosaccharides consisting of D-glucose units linked by α (1–4) interglucose bonds (Figure 1). Four species of cyclodextrins are known with rings comprising from 6 to 9 glucose units: α -CD (6 units), β -CD (7 units), γ -CD (8 units) and δ -CD (9 units). During the past 30 years CDs have extensively been investigated in order to understand their structural, transport, and enzymatic properties and to develop various applications in chemistry and biological sciences [1, 2].

All CDs possess a barrel shape resembling that of a truncated cone (Figure 2). Its inner portion may be occupied by water and/or other *guest* molecules (or part of them) which form inclusion complexes with the CDs. The CDs own their importance to the fact that these complexes are soluble in water and that we may make and break them quite easily. In fields as different as biology, agriculture, or pharmacology, we may thus control activity and biological availability of the complexed molecules. Furthermore, the CDs are relatively simple molecules which bind to complex molecules such as vitamins, hormones, or benzenoid systems.

They are also good model systems to investigate the water–biomolecule interactions.

Since most of the reactions involving CDs take place in an aqueous environment, the interaction between cyclodextrins and water is of fundamental importance. In various experiments the hydration/dehydration processes of crystalline CDs have been studied, which appear to be almost perfectly reversible [3–5]. Less attention has been devoted to CD molecules in aqueous solution.

In this article we present molecular dynamics simulation (MD) results of the hydration of the best known member of the CD family, namely β -CD. We performed MD simulations of a single β -CD molecule exposed to various amounts of water molecules between 12 and 258, corresponding to a low-hydrated, highly hydrated, and a β -CD molecule in solution, respectively. Moreover, we investigated the hydration properties of a CD molecule at various temperatures, in particular at room temperature. Experiments are usually performed at rather low temperatures (e.g., 120 K) on crystals. Our simulations provide insight on how the hydration properties in solution change with temperature.

Previous molecular mechanics (MM) calculations of a β -CD in aqueous solution [6, 7] showed an over-

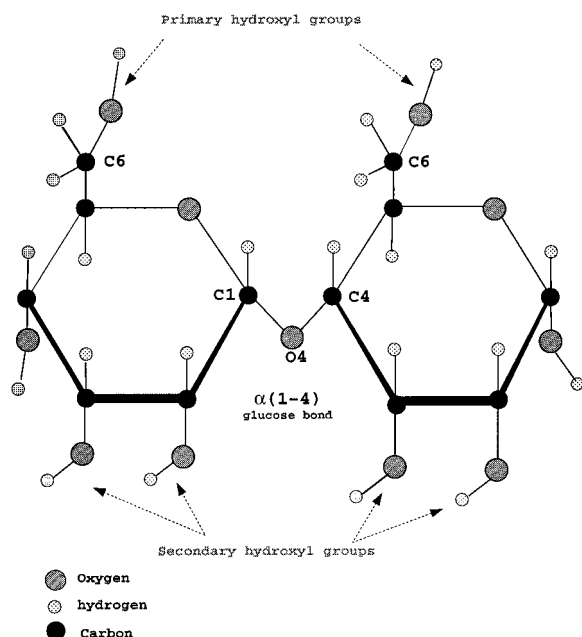


Figure 1. Schematic representation of an interglucose $\alpha(1-4)$ bond between two glucose units. Primary and secondary groups are indicated at the two opposite sides of the glucose units.

all hydrophobic behavior of the β -CD, both inside and outside the cavity. This would imply a negligible tendency to hydration, in apparent contrast with the experimental evidence of a spontaneous hydration of CD-crystals in wet atmosphere. The hydration properties of CD molecules are crucial for their application in biological systems. To clarify this point, we performed MD simulations of a system similar to that of the MM calculations and compared our results with these results and crystallographic data, respectively. Our simulations suggest that the cavity is hydrophobic and that the external part of the molecule, particularly the top and bottom regions, is hydrophilic, in agreement with the structure of solid hydrates and in apparent disagreement with the MM results.

The hydration properties of α -CD in aqueous solution have been studied in Reference 8. The molecular dynamics simulations exhibit a different hydration behavior of the α -CD in water and in a crystal. In solution a number of hydrogen bonds are observed, which are not present in a crystal. Simulations of complexes between α -CD and various hydrophobic guest molecules in aqueous solution show that hydrogen bonds between these compounds are rarely formed [9, 10]. We will briefly compare these results with our simulations of β -cyclodextrin.

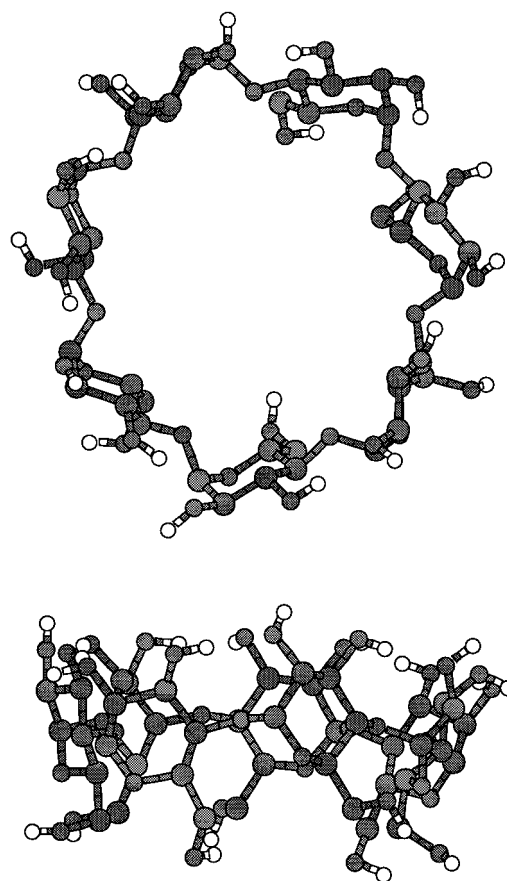


Figure 2. Top and front view of β -CD. The height of the barrel is 7.9 Å. The diameter of the outer periphery is 15 Å. The cavity diameter (calculated considering the van der Waals radius of every atom) is between 6.0 and 6.5 Å.

The paper is organized as follows: in the second section, we explain the model and the computational procedure. In the third section the results for the various amounts of water and the various temperatures are presented. The fourth section summarizes our findings.

Model and computational procedure

The structure of hydrated β -CD is well known from X-ray [11] and neutron diffraction studies [12]. β -CD crystallizes from water in hydrated form, dodecahydrated or undecahydrated. Approximately 6.5 water molecules are distributed over eight different sites within the cavity. The rest of the hydration water is outside of the molecule and fills the interstices between CDs. At low temperature (120 K) five water

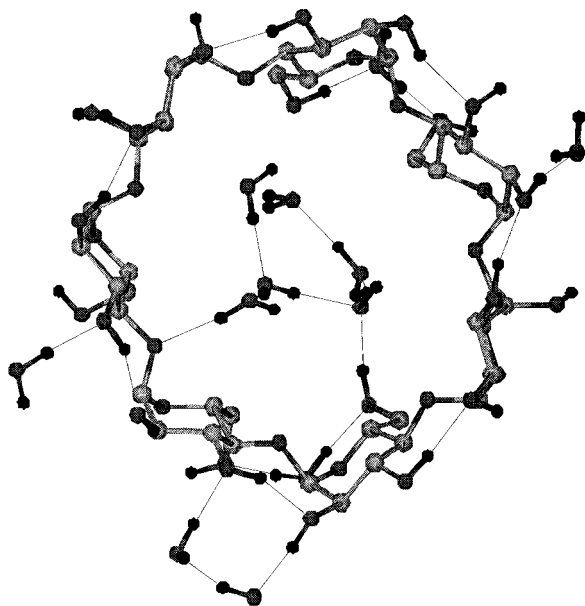


Figure 3. Configuration of a β -CD+12H₂O from neutron diffraction data at 120 K. H-bonds are represented as dotted lines. In the cavity the homodromic pentagonal ring of H-bonds between 5 of the 6 internal water molecules is clearly evident, while on the outer side of the β -CD the 6 external water molecules form hydrogen-bonds with both primary and secondary hydroxyl groups. Intramolecular H-bonds between secondary hydroxyl groups are also evident around the macrocycle.

molecules in the cavity arrange themselves in a pentagonal homodromic ring of H-bonds¹, which shows a hydrophobic behavior. The water molecules outside the cavity, on the other hand, create an H-bond network with the hydroxyl groups of the β -CD. At low temperatures all water sites but one are fully occupied [11] and one can clearly see highly ordered configurations through the whole crystal structure.

The initial configuration of the β -CD molecule for our simulations has been taken from neutron diffraction crystallographic data of a dodecahydrated β -CD crystal at 120 K [12] (Figure 3). The molecule is highly symmetrical. The secondary hydroxyl groups, which are directly bound to the second and third carbons of the glucose units and are all located on one side of the barrel (see Figure 1), are hydrogen bonded with the secondary hydroxyl groups of contiguous

¹The homodromic ring is a sequence of hydrogen-bonds in which the hydroxyl groups point all in the same direction ($\cdots O - H \cdots O - H \cdots O - H \cdots$); if an oxygen atom is donor for an H-bond of the ring, it can only be acceptor for the next, and vice versa. Different possible orientations of hydroxyl groups in rings composed of H-bonds define antidromic and eterodromic structures [12].

glucose units, permitting only limited rotation of the glucoses in the cyclic molecule. On the other hand, the primary hydroxyl groups, bonded to the sixth carbon of the glucose group (see Figure 1), are free to rotate and interact with the external and/or the internal water molecules partially blocking the cavity.

In our model all atoms are treated explicitly, with the exception of hydrogen atoms belonging to the carbons of sugar units. These are incorporated with the respective carbon atom into a ‘pseudo-carbon atom’ or ‘united atom’. The van der Waals and electrostatic parameters of these groups are modified to take the hydrogens into account. The biomolecular force field, i.e., the effective potential for the β -CD, includes the ordinary Lennard-Jones potential as well as charge-charge interactions (which represent dispersion-repulsion and electrostatic terms, respectively) and several intramolecular terms:

$$\begin{aligned}
 V_{\beta\text{-CD}} = & \sum_{\text{bonds}} \frac{1}{2} K_b (b - b_0)^2 + \sum_{\text{angles}} \frac{1}{2} K_\theta (\theta - \theta_0)^2 \\
 & + \sum_{\text{improper dihedrals}} \frac{1}{2} K_\xi (\xi - \xi_0)^2 \\
 & + \sum_{\text{proper dihedrals}} K_\phi (1 + \cos(n\phi)) \\
 & + \sum_{\text{non-excluded pair}(i,j)} \left(\frac{C'_{12}}{r_{ij}^{12}} - \frac{C'_6}{r_{ij}^6} + \frac{q_i q_j}{4\pi\epsilon r_{ij}} \right). \quad (1)
 \end{aligned}$$

In the above equation, the first term represents covalent bond stretching, the second describes bond angle bending (three-body term), while the third and fourth term are the (four-body) dihedral angle interactions. The torsional term is divided in two classes: improper torsion, which keeps certain atoms in specific tetrahedral configurations, i.e., carbons in the glucose rings, and proper torsion, which maintains the geometrical configuration of the whole molecule. The last term of the potential energy describes the non-bonded interactions: van der Waals and electrostatic contributions. First and second neighbors are excluded from non-bonded interactions, while third neighbors contribute to the overall energy, however, with specific smaller van der Waals parameters. For force fields of biomolecules in aqueous environment, a value of the dielectric constant $\epsilon = 1$ is a reasonable choice [13].

The intermolecular potential function characterizing the interaction between solute and solvent has the following atom-atom expression:

$$V_{\text{H}_2\text{O}, \beta\text{-CD}} = \sum_{\text{pair}(i,j)} \left(\frac{C_{12}}{r_{ij}^{12}} - \frac{C_6}{r_{ij}^6} + \frac{q_i q_j}{4\pi\epsilon r_{ij}} \right). \quad (2)$$

The water molecules are described by a simple rigid three point charge (SPC) model, which adequately describes properties of a bulk solvent [14, 15]. The rigidity is granted by bond constraints between the three atoms using the SHAKE method [16].

We used periodic boundary conditions (PBC) and chose the spherical cutoff radius for the short range interactions as half the value of the smallest edge of the computational box. Ewald summation was included to calculate long range Coulomb contributions. The system was coupled to a thermal bath using a Nosé-Hoover algorithm [17–19].

The molecular dynamics simulations were performed using the DL_POLY [20] code with the GROMOS [21] parameters for the force field. All parameters of the GROMOS force field that we used are summarized in Tables 1 and 2 of Reference 22.

The DL_POLY integration algorithm is based on the Verlet leapfrog scheme, which is time reversible and simple in nature [23]. The coupling of the system to a heat bath via the Nosé-Hoover scheme is controlled by a time constant, which was set equal to 0.5 ps. The integration time step was 1 fs. The force field input of DL_POLY and the topology of the cyclodextrin molecule were constructed using DL_GEN [24]. This program is able to generate the input files for DL_POLY from a PDB (Protein Data Bank) file.

To build the initial configurations for the various hydrated cyclodextrin molecules, we used a routine that is able to add solvent to a simulation box containing a solute molecule [20]. A system of equilibrated water at normal conditions is superposed on the box containing the solute. The positions of every water molecule and any solute atom are compared. A water molecule is rejected whenever the oxygen is closer than a prefixed minimum solute-solvent distance for any solute atom. If this is not the case, the water molecule is added to the simulation box. If the dimension of the solute box is larger than the solvent box, the water configuration is translated in the three spatial directions of the box in order to fill completely the solute molecule box. In this way we constructed the various hydrated forms of our solute.

Starting from these initial configurations, we performed equilibration runs lasting for 10–20 ps. To control the equilibration process, we calculated and monitored various quantities like kinetic energy, total energy, mean square displacement of the water molecules, etc. When these quantities assumed stationary values we considered the system as equilibrated.

Results and discussion

In this section we present results of the three different studies on the water/ β -cyclodextrin system: a dodecahydrated β -CD, a cluster of highly hydrated β -CD, and a β -CD in diluted solution. Each system was simulated at low (120 K) and at high (293 K) temperature.

The dodecahydrated β -cyclodextrin

We first simulated a cyclodextrin molecule with 12 water molecules attached at 120 K in order to compare the simulation results with neutron diffraction data. Accordingly, we used a semiempirically optimized hydrated cyclodextrin conformation extracted from the neutron diffraction data. The system was placed in a $25 \times 25 \times 25 \text{ \AA}^3$ simulation box and the simulation lasted for 1 ns. The box is large enough, compared to the system size, to represent a simulation in vacuum. We did not observe any evaporation on the time scale of our simulation. Comparing the averaged positions of the atoms of the CD molecule and the water molecules obtained in our simulation with the corresponding positions measured by neutron scattering, we observed very good agreement. Hence, the applied force field yields an adequate representation of a β -cyclodextrin, which is in agreement with simulations of α -cyclodextrin [9, 28].

In the main studies, we focused our attention essentially on the water behavior and on its distribution around the β -cyclodextrin. Already after a few picoseconds simulation time, the water distribution around the β -CD was very stable and reflected the hydrophilicity of the solute molecule exterior and the hydrophobicity of the cavity. The water molecules moved from their initial positions faster than β -CD, but never diffused in the whole simulation box. The diffusion seemed to be driven by exchanges within the hydration shells rather than by long range motion. This kind of very high water mobility fully agrees with the NMR findings [3], but does not imply the concept of long-range water motion in the crystal, as suggested by Steiner et al. [25]. To understand the dehydration process of a hydrated crystal, explicit simulations of such a system are required. We, however, can conclude from our simulation that β -CD is a partial hydrophilic molecule with hydrophobic cavity.

To give a more precise meaning of the above statement, we computed the number of water molecules as a function of their separation from the β -CD molecule

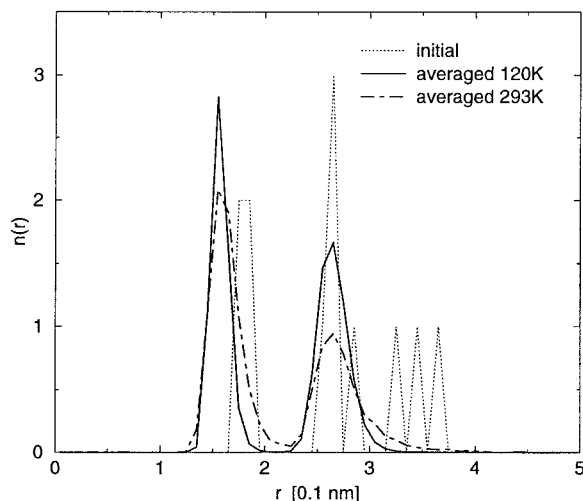


Figure 4. Initial and averaged number of water molecules at a distance r from the solute. The distance is the minimum distance between the centre of mass of the water molecules and any CD atom. (Solid line: $T = 120$ K; dashed line: $T = 293$ K).

(class interval 0.1 \AA). The distance has to be understood as the closest distance between the water centre of mass and any cyclodextrin atom. The number has been averaged over configurations taken every 0.2 ps (see Figure 4). The peak (solid line) between 1.4 and 1.9 \AA of Figure 4 reflects the presence of hydrogen bonds between water molecules and hydroxyl groups of the CD-molecule: it is the first hydration shell. The second peak, on the other hand, shows the presence of water molecules too far apart to form H-bonds with the solute molecule hydroxyls. Our simulations show that the water molecules belonging to the first hydration shell are all outside the CD molecule, while the internal water molecules arrange themselves in the cavity interacting only with each other. This demonstrates the apolarity of the cavity with respect to water, i.e., the hydrophobicity².

To localize the position of water molecules relative to the CD molecule, we changed from the laboratory frame to a Cartesian coordinate system attached to the β -CD molecule with the origin in its center of mass. We aligned the z -axis of this new reference frame parallel to the longest of the main axes of inertia of the molecule. The x -axis points through the projection of a particular CD oxygen atom between two glucose

²The term hydrophobicity normally refers to the tendency of non-polar groups to interact with each other rather than with water and to the consequent water-ordering effect: the formation of tightly packed H-bond networks among water molecules around the nonpolar surface.

units on the plane perpendicular to the z -axis, and the y -axis was chosen perpendicular to the other two axes. In this frame we calculated the water density by cutting the cyclodextrin in 1 \AA thick slices parallel to the xy -plane. From this study it is evident that external water molecules outside of the CD molecule are H-bonded only to primary and secondary hydroxyl groups of the sugar units, i.e., in the upper and lower part of the cyclodextrin. The water molecules in the cavity were ordered but did not create H-bonds with the β -CD molecule. In agreement with experimental results, we find on average 5.5 water molecules outside the cavity and 6.5 inside. This is close to the maximum number of water molecules that the cavity may accommodate.

It is not obvious whether the applied force field yields a reasonable description of the CD molecule at a temperature much higher than 120 K . In our simulation at 293 K , the molecule remained stable. The positions of the individual atoms, however, were less well defined. The molecule deformed much stronger by thermal motion at the higher temperature (see below). Larger variations in the mean position of an individual atom were also found in molecular dynamics simulations of α -cyclodextrin in aqueous solution compared to the crystal structure [28]. We expect the β -cyclodextrin to exhibit even larger fluctuations than the α -cyclodextrin, since adding a glucose unit increases the ring diameter and hence the flexibility of the whole molecule.

Correspondingly, the water distribution around the molecule is also broadened compared to the distribution at lower temperature. This is obvious from the dashed line in Figure 4, representing the number of water molecules next to the CD molecule. But there is still a pronounced peak corresponding to hydrogen bonds. Since the interaction between the water molecules and the CD molecule is decreasing with increasing temperature, we observe a broadening of the second peak in Figure 4. From our simulations we conclude, however, that the outer part of the CD molecule is still hydrophilic and the internal part remains hydrophobic.

The β -CD in water cluster

We now present results of NVT simulations of β -cyclodextrin hydrated with 47 water molecules (including the 12 crystalline water molecules).

We generated the initial configuration by adding solvent to a small box of $17.5 \times 16 \times 10 \text{ \AA}^3$. A

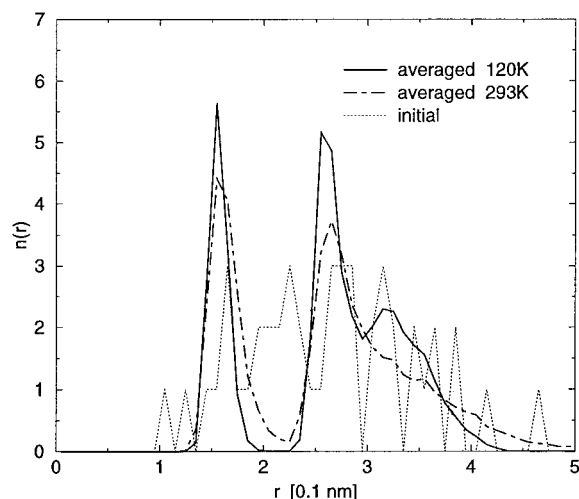


Figure 5. Initial and averaged number of water molecules at distance r (Å) from the β -cyclodextrin molecule hydrated with 47 water molecules (class interval of 0.1 Å). The distance is the minimum distance between the centre of mass of the water molecules and any CD atom (Solid line: $T = 120$ K; dashed line: $T = 293$ K).

dense cluster was created in order to study the variation of the water distribution around cyclodextrin and the diffusion of the non-crystalline water. The dimensions of the simulation box itself were chosen as $40 \times 40 \times 40 \text{ Å}^3$, which is large enough to model the vacuum condition. The simulation lasted for 500 ps at a temperature of 120 K. Within a few picoseconds the water molecules distributed around the CD and reached a state which was maintained through the rest of the run (Figure 5). Similar to the dodecahydrated β -CD system, water molecules did not evaporate. Also no relevant differences have been noted between the mobility of water molecules present in a crystal and the additional water. In Figure 5, the peak (solid line) between 1.4–1.9 Å identifies the first hydration shell. Approximately 13 water molecules contribute to this shell and are bound by H-bonds to the top and bottom external sides of the CD. The cavity appears again as a nonpolar environment where the water molecules interact with each other to form an H-bond network.

To obtain a deeper insight into the distribution of water molecules we calculated in the β -CD reference frame the averaged radial densities of water molecules and the CD itself in 1 Å thick slices perpendicular to the longest main axis of inertia of the molecule. In the presentation of Figure 6 we divided the system in three parts: $z \leq -3 \text{ Å}$, $-3 \text{ Å} < z \leq 3 \text{ Å}$, and $z > 3 \text{ Å}$. The hydration of the CD molecule is evident. Moreover, the water density close to the secondary hydroxyl

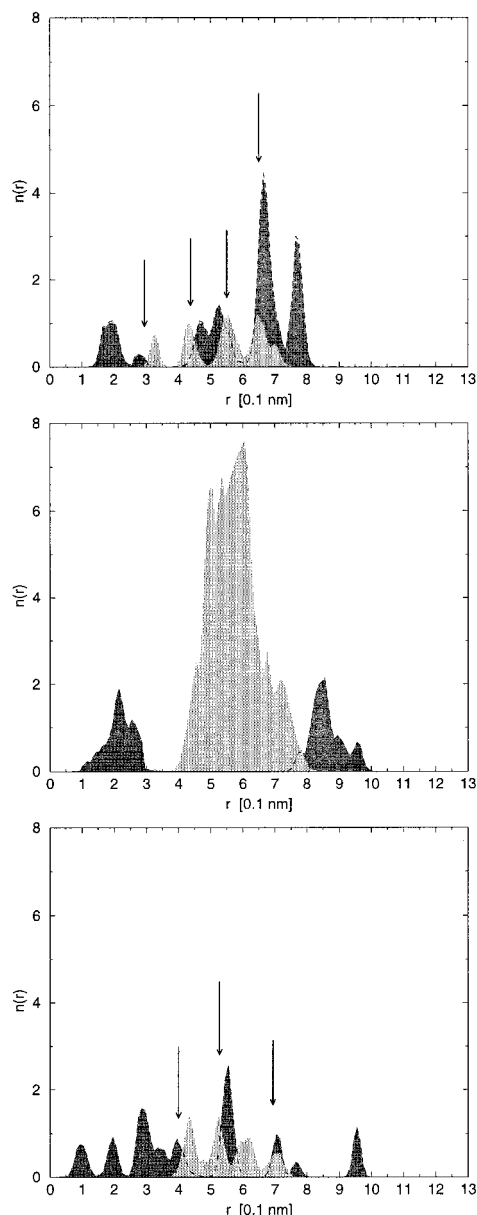


Figure 6. Cylindrical radial densities of water (dark) and cyclodextrin (light) calculated in slices of 1 Å thickness for the three cases: $z \leq -3 \text{ Å}$, $-3 \text{ Å} < z \leq 3 \text{ Å}$, and $z > 3 \text{ Å}$, respectively, from top to bottom. The arrows indicate the presence of hydrogen bonds. Case: CD+47 water molecules at 120 K.

groups ($z > 3 \text{ Å}$ in Figure 6) is 75% lower than the density close to primary groups ($z \leq -3 \text{ Å}$). This fact strongly supports the concept that secondary hydroxyl groups interact more with each other than with water, thus reducing the solubility of the molecule. In fact, β -CD is much less soluble than the other species of the CD family, where the secondary groups are more

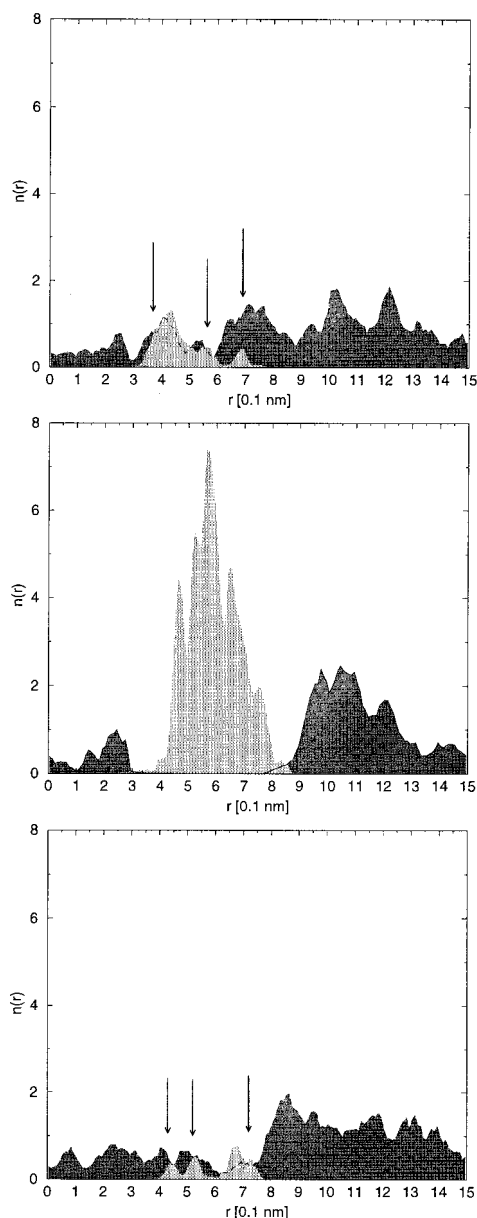


Figure 7. Cylindrical radial densities of water (dark) and cyclodextrin (light) calculated in slices of 1 Å thickness for the three cases: $-6 \text{ Å} < z \leq -3 \text{ Å}$, $-3 \text{ Å} < z \leq 3 \text{ Å}$, and $3 \text{ Å} < z \leq 6 \text{ Å}$, respectively, from top to bottom. The arrows indicate the presence of hydrogen bonds. Case: CD+258 water molecules at 120 K.

mobile and, due to the geometry of the molecule, may more freely interact with water [7].

A temperature change from 120 K to 300 K in steps of 20 K does not yield any qualitative difference in the results. The simulation at 293 K exhibits a broadening of the peaks in the distribution of the water molecules, as obvious from Figure 5 (dashed line). The conclu-

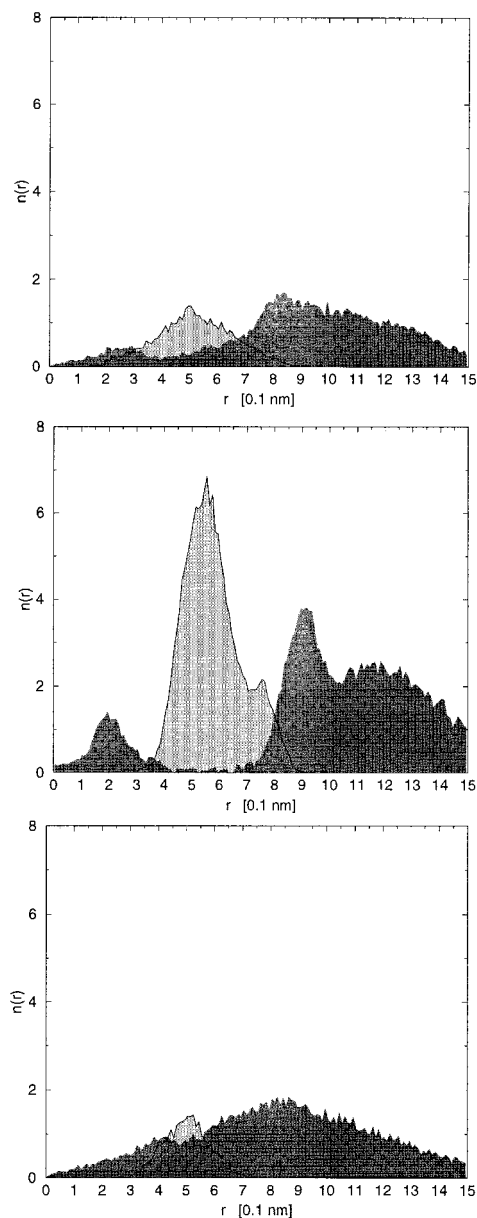


Figure 8. Cylindrical radial densities of water (dark) and cyclodextrin (light) calculated in slices of 1 Å thickness for the three cases: $-6 \text{ Å} < z \leq -3 \text{ Å}$, $-3 \text{ Å} < z \leq 3 \text{ Å}$, and $3 \text{ Å} < z \leq 6 \text{ Å}$, respectively, from top to bottom. Case: CD+258 water molecules at 293 K.

sions are similar to those of the simulation with 12 water molecules (the dodecahydrated β -cyclodextrin).

β -CD molecule in diluted water solution

The molecular mechanics (MM) calculations presented by Margheritis and Sinistri [6] yield a com-

pletely hydrophobic behavior of the β -CD molecule. Our molecular dynamics simulations of such a molecule exposed to a small number of water molecules, however, exhibit hydrophilic parts, as discussed in the last sections. To elucidate the dependence of the water affinity of the molecule on the amount of the surrounding water, we placed a β -cyclodextrin molecule in a simulation box filled with water, corresponding to a CD molecule in solution. We generated a system similar to the one constructed in Reference 6. A β -cyclodextrin molecule was placed in the periodic box of $25 \times 25 \times 18 \text{ \AA}^3$ size initially filled with water. By eliminating all water molecules at distances smaller than 1.9 \AA , we obtained a system containing 258 water molecules. Our MD simulation lasted for 500 ps at temperatures of 120 K and 293 K.

As in the above hydration studies, we analyzed the water molecule distribution around the CD molecule (see Figures 4–8). The results are similar and consistent with our MD calculations of β -CD at low and high hydration conditions. This supports the picture that water molecules inside the cavity possess a minimal distance from the β -CD of approximately 2.4 \AA and assume an ordered configuration (hydrophobic water-ordering effect). In contrast, the external water molecules are distributed in shells. The first shell consists of water molecules forming H-bonds with the top and bottom hydroxyl groups of β -CD (see Figure 5). The other shells originate from the packing of the water molecules around the solute.

There is a major discrepancy between MM and MD results because no tendency to form H-bonds in the first hydration shell is revealed by MM calculations. We decreased the temperature of the simulation to 20 K without observing any change in the water distribution around the β -cyclodextrin.

The increase of the temperature to 293 K influences the distribution of the water molecules. However, hydrogen bonds are still present similar to the low temperature situation. As is obvious from Figures 7 and 8, the peaks at the higher temperature are broader and there is a small probability for an exchange of water molecules between the hydrogen bonded ones and the ones bonded by van der Waals forces ($r \approx 2.2 \text{ \AA}$, see Figure 5). At larger distances, we find a nearly constant density of water molecules. Figure 8 illustrates the larger fluctuations of the CD molecule as well as those of the water molecules at the higher temperature. Moreover, the top and bottom hydroxyl groups are more mobile. Despite that, the CD molecule still exhibits hydrophilic parts.

With an increasing amount of water, the number of water molecules participating in hydrogen bonds increases. This is consistent with the observation that additional hydrogen bond configurations exist in solution of α -CD compared to its crystalline state [28]. Hence, our simulations underline the presence of many more hydrogen bond configurations in aqueous solution than in a crystal.

The results confirm that the GROMOS force field gives a good model for studying biological systems, including cyclodextrins (at least at low temperatures) [7, 27, 28]. To which extent, however, the parameters and hence the simulation results of a CD molecule in solution are correct, remains to be clarified experimentally.

Conclusions

We have presented MD simulation results for the hydration of a single β -CD molecule. Three different systems have been studied in order to shed some light on the water/ β -CD interaction. For different amounts of water added to a single CD molecule, the simulations have consistently given evidence of predominant hydrophilic interactions outside the molecule and of hydrophobic interactions inside. This result agrees with the generally accepted idea of CDs as being a family of molecules interacting hydrophilically on the outer side, and hydrophobically in the internal cavity. The relatively low solubility of β -CD has been explained in terms of interactions between adjacent secondary hydroxyl groups. In contrast with MM results, we find evidence of hydrophilic interactions in a first hydration shell, particularly at the top and bottom part of the molecule. The hydration properties are independent of the temperature. Our simulations reveal the formation of hydrogen bonds at low (120 K) as well as at room temperature.

MD simulations are also required for a better understanding of the general hydration and dehydration behavior of β -CD crystals, which have been carefully investigated with thermal and diffraction techniques [3]. Work is in progress in this direction.

Acknowledgements

The major part of this work was performed during the visit of S.F. at University of Ulm. S.F. is grateful to

Prof. P. Reineker for the kind hospitality and to the Vetter Foundation for financial support.

References

1. Szejtli, J., Cyclodextrin Technology, Kluwer Academic Publishers, Dordrecht, 1988.
2. Bender, M.L. and Komiyama, M., Cyclodextrin Chemistry, Springer-Verlag, New York, NY, 1978.
3. Marini, A., Berbenni, V., Bruno, G., Massarotti, V., Mustarelli, P. and Villa, M., J. Chem. Phys., 103 (1995) 1532.
4. Marini, A., Berbenni, V., Bruni, G., Giordani, F. and Villa, M., Thermochim. Acta, 279 (1996) 27.
5. Steiner, T. and Koellner, G., J. Am. Chem. Soc., 116 (1994) 5122.
6. Margheritis, C. and Sinistri, C., Z. Naturforsch., 51a (1996) 950.
7. Margheritis, C. and Sinistri, C., Il Farmaco, 52 (1997) 435.
8. Koehler, J.E.H., Saenger, W. and van Gunsteren, W.F., J. Mol. Biol., 203 (1988) 241.
9. van Helden, S.P., van Eijck, B.P. and Janssen, L.H.M., J. Biomol. Struct. Dyn., 9 (1992) 1269.
10. Mark, A.E., van Helden, S.P., Smith, P.E., Janssen, L.H.M. and van Gunsteren, W.F., J. Am. Chem. Soc. Mol., 116 (1994) 6293.
11. Zabel, V., Saenger, W. and Mason, S.A., J. Am. Chem. Soc., 108 (1986) 3664.
12. Betzel, C., Saenger, W., Hingerty, B.E. and Brown, G., J. Am. Chem. Soc., 106 (1984) 7545.
13. Robinson, G.W., Zhu, S., Singh, S. and Evans, M.W., Water in Biology, Chemistry and Physics, World Scientific, Singapore, 1996.
14. Jorgensen, W.L., J. Chem. Phys., 79 (1983) 926.
15. Watanabe, Y. and Klein, M., J. Chem. Phys., 131 (1987) 157.
16. Ryckaert, J.P., Ciccotti, G. and Berendsen, H.J.C., J. Comput. Phys., 23 (1977) 327.
17. Nosé, S., Mol. Phys., 57 (1986) 187.
18. Hoover, W.G., Phys. Rev. A, 31 (1985) 1695.
19. Nosé, S., J. Chem. Phys., 81 (1984) 511.
20. Smith, W. and Forester, T.R., DL_POLY: A molecular dynamics simulation package, CCLRC, Daresbury Laboratory, UK, 1994.
21. van Gunsteren, W.F. and Berendsen, H.J.C., Groningen Molecular Simulation (GROMOS) Library Manual, Biomos, Groningen, 1987.
22. Koehler, J.E.H., Saenger, W. and van Gunsteren, W.F., Eur. Biophys. J., 15 (1987) 197.
23. Allen, M.P. and Tildesley, D.J., Computer Simulation of Liquids, Clarendon Press, Oxford, 1989.
24. Melchionna, S., Cozzini, S., Venturoli, M. and Luise, A., In Voli, M. (Ed.), Science and Supercomputing at Cineca: Report 97, 1998.
25. Steiner, T., Koellner, G., Ali, S., Zakimand, D. and Saenger, W., Biochem. Biophys. Res. Commun., 188 (1992) 3, 1060.
26. Shied, W.J. and Hedges, A.R., J. Macromol. Sci. – Pure Appl. Chem., A33 (1996) 673.
27. Koehler, J.E.H., Saenger, W. and van Gunsteren, W.F., Eur. Biophys. J., 15 (1987) 211.
28. Koehler, J.E.H., Saenger, W. and van Gunsteren, W.F., Eur. Biophys. J., 16 (1988) 153.