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Molecular dynamics simulation of crystalline β-cyclodextrin dodecahydrate at 293 K and 120 K

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Abstract. Molecular dynamics (MD) simulations for crystalline β-cyclodextrin dodecahydrate (β-CD) at two different temperatures, 293 K and 120 K, have been performed using the GROMOS program package. The calculated structural properties are compared to those obtained from neutron diffraction studies of this system at the quoted temperatures. The simulation was carried out over a period of 20 ps on four unit cells containing 8 β-CD molecules and 96 water molecules, whereby all atoms were allowed to move.

At room temperature, the experimental positions of the (non-hydrogen) glucose atoms are reproduced within 0.034 nm, a value which is smaller than the experimental (0.041 nm) or simulated (0.049 nm) overall root mean square (rms) positional fluctuation. The corresponding numbers for the low temperature study are 0.046 nm, 0.019 nm and 0.022 nm. At both temperatures the experimentally observed degree of anisotropy of the atomic motions is also found in the simulations.

The comparison of a variety of structural properties leads to the conclusion that the molecular model and force field used are able to simulate the cyclodextrin system very well. Experimentally observed differences in properties as a function of number of glucose units in the CD molecule (α-CD, 6 versus β-CD, 7) and as a function of temperature are qualitatively reproduced by the simulations.

Key words: β-cyclodextrin dodecahydrate, molecular dynamics simulation, hydrogen bonds, empirical force field, water molecule diffusion, positional disorder

Introduction

β-Cyclodextrin (β-CD) is the seven membered species in the class of cycloamyloses consisting of 6 to 8 glucose units linked by α(1 → 4) glucosidic bonds. The central cavity of this torus shaped molecule has a diameter of about 0.6 to 0.64 nm and is able to include various organic and inorganic guest molecules. Therefore cyclodextrins and their inclusion compounds have found applications as molecular capsules in the pharmaceutical, agricultural and food industries. In research they have been used as models to study intermolecular interactions and enzymatic reaction mechanisms (Saenger 1980; Van Etten et al. 1967; Tutt and Schwartz 1970; Bender and Komiyama 1978; Szejtli 1982; Atwood et al. 1984) and they are promising materials in gelinclusion-chromatography (Szejtli et al. 1978).

Two high resolution neutron diffraction studies of crystalline β-CD dodecahydrate have been performed, one at 293 K (Betzel et al. 1984), and the other at 120 K, well below a phase transition occurring at 227 K (Zabel et al. 1986). The crystals have identical space groups and, because of temperature effects, slightly different cell dimensions. The main difference lies in the observed hydroxyl and hydroxymethylene [1] and water molecule positions: at low temperature almost all positions are fully occupied, whereas at room temperature there are many partially occupied atomic sites, especially for water molecules. The latter form extended flip-flop hydrogen bond networks with each other and with the hydroxyl groups of the sugar molecules (Saenger et al. 1982), most of which do not exist at low temperature.

The flip-flop hydrogen bonds are of the form O–H ... H–O with the hydrogen atoms so close together (~ 1 Å) that they can only alternatively be present, and their occupation is, in fact, close to 0.5. This indicates that we observe an average over two states O–H ... O and O ... H–O which are in dynamic equilibrium in the room temperature crystals. Below the phase transition temperature of 227 K, these

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1 "short" (O2–H and O3–H hydroxyls) and "long" (CH_2–O 6–H) side chains

flip-flop hydrogen bonds freeze in one or the other state so that extended networks of O–H ... O hydrogen bonds are formed where all the hydrogen bonds point in the same direction (homodromic) O–H ... O–H ... O–H .... This scheme indicates the dominating influence of the cooperative effect which is due to the charge polarization of the O–H group when it is involved in hydrogen bonding (Betzel et al. 1984).

The purpose of this molecular dynamics study of crystalline β-CD dodecahydrate at 293 K and at 120 K is threefold. *First* the interatomic potential function as it is used in the GROMOS package is tested by comparison of the simulated results with available high resolution neutron diffraction data of β-CD · $12H_2O$, as described in the previous paper for the α-CD · $6H_2O$ crystal at 293 K (Koehler et al. 1987a). β-CD · $12H_2O$ contains more water molecules per glucose than α-CD · $6H_2O$, most of which are disordered. Moreover, the β-CD molecule is conformationally more regular than α-CD, where the torus is distorted by rotation of one glucose. Therefore, β-CD forms another sensitive test case for computer simulation studies. *Second*, it will be examined how well the potential function, which is an effective force field developed for application at room temperature, functions at 120 K. *Third*, it is a challenge to find out whether the dynamic flip-flop hydrogen bond phenomenon observed in β-CD · $12H_2O$ can be reproduced in the obtained MD trajectories; all results concerning this subject will be discussed in the following paper (Koehler et al. 1987b).

Methods and computational details

The experimental data referred to in this paper are the neutron diffraction studies of β-CD · $11H_2O$ at 293 K (Betzel et al. 1984), and of β-CD · $11.6H_2O$ at 120 K (Zabel et al. 1986). The nomenclature of the various glucose and water atoms is given there and in (Koehler et al. 1987a).

At both temperatures the space group is $P2_1$ and the number of molecules per unit cell $Z = 2$. The cell dimensions are comparable:

1) 293 K: $a = 2.1261$ nm, $b = 1.0306$ nm, $c = 1.5123$ nm, $\beta = 112.3°$
2) 120 K: $a = 2.1617$ nm, $b = 1.0026$ nm, $c = 1.4891$ nm, $\beta = 112.52°$.

The R-values from the full-matrix least-squares refinement are $R = 6.8\%$ (293 K) and $R = 4.9\%$ (120 K).

The 11 ± 0.5 crystal water molecules were found to be distributed over 16 oxygen and 37 hydrogen positions at room temperature (12 oxygen and 26 hydrogen positions at 120 K), implying that some sites have occupancy factors less than 1.

The molecular dynamics calculations were carried out using the GROMOS (Groningen Molecular Simulation Library) program package. The molecular model, the force field, the algorithm and computational details were given in the preceding paper on α-CD (Koehler et al. 1987a) and we discuss here where the treatment of β-CD differs. For the molecular dynamics simulations, "computational boxes" have been chosen with dimensions:

1) 293 K: $a = 2.1261$ nm, $2b = 2.0612$ nm, $2c = 3.0246$ nm, $\beta = 112.3°$
2) 120 K: $a = 2.1617$ nm, $2b = 2.0052$ nm, $2c = 2.9782$ nm, $\beta = 112.52°$.

All atoms of the cyclodextrin and the water molecules have been treated explicitly, with the exception of hydrogen atoms covalently bound to carbon atoms. They are combined with the latter, as described by Koehler et al. (1987a). For each β-CD molecule twelve water molecules have been selected in both the room and low temperature MD simulation studies to model the experimental multiple water sites. This is close to the 11.6 water molecules found in the 120 K neutron study and to the 11.0 water molecules in the 293 K analysis where, as outlined in Zabel et al. (1986), some of the more spurious water sites were probably not seen in the Fermi density maps. The initial water sites were selected according to occupancy and hydrogen bonding. Application of the symmetry transformations led to boxes containing 8 β-CD molecules (784 atoms) and 8×12 water molecules (288 atoms).

With these 1072 atoms at their experimental positions, an energy minimization was performed over 20 steps in which all β-CD and water molecules were allowed to move. The molecular dynamics simulation was carried out in periods ranging from 0 to 19 picoseconds (ps) for 293 K and from 0 to 20 ps for 120 K. Periodic boundary conditions were applied, and the volume was held constant. The starting velocities for all atoms were chosen from a Boltzmann distribution at 293 K (120 K), and the equations of motion were integrated with time steps of $\Delta t = 0.002$ ps.

Although all the eight β-CD molecules had identical geometries at the beginning of the simulation, they experienced different development during the MD-simulation, because their atoms had different Boltzmann distribution velocities. The eight molecules follow individual trajectories. These indicate the flexibility of the molecules at certain temperatures.

In order to compare the obtained molecular dynamics structures of β-CD with the experimental one, they were averaged over the last 15 ps of the MD run and over all eight molecules (M), referred to as $\langle M1\text{–}8; 4\text{–}19\,\text{ps}\rangle$ for 293 K, and $\langle M1\text{–}8; 5\text{–}20\,\text{ps}\rangle$ for 120 K. Root mean square differences between experimental and averaged MD atomic positions are

($rms_{pos\ diff.}$) given in terms of an expression as defined in the preceding paper, and a quantitative measure for the flexibility of the β-CD molecule during the MD simulation is the root mean square fluctuation $rms_{pos.fluc.}$ as introduced in (Koehler et al. 1987a). These fluctuations can be compared with the experimentally obtained isotropic temperature factors B (Å^2), although their physical meaning is slightly different (Koehler et al. 1987a), using the formula

$$rms_{pos.fluc.} = [3\,B_{iso}/(8\,\pi^2)]^{1/2}\,.$$

Results and discussion

Atomic positions and fluctuations

When the averaged MD structure of β-CD at room temperature $\langle M1-8;\,4-19\ ps\rangle$ is compared to the experimentally determined one, the root mean square positional difference for all atoms is 0.048 nm and for all atoms except the hydrogens it is 0.034 nm. These data are entered in the first column of Table 1 a which gives these differences for the individual atoms aver-

Table 1 a. rms positional differences and simulated fluctuations between various β-cyclodextrin structures at 293 K and 120 K. The experimental structure is denoted by X_{exp}. Averages over simulated structures are denoted by the symbol $\langle\ldots\rangle$, where M refers to one or more of the 8 molecules in the computational box and the time span over which is averaged is given in picoseconds. The mean is over the 7 glucose units of the β-cyclodextrin molecule. The values are given in nm. Experimental rms fluctuations have been calculated using the formula $rms_{pos.fluc.} = [3 \cdot B_{iso}/(8\,\pi^2)]^{1/2}$, where the isotropic B-factor is denoted by B_{iso}. The ratio between the shortest and longest axes of the thermal ellipsoids are given

	$X_{exp}/\langle M1-8;\,15\ ps\rangle$										$X_{exp}/\langle M1-8;\,10\ ps\rangle$		$X_{exp}/\langle M1;\,15\ ps\rangle$		$\langle M1;\,15\ ps\rangle/\langle M5;\,15\ ps\rangle$	
	rms differences		Isotropic rms fluctuations				Anisotropic ratio between shortest and longest axes of thermal ellipsoids									
	293 K	120 K	293 K		120 K		293 K		120 K		293 K	120 K	293 K	120 K	293 K	120 K
	exp/ MD	exp/ MD	exp	MD	exp	MD	exp	MD	exp	MD	exp/ MD	exp/ MD	exp/ MD	exp/ MD	exp/ MD	exp/ MD
C_1	0.037	0.046	0.037	0.042	0.017	0.021	0.67	0.68	0.53	0.72	0.034	0.046	0.052	0.041	0.039	0.002
C_2	0.033	0.049	0.038	0.042	0.018	0.019	0.68	0.66	0.66	0.67	0.030	0.048	0.049	0.044	0.039	0.003
C_3	0.022	0.043	0.036	0.041	0.017	0.019	0.70	0.72	0.69	0.66	0.018	0.043	0.035	0.037	0.033	0.003
C_4	0.026	0.039	0.035	0.039	0.017	0.019	0.73	0.70	0.72	0.61	0.023	0.039	0.040	0.033	0.034	0.003
O_4	0.026	0.037	0.036	0.041	0.018	0.020	0.68	0.68	0.59	0.60	0.023	0.037	0.039	0.032	0.035	0.002
C_5	0.034	0.039	0.037	0.043	0.018	0.020	0.72	0.66	0.65	0.65	0.031	0.039	0.050	0.034	0.042	0.002
O_5	0.038	0.044	0.039	0.045	0.019	0.022	0.67	0.63	0.66	0.64	0.035	0.043	0.055	0.039	0.044	0.002
O_2	0.039	0.067	0.043	0.051	0.021	0.023	0.66	0.60	0.52	0.62	0.035	0.066	0.053	0.062	0.045	0.003
H_2	0.088	0.051	0.046	0.089	0.030	0.025	0.64	0.39	0.71	0.57	0.086	0.050	0.101	0.045	0.081	0.004
O_3	0.019	0.050	0.044	0.053	0.021	0.024	0.57	0.56	0.59	0.57	0.015	0.050	0.036	0.044	0.040	0.003
H_3	0.082	0.091	0.046	0.088	0.030	0.031	0.67	0.40	0.69	0.52	0.078	0.090	0.094	0.086	0.073	0.010
C_6	0.043	0.042	0.045	0.055	0.022	0.023	0.63	0.50	0.63	0.47	0.040	0.041	0.065	0.038	0.059	0.002
O_6	0.050	0.045	0.058	0.076	0.024	0.027	0.56	0.42	0.51	0.61	0.047	0.045	0.073	0.041	0.079	0.003
H_6	0.069	0.041	0.054	0.107	0.033	0.027	0.56	0.39	0.71	0.61	0.063	0.041	0.096	0.037	0.116	0.003
All atoms excl. H	0.034	0.046	0.041	0.049	0.019	0.022	0.66	0.62	0.61	0.62	0.031	0.046	0.051	0.041	0.046	0.002
All atoms	0.048	0.051	0.043	0.062	0.022	0.023	0.65	0.57	0.63	0.61	0.045	0.050	0.064	0.046	0.059	0.004

Table 1 b. rms positional differences between experimental and simulated structures at 293 and 120 K. The MD time average is over the final 15 ps of each simulation. The rms differences in the lower left triangle of the table have been obtained after superposition of the centers of mass of the structures. The upper right triangles contain values obtained without any fitting

		All atoms excluding H-atoms				All atoms			
		X_{exp}		$\langle M1-8\rangle_{MD}$		X_{exp}		$\langle M1-8\rangle_{MD}$	
		120 K	293 K	120 K	293 K	120 K	293 K	120 K	293 K
X_{exp}	120 K	0.0	0.050	0.046	0.052	0.0	0.070	0.051	0.068
	293 K	0.031	0.0	0.074	0.034	0.058	0.0	0.091	0.048
$\langle M1-8\rangle_{MD}$	120 K	0.034	0.049	0.0	0.066	0.039	0.073	0.0	0.077
	293 K	0.038	0.024	0.052	0.0	0.057	0.039	0.068	0.0

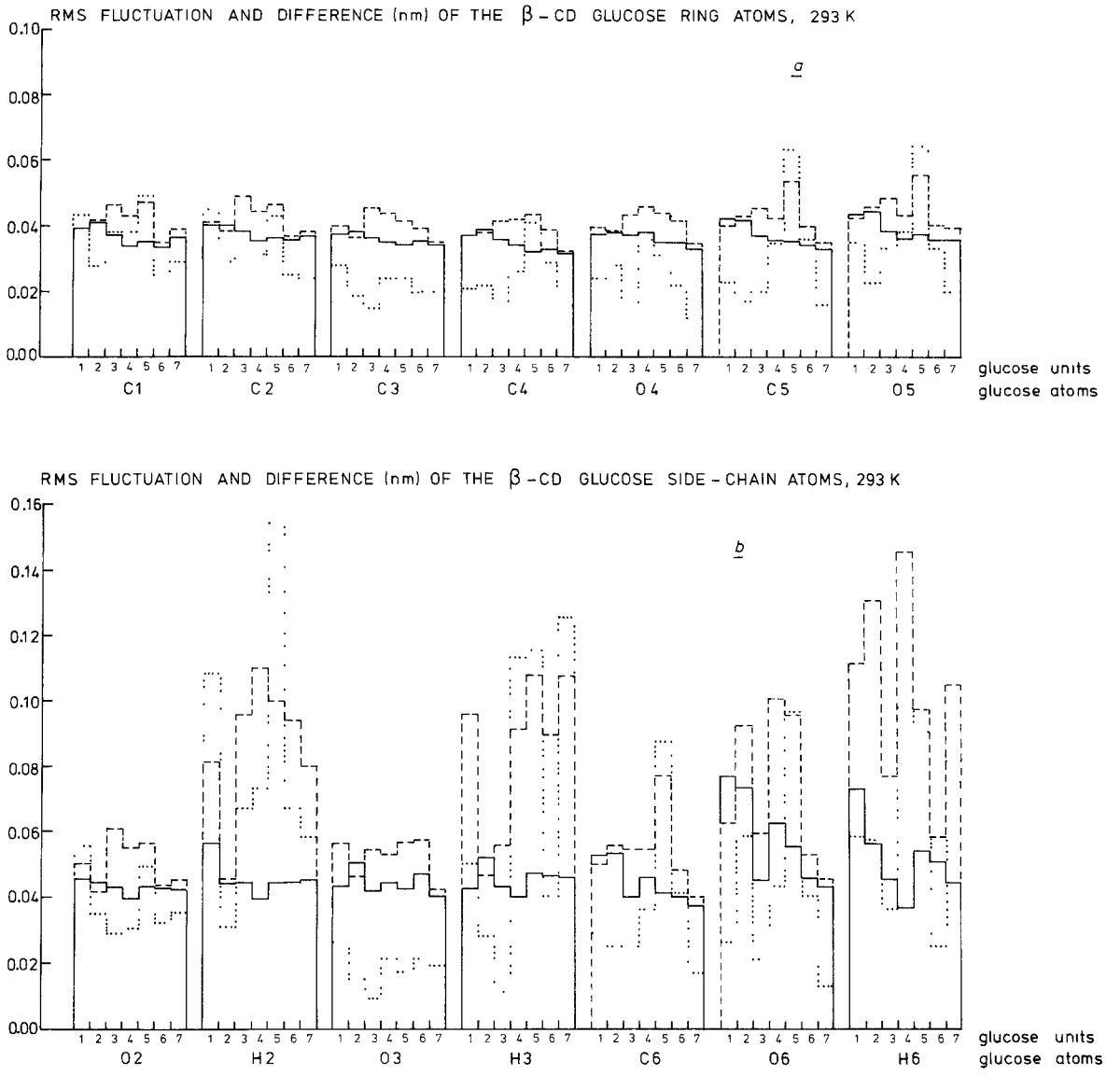

Fig. 1 a and b. rms positional fluctuations and differences (in nm) of main chain atoms (**a**) and side-chain atoms (**b**) in the 7 glucose units of β-cyclodextrin at 293 K. The atom numbering is defined in Fig. 1 of the preceding paper (Koehler et al. 1987 a). *Solid line*: fluctuation as derived from experimental isotropic *B*-factors; *dashed line*: fluctuation obtained from the MD simulation $\langle M1-8; 4-19\,\text{ps}\rangle$; *dotted line*: difference between the MD averaged structure and the experimental one

aged over all seven glucose units. The same values for an individual atom in a specific glucose unit are displayed in Fig. 1 a for the pyranose ring atoms and in Fig. 1 b for the side chain atoms (dotted lines). Averaging over a shorter time period $\langle M1-8; 4-14\,\text{ps}\rangle$ gives almost the same values for all atoms, 0.045 nm, except hydrogen 0.031 nm. rms positional differences of an arbitrarily chosen molecule $M1$ and its experimental equivalent can be larger, 0.064 nm, as well as the difference between two molecules, $M1$ and $M5$, which is in this case about 0.059 nm. These data demonstrate the different behaviour of individual molecules during the simulation.

rms fluctuations for pyranose ring atoms and the O2 and O3 atoms of the O2, O3 hydroxyl groups obtained from the simulation (Table 1 a, Fig. 1 a, b (dashed lines)) are slightly higher than the corresponding experimental fluctuations as derived from isotropic *B*-factors (Table 1 a and Fig. 1 a, b (solid lines)). The C6 and O 6 atoms and the hydrogen atoms show even larger MD fluctuations.

For the hydrogen atoms the MD fluctuations are twice as large as the experimental ones. This is to be expected since the crystallographic refinement procedure adheres *B*-factors or fluctuations to atomic *sites*, whereas the MD average is taken over the *trajectory* of

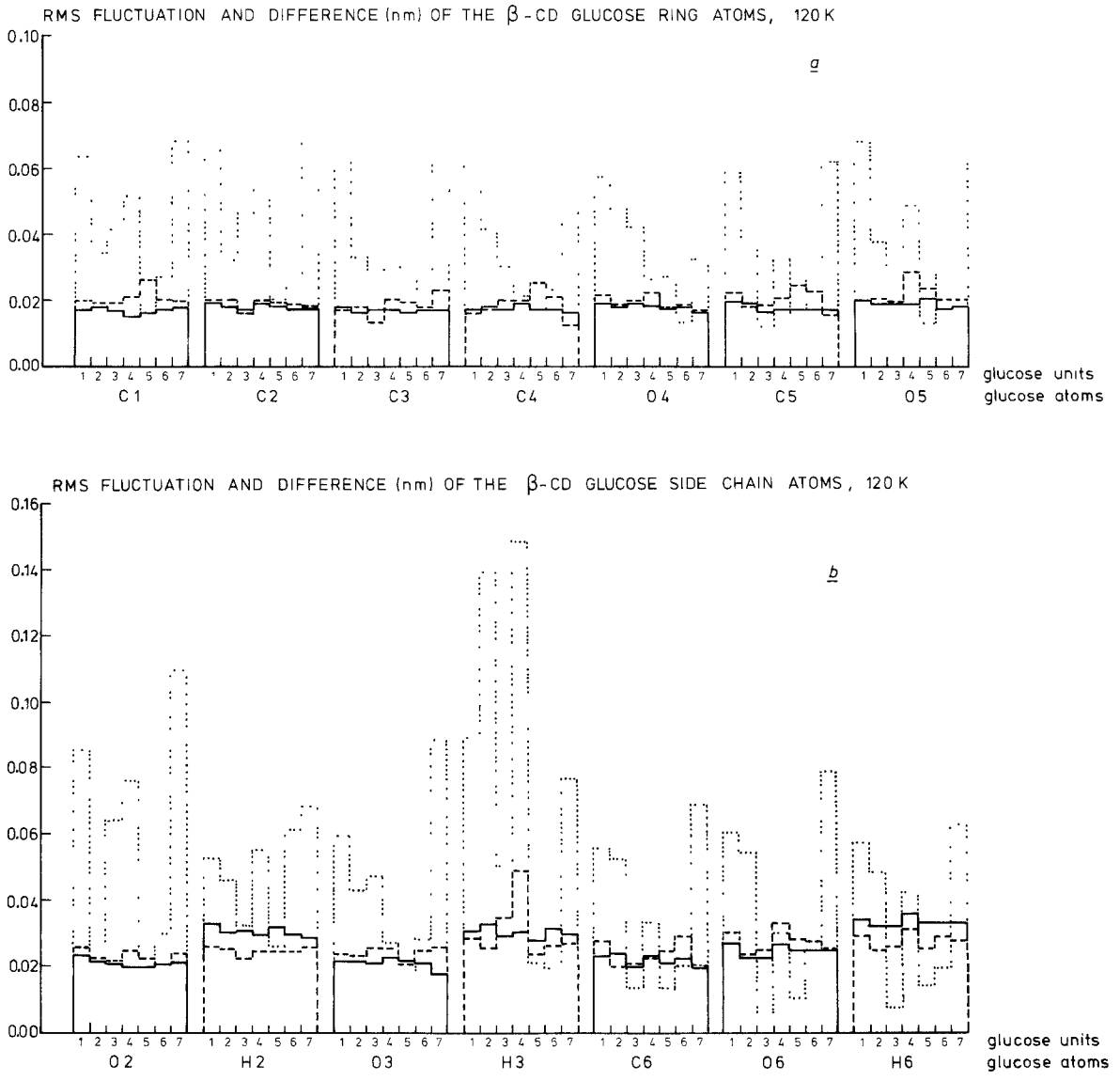

Fig. 2. As Fig. 1, but for 120 K

an individual atom, not a site. When the refinement procedure generates more than one site (with occupancy lower than 1) for an atom, the corresponding *B*-factors should therefore be smaller than the MD values which are summed over all sites that are accessible to an individual atom.

Most of the rms positional differences are smaller than the experimentally derived fluctuations especially for the non-hydrogen atoms. This means, that the averaged MD structure and the experimental structure are very close to each other, see Fig. 1 a, b. This was also true for α-CD · $6H_2O$ (Koehler et al. 1987a) where the α-CD molecule displays slightly smaller rms positional fluctuations than β-CD at room temperature (0.049 nm compared to 0.062 nm). These data indicate that the molecular structure of α-CD is more rigid compared to the larger β-CD.

At low temperature the rms positional differences between the experimental and the averaged MD structure $\langle M1-8; 5-20\,\text{ps}\rangle$ become somewhat larger (0.051 nm all atoms; 0.046 nm excluding hydrogens, Table 1 a, Fig. 2 a, b (dotted lines). The simulation period was long enough to let the molecule relax, because the values for $\langle M1-8; 5-15\,\text{ps}\rangle$ are almost identical (0.050 nm; 0.046 nm, Table 1 a). The difference between experiment and $\langle M1; 5-20\,\text{ps}\rangle$ is smaller (0.046 nm) compared to the room temperature result, and there is almost no geometrical difference between two molecules (0.004 nm).

rms fluctuations obtained from the simulation are very close to those derived from experimental temperature factors, see Table 1 a, Fig. 2 a, b (dashed and solid lines). Their values are half as large as their room temperature analogs, which illustrates that the mobility of

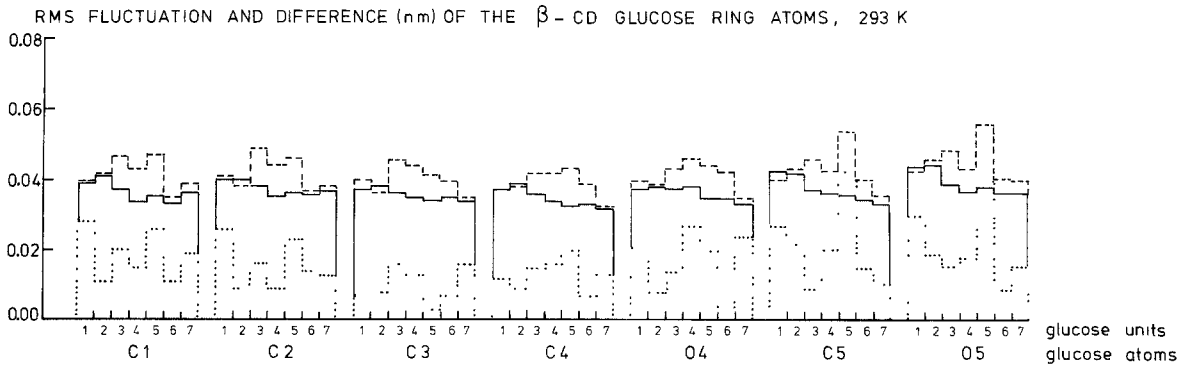

Fig. 3. As Fig. 1 a, but the difference between the MD averaged structure and the experimental one is plotted (*dotted line*) after the centres of mass of both structures have been superimposed

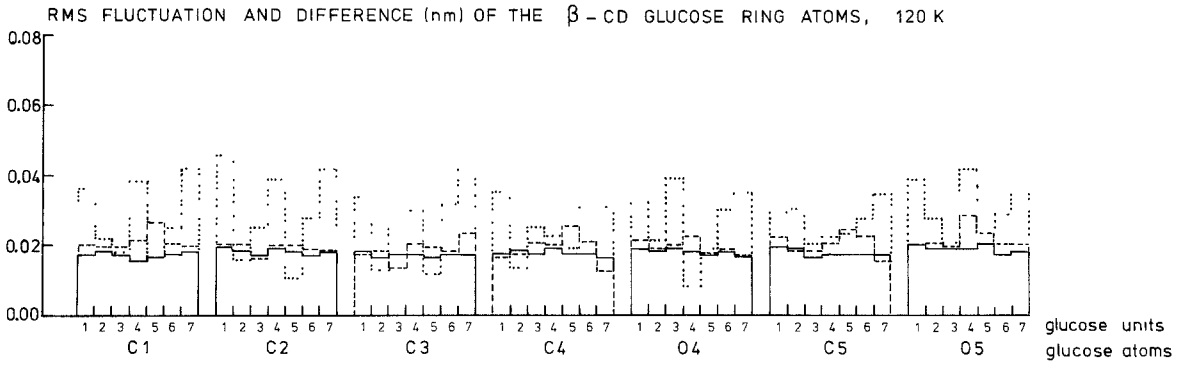

Fig. 4. As Fig. 3, but for 120 K

the molecules is more limited at lower temperatures. The similarity of experimental and MD fluctuations shows that MD can account for the difference in molecular flexibility at 293 K and 120 K. This is remarkable because the force field is an affective empirical potential, which was designed to model chemical systems at room temperature.

At low temperature the rms positional deviation of the MD averaged atomic positions with respect to the experimental ones is larger than both the experimental and the simulated atomic fluctuations. The larger deviation is partly due to displacements of complete β-CD molecules. As described in (Koehler et al. 1987 a) it is possible to superimpose the centres of mass of two β-CD structures before comparing them. When this translational fit procedure is applied, the deviation from the experimental structure of β-CD is reduced by about 0.02 nm, as can be seen by comparing Fig. 3 (translational fit, 293 K) with Fig. 1 a (no fit, 293 K) and comparing Fig. 4 (translational fit, 120 K) and Fig. 2 a (no fit, 120 K). For α-CD the corresponding reduction is about 0.01 nm (Koehler et al. 1987 a), which reflects the more tight packing of molecules in the α-CD crystal. An impression of the agreement between the simulated and the experimental β-CD structure can be obtained from Fig. 5.

Structural differences between β-CD at 120 K and 293 K are given in Table 1 b. Experimentally the rms positional difference between β-CD at 120 K and 293 K is 0.070 nm when averaged over all atoms and 0.050 nm for the non-hydrogen atoms. The two MD average structures at these temperatures differ by 0.077 nm (all atoms) and 0.066 nm (no H-atoms).

These values show that MD can account for the size of the structural differences at 120 K and 293 K. We note that the deviation of the MD structures from the experimental ones at corresponding temperatures is smaller than the structural difference as a function of temperature both for experiment and simulation. This picture is not changed when the translational fit procedure is applied before comparing various structures (lower-left part of Table 1 b).

Anisotropic atomic motion

Ratios between shortest and longest axes of thermal ellipsoids have been calculated from experimentally obtained anisotropic *B*-factors and from the (anisotropic) MD-fluctuations, see Table 1 a, b. A value of 1.0 represents completely isotropic fluctuations. The degree of anisotropy of the atomic motion that is ob-

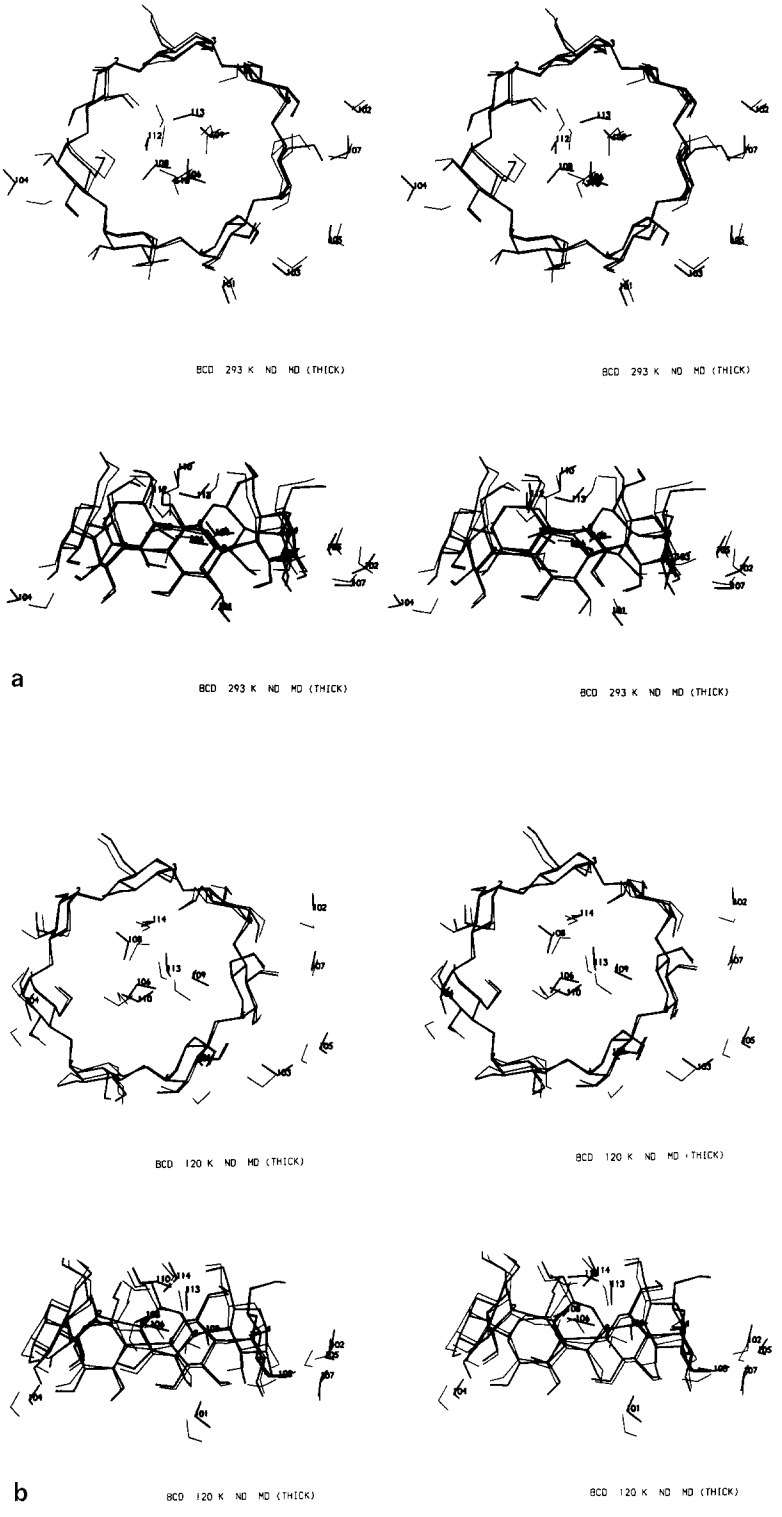

Fig. 5a and b. β-cyclodextrin at 293 K (**a**) and at 120 K (**b**). *Thin lines:* neutron diffraction structure; *thick lines:* averaged MD structure

served experimentally is generally well reproduced by the simulations. For the more mobile atoms like O6 and the hydrogen atoms at room temperature, the simulation shows a larger than average anisotropy. This can be understood from the occurrence of multiple sites for these atoms. When an atom is distributed over two sites, the atomic positional fluctuation along the line connecting the two sites will be much larger than in the directions perpendicular to it. If an atom occupies multiple sites, the MD anisotropy, which covers all sites, will in general be larger than the experimental anisotropy of each of the sites.

Bond angles and torsion angles

rms differences and fluctuations of bond angles in β-CD are larger at room temperature (5.4° and 4.1°), than at 120 K (2.9° and 2.5°), see Table 2 a, b. The angles that contain hydroxyl hydrogen atoms have a major influence upon the deviation from the experimental values at room temperature: excluding these bond angles, the deviation is only 2.8° which is even smaller than the value of 3.0° at 120 K.

The glucose ring torsion angles have rms differences of 3.2° at 293 K and 4.7° at low temperature. rms fluctuations are higher at room temperature, 6.3°, compared to low temperature values, being 3.7° on average, see Table 2 b.

When the temperature is lowered from 293 K to 120 K the simulation shows the correct direction of change for the torsion angles. All glycosidic bond torsion angles have similar values around 130°, Table 2 c. Their fluctuations are higher than those of the more rigid glucose ring torsion angles; they are about 9° at 293 K and about 4.5° at 120 K. As for α-CD (Koehler et al. 1987 a), the variation of the glycosidic bond torsion angles along the β-CD chain is reasonably well reproduced by the simulation.

β-CD torsion angles involving the glucose ring atoms and the non-hydrogen atoms in the short side chains are very similar in the experimental structure and in the averaged MD structure ⟨*M*1–8; 15 ps⟩ at high and low temperature, as can be seen from

Table 2 a–c. Bond- and torsion angles (in degrees), averaged over the corresponding angles in the 7 glucose units for the experimental structure and for the MD structure (⟨*M*1–8; 4–19 ps⟩ at 293 K, and ⟨*M*1–8; 5–20 ps⟩ at 120 K, are given in the first two columns. The third column gives the rms difference between the experimental and MD values. The last column contains the rms dynamical fluctuation of the angles as observed in the simulation

a) β-cyclodextrin, bond-angles

	293 K				120 K			
	Bond angle		Difference	Fluctuation	Bond angle		Difference	Fluctuation
	exp	MD	exp/MD	MD	exp	MD	exp/MD	MD
$C_2-C_1-O_4$	108.5	114.3	5.8	3.9	107.9	113.8	6.0	2.5
$C_1-O_5-C_5$	114.5	115.0	0.9	3.4	114.4	114.4	0.6	2.1
$C_5-C_6-O_6$	112.0	114.4	2.9	4.9	111.8	113.3	2.8	2.9
$C_2-O_2-H_2$	113.6	122.6	13.4	4.5	108.9	110.3	2.3	2.9
$C_3-O_3-H_3$	112.0	119.9	10.7	4.5	108.2	110.2	3.0	2.9
$C_6-O_6-H_6$	106.3	116.1	13.6	4.5	108.4	108.0	1.2	2.8
All atoms excl. H			2.8	4.0			3.0	2.4
All atoms			5.4	4.1			2.9	2.5

b) β-cyclodextrin glucose ring torsion angles

	293 K				120 K			
	Torsion angle		Difference	Fluctuation	Torsion angle		Difference	Fluctuation
	exp	MD	exp/MD	MD	exp	MD	exp/MD	MD
$C_1-C_2-C_3-C_4$	−53.6	−53.6	2.1	6.4	−52.6	−51.4	5.0	3.7
$O_5-C_1-C_2-C_3$	+56.2	+56.0	1.1	5.6	+55.5	+55.0	4.6	3.4
All			3.2	6.3			4.7	3.7

c) β-cyclodextrin glucoside bond torsion angles

	293 K			120 K		
	Torsion angle		Fluctuation	Torsion angle		Fluctuation
	exp	MD	MD	exp	MD	MD
$C_{32}-C_{42}-O_{42}-C_{11}$	+133.9	+125.0	8.3	+129.8	+122.9	4.4
$C_{33}-C_{43}-O_{43}-C_{12}$	+140.3	+130.1	7.5	+143.4	+131.4	4.4
$C_{34}-C_{44}-O_{44}-C_{13}$	+114.2	+108.0	9.2	+114.4	+104.7	5.0
$C_{35}-C_{45}-O_{45}-C_{14}$	+130.1	+125.9	8.0	+129.6	+121.0	4.3
$C_{36}-C_{46}-O_{46}-C_{15}$	+125.1	+115.4	10.0	+125.6	+119.1	4.4
$C_{37}-C_{47}-O_{47}-C_{16}$	+129.4	+119.9	7.5	+134.3	+121.7	4.3
$C_{31}-C_{41}-O_{41}-C_{17}$	+118.9	+114.3	7.8	+120.3	+112.7	4.8
All $C_3-C_4-O_4-C_1$	+127.4	+119.8	8.4	+128.2	+119.1	4.5

Table 3 a, b. Glucose ring side-chain torsion angles (in degrees) in the 7 glucose units for the experimental structure and from the MD simulation ⟨*M*1–8; 4–19 ps⟩ at 293 K, Table 3 a, and ⟨*M*1–8; 5–20 ps⟩ at 120 K, Table 3 b. The last column gives the rms dynamical fluctuations of the torsion angles as observed in the simulation

a) β-cyclodextrin glucose ring torsion angles involving exocyclic atoms, 293 K

Glucose unit	$O_5-C_1-C_2-O_2$			$C_1-C_2-C_3-O_3$			$C_4-C_5-C_6-O_6$		
	Torsion angle		Fluctuation	Torsion angle		Fluctuation	Torsion angle		Fluctuation
	exp	MD	MD	exp	MD	MD	exp	MD	MD
1	+177.7	+178.8	7.4	−171.5	−170.0	9.4	−174.2	−166.7	14.3
2	+174.6	+177.2	7.2	−174.8	−170.9	8.1	−169.1	−145.6	33.8
3	−178.7	−176.7	7.9	−175.8	−176.1	7.8	+ 57.3	+ 56.8	9.2
4	+180.0	−175.5	7.5	−176.5	−177.0	7.3	−177.4	−177.8	44.2
5	−179.7	−179.7	8.2	−176.6	−178.7	9.2	+ 50.5	+ 55.5	11.5
6	−180.0	−179.4	7.7	−174.8	−174.8	9.2	+ 58.5	+ 64.2	8.8
7	−179.4	−175.2	7.1	−174.8	−179.5	6.9	+ 59.9	+ 63.0	9.6
All	+179.2	−178.6	7.6	−175.0	−175.3	8.3	+112.2	+118.5	22.9
Glucose unit	$C_1-C_2-O_2-H_2$			$C_2-C_3-O_3-H_3$			$C_5-C_6-O_6-H_6$		
	Torsion angle		Fluctuation	Torsion angle		Fluctuation	Torsion angle		Fluctuation
	exp	MD	MD	exp	MD	MD	exp	MD	MD
1	+119.1	+157.1	32.1	+176.1	−149.8	57.1	− 82.6	− 69.9	84.5
2	+155.7	+154.3	13.4	+161.2	+173.3	15.4	+ 61.7	+ 50.5	163.6
3	+131.8	+127.3	245.9	+153.4	+161.9	18.5	−177.8	+179.3	24.7
4	− 46.7	− 42.7	118.8	+177.6	−100.1	51.3	+ 98.4	+125.0	171.4
5	− 37.8	+168.5	125.9	+153.8	− 97.1	74.3	+ 67.8	+ 71.1	27.8
6	+177.8	−123.0	185.5	+166.7	+173.6	47.7	+ 89.0	+ 92.0	16.4
7	+ 99.9	+114.0	48.8	+ 58.4	−141.1	82.9	+ 61.8	+118.7	111.4
All	+ 85.7	−177.8	135.5	+149.6	−151.3	54.9	− 34.6	− 73.3	105.1

b) β-cyclodextrin glucose ring torsion angles involving exocyclic atms, 120 K

Glucose unit	$O_5-C_1-C_2-O_2$			$C_1-C_2-C_3-O_3$			$C_4-C_5-C_6-O_6$		
	Torsion angle		Fluctuation	Torsion angle		Fluctuation	Torsion angle		Fluctuation
	exp	MD	MD	exp	MD	MD	exp	MD	MD
1	−175.9	+175.9	5.2	−176.1	−163.2	4.6	−172.1	−163.4	5.3
2	+170.9	+177.2	4.4	−171.0	−173.0	4.6	+ 52.2	+ 59.6	4.8
3	+178.9	−174.4	4.1	−174.4	−174.1	4.4	+ 55.2	+ 58.9	5.5
4	−178.9	+176.7	4.6	−176.4	−170.7	4.5	−177.2	−158.4	5.5
5	+178.5	−175.8	4.6	−176.6	−175.8	4.1	+ 46.0	+ 55.5	5.1
6	+179.7	−178.9	4.6	−173.5	−173.2	4.8	+ 54.8	+ 60.5	4.4
7	+177.4	−168.2	4.4	−175.9	−173.4	4.9	+ 55.6	+ 67.5	6.2
All	+178.6	−179.4	4.6	−174.9	−171.9	4.6	+ 90.6	+100.0	5.3
Glucose unit	$C_1-C_2-O_2-H_2$			$C_2-C_3-O_3-H_3$			$C_5-C_6-O_6-H_6$		
	Torsion angle		Fluctuation	Torsion angle		Fluctuation	Torsion angle		Fluctuation
	exp	MD	MD	exp	MD	MD	exp	MD	MD
1	− 40.5	− 19.4	10.8	+179.1	−121.4	13.4	− 71.4	− 82.7	8.0
2	+156.7	+167.3	6.7	+ 53.8	− 29.1	9.3	+ 70.5	+ 70.0	8.0
3	+122.1	+ 89.0	9.0	+152.1	+157.6	13.4	+178.1	−174.3	7.3
4	− 46.2	− 26.3	8.5	+177.8	− 56.2	26.1	+ 79.5	+ 73.5	7.8
5	−164.2	−172.8	7.7	+ 42.5	+ 43.5	8.1	+ 64.4	+ 59.5	7.6
6	− 37.7	− 16.0	9.8	+171.1	+179.6	8.0	+ 88.7	+ 82.6	7.2
7	+ 91.6	+ 58.8	8.8	+ 63.2	− 21.4	9.9	+166.2	+174.6	7.6
All	− 39.7	− 39.9	8.8	+119.9	− 81.1	13.9	− 20.6	− 20.9	7.6

Table 3 a, b. Their fluctuations are about 8° at 293 K and about 4.6° at 120 K. The torsion angles C4–C5–C6–O6 show larger differences and larger fluctuations: about 23° difference at 293 K and about 5.3° at 120 K. Especially glucose units 4 and 2 show high fluctuation values at room temperature (44.2° and 33.8°). The same trend is also reflected in large fluctuations for the torsion angles C5–C6–O6–H6 of these two glucoses, 171.4° and 163.6°, Table 3 a, which means that these atomic groups are extremely flexible at room temperature. This is also true for the C1–C2–O2–H2 torsion angles which show much larger fluctuations than angles C2–C3–O3–H3.

At low temperature the mobility decreases substantially, as can be seen from the C1–C2–O2–H2, C2–C3–O3–H3 and C5–C6–O6–H6 fluctuations. Here the groups involving O3 tend to show the largest flexibility. The torsion angles obtained from the MD averaged structure differ more significantly from those observed in the experimental structure for C4–C5–C6–O6, C1–C2–O2–H2, C2–C3–O3–H3, C5–C6–O6–H6 at 293 K and C2–C3–O3–H3 at 120 K. This is based on 120° rotational transitions which are observed for just these torsion angles. For the eight β-CD molecules over a period of 15 ps the following number of 120° transitions have been observed: C4–C5–C6–O6, 293 K: 10 transitions, 120 K; none; C1–C2–O2–H2, 293 K: 60 transitions, 120 K: none; C2–C3–O3–H3, 293 K: 75 transitions, 120 K: 4 transitions; C5–C6–O6–H6, 293 K: 57 transitions, 120 K: none.

Occupancy factors of glucose atom sites

The neutron diffraction work at 293 K shows that one O6 hydroxyl group and 16 hydrogen atoms occupy two alternative sites. These are listed together with their experimental occupancy factors in Table 4. In order to compare simulation with experiment, the electron density map should be calculated directly from the MD atom trajectories, as in (van Gunsteren et al. 1983). However, both electron densities were calculated in the crystal coordinate system, and difficulties arose by overall translocations of the molecules in the unit cell.

Here we use a different approach to eliminate the effects of overall translations of the molecules in the calculation of the MD occupancy of specific atomic sites. First, the position of the reference experimental site is expressed with respect to the experimental positions of three reference glucose atoms. E.g. the position of site HO21A is determined with respect to the positions of reference atoms C11, C21 and C31. Second, we probe for each MD time frame (structure) whether a specified atom, say atom HO21, lies within a distance σ or 2σ from the reference position that is constructed using the actual MD coordinates of the three reference atoms. For the distance σ we have chosen the experimental rms fluctuation derived from the *B*-factor of a site. The occupancy factor that is determined in this way (Table 4) measures the occupancy relative to the positions of the three reference atoms, i.e. the occupancy with respect to a local coordinate system.

Applying the stringent criterion of only 1σ deviation, a non-zero occupancy is observed for 84% of the glucose hydrogen atom sites. For 62% of the hydrogen atoms the relative occupancy of the two alternative sites is qualitatively reproduced by the simulation. The results of Table 4 a match with those shown in Table 3 a; the hydrogen atoms that show a strong preference for a single site, like HO32A (occupancy = 92%), HO22A (occupancy = 83%), and HO33A (occupancy = 80%) exhibit the smallest torsion angle fluctuations in Table 3 a, viz. 15.4° (H32), 13.4° (H22) and 18.5° (H33).

At low temperature, alternative sites have been observed experimentally for the two β-CD hydrogen atoms HO24 and HO35 (Zabel et al. 1986) which are part of a 4-membered flip-flop ring. The low temperature MD simulation only populates one of the alternative sites (Table 4 b) corresponding to one homodromic arrangement in the 4-membered hydrogen bond ring (O24 → O35 → OW7 → OW2 → O24).

Position and mobility of water molecules

At low temperature, the refinement of the crystal structure using neutron diffraction data shows that all 12 water oxygen sites are fully occupied except for OW8, which has an occupancy of 64%. The majority (83%) of the hydrogen atom sites are fully occupied, indicating that the water structure is rather stable. This picture also emerges from the simulation. The simulated and experimental rms positional fluctuations of the water atoms are comparable. Half of the water sites are reproduced within 0.03 nm, 80% within 0.06 nm. Only two water molecules have substantially moved away from their initial experimental positions: W1 (0.15 nm) and W4 (0.08 nm). In Fig. 6 b the deviations of the time-averaged MD water positions from the experimental ones is shown for the individual water oxygen atoms. The distributions do not show much spread, that is, the water molecules in the different asymmetric units show very similar behaviour. Summarizing we conclude that with the exception of water W1, the position and mobility of the water molecules are well reproduced by the simulation at 120 K.

At room temperature, the crystallographic refinement yields 16 oxygen and 37 hydrogen sites which are

Table 4 a, b. The names and experimental occupancy factors have been taken from (a) Betzel et al. 1984 and (b) Zabel et al. 1986. MD occupancy factors are calculated using a radius σ or 2σ around the locally defined experimental atom site. The value of σ is chosen equal to $(3 \cdot B_{iso}/8\pi^2)^{1/2}$

a) at 293 K

Name of site	exp	MD		Name of site	exp	MD		Name of site	exp	MD	
		σ	2σ			σ	2σ			σ	2σ
HO21A	0.66	0.39	0.91	OW1	1.00	0.25	0.69	OW6	0.88	0.08	0.32
HO21B	0.34	0.00	0.23	HW1A	0.50	0.18	0.59	HW6A	0.87	0.03	0.26
HO31A	0.58	0.45	0.66	HW1B	0.60	0.15	0.45	HW6B	0.95	0.07	0.41
HO31B	0.30	0.20	0.33	HW1C	0.49	0.16	0.42				
				HW1D	0.34	0.01	0.19	OW7	0.78	0.09	0.35
								HW7A	0.83	0.11	0.65
HO22A	0.60	0.83	1.00	OW2	1.00	0.25	0.68	HW7B	0.80	0.13	0.37
HO22B	0.37	0.00	0.62	HW2A	0.99	0.40	0.88				
HO32A	0.65	0.92	1.00	HW2B	0.44	0.14	0.48	OW8	0.53	0.00	0.02
HO32B	0.38	0.00	0.02	HW2C	0.49	0.03	0.14	HW8A	0.52	0.01	0.07
HO62A	0.53	0.43	0.69					HW8B	0.61	0.01	0.05
HO62B	0.43	0.00	0.04	OW3A	0.71	0.04	0.24				
HO23A	0.48	0.22	0.61	HW3A1	0.12	0.01	0.07	OW9	0.72	0.04	0.26
HO23B	0.54	0.21	0.49	HW3A2	0.37	0.05	0.42	HW9A	0.63	0.03	0.18
HO33A	0.61	0.80	0.99	HW3A3	0.46	0.09	0.42	HW9B	0.75	0.03	0.28
HO33B	0.37	0.00	0.27	HW3A4	0.35	0.04	0.25				
HO24A	0.43	0.38	0.59					OW10	0.81	0.04	0.26
HO24B	0.53	0.21	0.26	OW3B	0.28	0.13	0.38	HW10A	0.81	0.08	0.55
HO34A	0.46	0.16	0.31	HW3B1	0.22	0.01	0.16	HW10B	0.82	0.03	0.30
HO34B	0.56	0.51	0.82	HW3B2	0.41	0.01	0.17				
HO25A	0.58	0.11	0.38					OW12	0.89	0.02	0.20
HO25B	0.42	0.59	0.85	OW4	1.00	0.14	0.55	HW12A	1.00	0.04	0.34
HO35A	0.57	0.22	0.66	HW4A	0.58	0.06	0.35	HW12B	0.84	0.07	0.59
HO35B	0.44	0.33	0.58	HW4B	0.49	0.16	0.81				
HO26A	0.52	0.34	0.51	HW4C	0.80	0.03	0.20	OW13	0.68	0.01	0.10
HO26B	0.48	0.29	0.61					HW13A	0.66	0.02	0.16
HO36A	0.42	0.60	0.85	OW5A	0.70	0.22	0.52	HW13B	0.68	0.04	0.31
HO36B	0.59	0.09	0.13	OW5B	0.13	0.02	0.14				
HO27A	0.58	0.35	0.78	OW5C	0.20	0.01	0.10	OW14	0.62	0.02	0.12
HO27B	0.34	0.05	0.49	HW5A	0.60	0.23	0.68	HW14A	0.64	0.01	0.06
HO37A	0.46	0.09	0.11	HW5B	0.51	0.21	0.65	HW14B	0.61	0.01	0.12
HO37B	0.47	0.44	0.68	HW5C	0.47	0.02	0.15				
HO67A	0.33	0.37	0.47	HW5D	0.21	0.03	0.20	*b) at 120 K*			
HO67B	0.62	0.36	0.51	HW5E	0.22	0.09	0.62				
								HO24A	0.49	0.00	0.00
								HO24B	0.49	0.64	0.99
								HO35A	0.49	0.00	0.00
								HO35B	0.48	0.87	1.00

occupied by 11 ± 0.5 water molecules (Betzel et al. 1984). Only three sites (W1, W2 and W4) are fully occupied. Therefore, it is not surprising that the simulation displays a large overall rms positional fluctuation for the water atoms of 0.10 nm. The value derived from the crystallographic *B*-factors is 0.05 nm. As was discussed for the glucose hydrogen atoms, the discrepancy between these values is explained by the occurrence of alternative sites which are accessible for one atom. The crystallographic refinement procedure treats different sites that are partially occupied by one atom separately with a *B*-factor for each site, whereas the MD values in Fig. 6 are time-averaged over the trajectories of individual atoms, irrespective of the different sites that are visited by one atom. Only when a water molecule is not diffused from one site to another in the simulation its time-averaged position can be compared to the experimental one. Only water W1 has a simulated mobility (0.040 nm) that is comparable to the experimental value (0.044 nm). The experimental position of this water molecule is nicely reproduced within 0.03 nm. For all other water molecules their diffusion does not allow a direct comparison of time-averaged and experimental positions. Figure 6 a shows how far the individual water molecules in the 8 symmetric units have been moved away from their initial (experimental) positions during the equilibration period of the MD simulation. In contrast with the low temperature result, the distribution for most water molecules is very wide; in one asymmetric unit a water

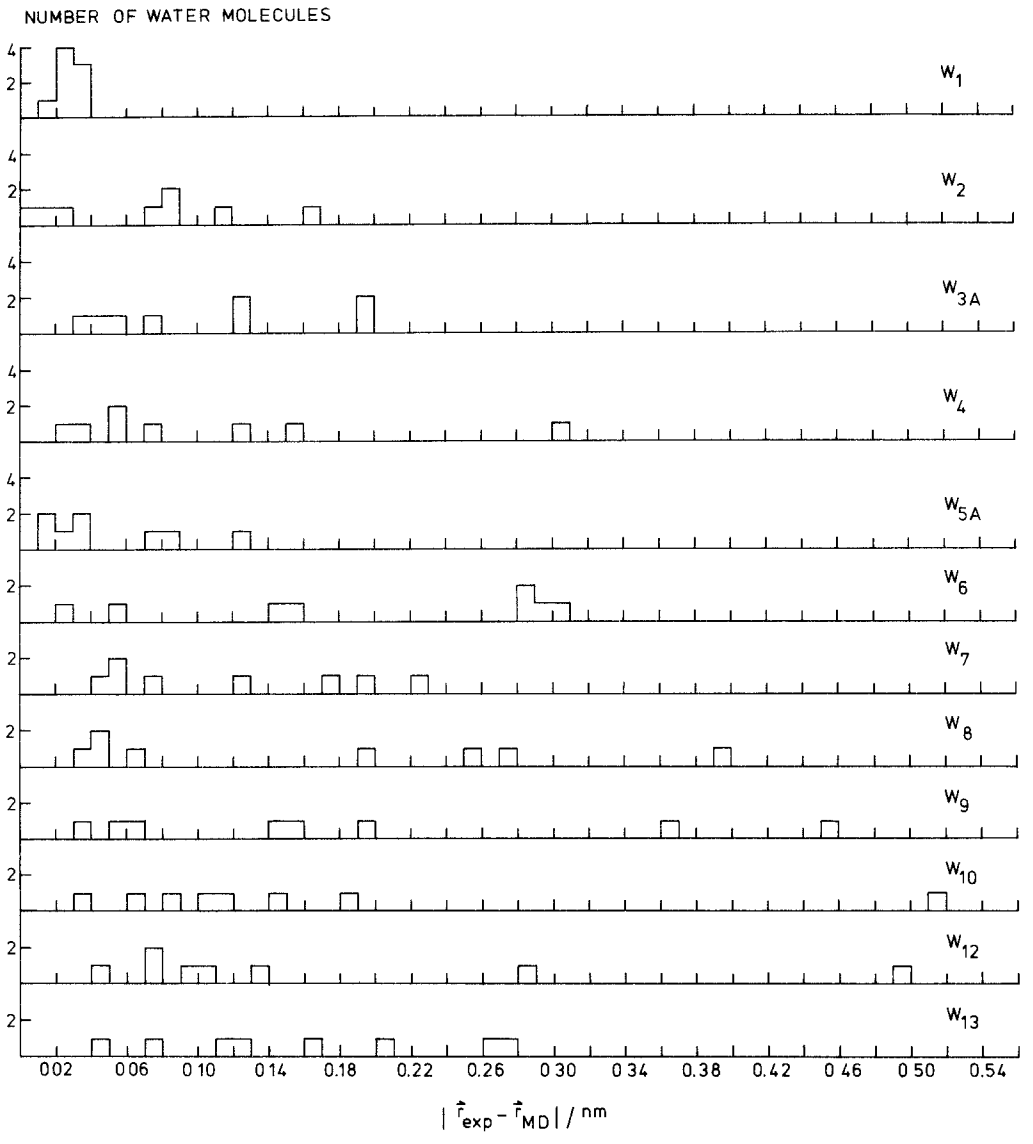

Fig. 6a

molecule may stay within 0.05 nm, whereas in another it may move by several Angstroms.

Because of the diffusion that is observed for the water molecules in the simulation process, we decided to perform a local occupancy factor analysis for the experimentally observed water sites, just as has been done for the alternative sites of the glucose hydrogen atoms. Here, a complication is the choice of the three reference atoms that define the local coordinate frame in which the local occupancy is calculated. For each water site the three non-hydrogen glucose atoms that are closest to it in the experimental structure have been taken as reference atoms. The calculated occupancy factors are low in the distance range 1 σ, see Table 4 a, because the σ-values are only 0.053 nm on average for both hydrogen and oxygen atoms. If the occupancy is measured with a radius of $2\sigma \approx 0.1$ nm around the experimental site, the occupancy factors increase considerably. Only W8 has an occupancy below 10%, which is also reflected in the neutron crystal structure analysis where this water displays the lowest occupancy. Apart from an overall normalization factor, the distribution of atom density over the various available sites as obtained from the simulation is comparable to the distribution resulting from the crystallographic refinement. It would be interesting to calculate structure factors directly from the MD atomic trajectories and to compare them to the observed ones.

Hydrogen bond network

Another way to describe the structure of β-CD and the water molecules in the crystal is to determine the hydrogen-bond network. This turns out to be partly stable and partly dynamic, giving rise to the phenome-

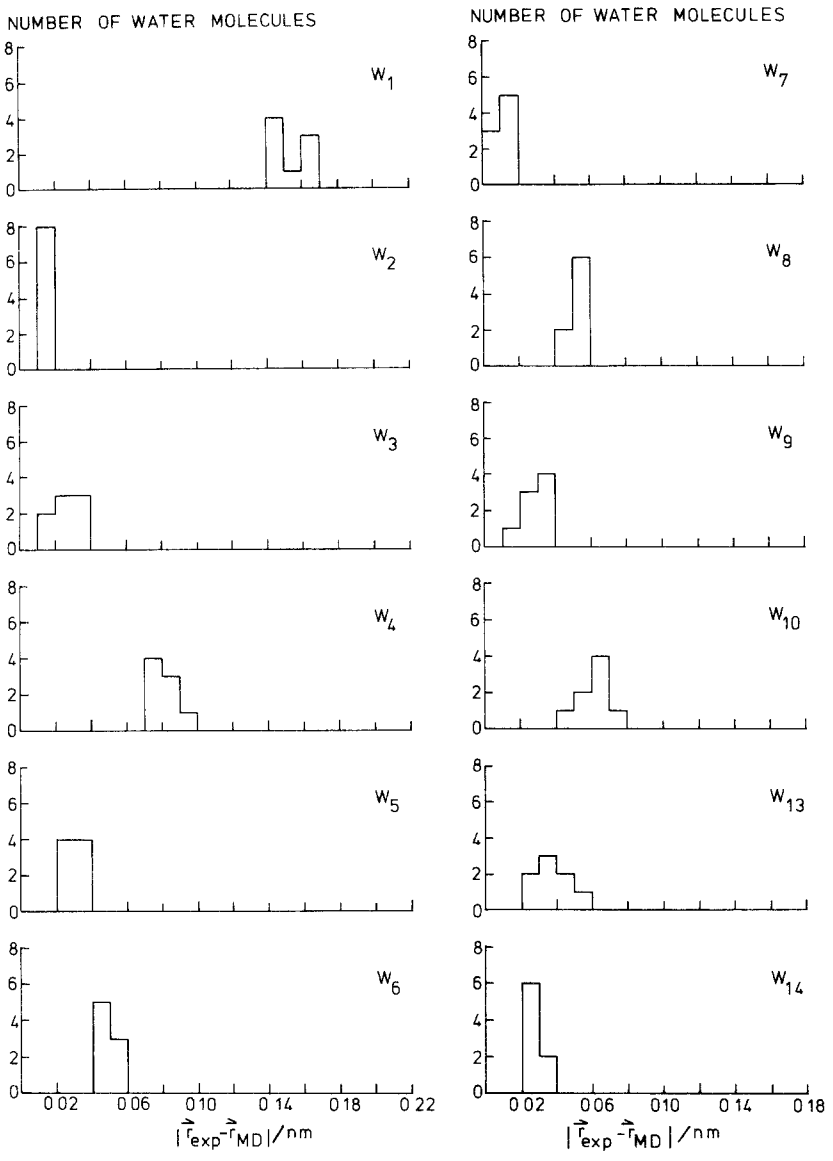

Fig. 6a and b. Distribution of the distance (in nm) between the MD time-averaged position and the experimental position for corresponding water molecules in the 8 asymmetric units. The distribution is given for each of the 12 water molecules present per asymmetric unit **a** at 293 K; **b** at 120 K

non of flip-flop hydrogen bonds. This will be discussed in the following paper (Koehler et al. 1987b).

Conclusions

Crystalline β-cyclodextrin dodecahydrate has been simulated at two different temperatures, 293 K and 120 K over a period of about 20 ps, using the molecular dynamics (MD) technique. Atomic coordinates derived from neutron diffraction studies (Betzel et al. 1984; Zabel et al. 1986) were used as initial positions. The system that is simulated consists of four unit cells containing 8 β-CD molecules and 96 water molecules. Crystalline periodic boundary conditions are applied. A period of 4 to 5 ps turned out to be long enough to reach a satisfactory degree of equilibrium. Analysis is performed for the final 15 ps of each MD simulation. Statistics is enhanced by averaging over the 8 asymmetric units.

At room temperature, the experimental positions of the (non-hydrogen) glucose atoms are reproduced within 0.034 nm. This value is smaller than the root mean square (rms) atomic fluctuation of 0.041 nm as derived from the crystallographic B-factors. The simulation shows an overall rms atomic fluctuation of 0.049 nm, slightly larger than the experimental value. At low temperature the experimental positions are reproduced within 0.046 nm. This value is larger than the rms atomic fluctuation of 0.019 nm (experiment) or 0.022 nm (MD). Both experiment and simulation show a reduction by a factor 2 of the atomic mobility when lowering the temperature from 293 K to 120 K. The larger deviation of the atomic positions from the experimental ones at low temperature might be due to the fact that the empirical interatomic potential func-

tion has been designed as an effective force field at room temperature.

In the average values of bond angles and torsion angles the slight changes that are experimentally observed when reducing the temperature, are qualitatively reproduced by the simulation. Also the variation of corresponding torsion angles along the cyclic chain of 7 glucose units in β-CD is qualitatively mirrored in the simulations. At both temperatures the degree of anisotropy in the atomic motions that is observed experimentally is also found in the simulations.

At room temperature, the neutron diffraction refinement yields alternative sites for 17 glucose atoms, one O 6 oxygen and 16 hydroxyl hydrogens. For two thirds of the atoms the relative occupancy of the alternative sites is qualitatively reproduced in the simulation. Although the β-CD crystal contains twice as many water molecules per CD molecule compared to the α-CD crystal and also shows more atomic disorder, the agreement with the experimental data for the glucose atoms is of the same quality in both studies (Koehler et al. 1987a).

At low temperature, for 10 out of 12 water molecules the simulated positions lie within 0.06 nm from the crystallographic positions. Only water W1 is consistently shifted away from its experimental position by 0.15 nm in all 8 asymmetric units. The simulated rms atomic fluctuations of the water molecules (0.03 nm) are slightly smaller than the experimental ones (0.04 nm). At room temperature, only one water molecule (W1) remains in a relatively fixed position close to the experimental one. The other water molecules show various degrees of diffusion through the crystal. Apart from an overall normalization factor, the simulated occupation of the water sites is comparable to the distribution observed in the crystal structure.

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