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The flip-flop hydrogen bonding phenomenon

Molecular dynamics simulation of crystalline β-cyclodextrin

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Abstract. In crystalline β-cyclodextrin undecahydrate (β-cyclodextrin · 11 H_2O), flip-flop hydrogen bonds $O-H\cdots O-H \rightleftharpoons H-O\cdots H-O$ have been detected by neutron diffraction studies. In this type of bond the directionality is inverted dynamically even in the crystalline state as could be shown by diffraction experiments carried out at 293 K and at 120 K. Molecular dynamics methods (MD) can be used to simulate the dynamics of molecular systems on a computer. In this paper, the atomic trajectories obtained by MD simulations, of β-cyclodextrin at 293 K and at 120 K and of α-cyclodextrin at 293 K, are analysed with respect to the occurrence of hydrogen bonds of flip-flop type. In all three simulations the hydrogen bonds with the highest percentage of occurrence correspond to the ones found in the neutron diffraction structure. In the simulation of crystalline β-cyclodextrin at 293 K over 19 ps, sixteen out of eighteen experimentally detected flip-flop bonds are reproduced. The other two hydrogen bonds are unidirectional, $O-H\cdots O$, i.e. they have a lifetime larger than 19 ps. The four experimentally observed flip-flops at 120 K are not seen in a 20 ps MD simulation. For α-cyclodextrin a flip-flop hydrogen bond is predicted with low population, which may be observed experimentally. The good agreement between MD calculations and neutron diffraction studies suggests that the force field used in the simulations yields a good description of cyclodextrin crystal structure at room temperature, and even the energetically delicate dynamic hydrogen bond flip-flop phenomenon is satisfactorily reproduced.

Key words: Flip-flop hydrogen bonds, neutron diffraction, molecular dynamics

Introduction

Cyclodextrins are cyclically closed oligosaccharides consisting of 6 (α-), 7 (β-) or 8 (γ-cyclodextrin) D-glucopyranose units covalently bound by α(1–4) linkages (Szejtli 1982; Saenger 1984). They exhibit a central cavity of 0.5 nm (α-cyclodextrin), 0.7 nm (β-cyclodextrin) or 0.8 nm (γ-cyclodextrin), and are able to include molecules small enough to fit in these cavities. The cyclodextrins can be crystallised as hydrates from water in the form of α-cyclodextrin · 6 H_2O (Klar et al. 1980; Chacko and Saenger 1981; Lindner and Saenger 1982), β-cyclodextrin · 11 H_2O (Betzel et al. 1984; Zabel et al. 1986), and γ-cyclodextrin · 19 H_2O (Lindner and Saenger 1980), where water molecules occupy voids between the cyclodextrin molecules and are included within their cavities.

The crystal structures of α-cyclodextrin · 6 H_2O and of β-cyclodextrin · 11 H_2O were determined by X-ray and neutron diffraction analysis (Klar et al. 1980; Betzel et al. 1984; Zabel et al. 1986). For β-cyclodextrin · 11 H_2O, a phase transition was observed (Fujiwara et al. 1983), when crystals were cooled below 227 K, and a subsequent neutron diffraction analysis carried out at 120 K, well below the transition temperature, revealed that several water molecules and O–H groups, which showed occupational or rotational disorder at room temperature (293 K), had moved into well-defined, fully occupied positions (Zabel et al. 1986).

Because each glucose contains two secondary O2, O3 and one primary O6 hydroxyl groups, which can act simultaneously as hydrogen bonding donor and as acceptor, and each water molecule also consists, formally, of two hydroxyl groups, a large number of $O-H\cdots O$ hydrogen bonds exist in the cyclodextrin hydrate crystal structures. They form extended networks displaying infinite chains and four-, five-, six-membered cycles (Saenger 1979) in which all hydrogen bonds point in the same direction $O-H\cdots O-H\cdots O-H$, called homodromic, a configuration which is stabilised by the influence of the cooperative effect (Lesyng and Saenger 1981; Koehler et al. 1987).

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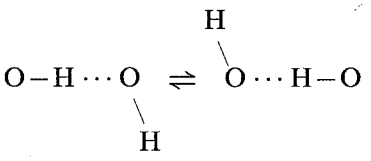
In β-cyclodextrin, besides homodromic arrangements of O−H ··· O hydrogen bonds, extended systems of hydrogen bonds of type O−H ··· H−O were observed by the neutron diffraction experiment. Because the H atom positions are only half filled and so close together, about 0.1 nm, that they cannot be occupied simultaneously, this was interpreted as time average between two configurations in dynamic equilibrium called flip-flop hydrogen bonds (Saenger et al. 1982; Betzel et al. 1984; Zabel et al. 1986).



Several O−H groups are involved in these flip-flop systems, and therefore the transition from one configuration to the other has to occur in a cooperative, concerted mode. The mechanism of the transition is still unknown. It could be either a rotation of hydroxyl groups from one into the other position, or a tunneling of H atoms along the hydrogen bond which, however, is unlikely to occur because the O−H ··· O hydrogen bonds in the flip-flops are unsymmetrical with distances O−H about 0.1 nm and H ··· O about 0.18 nm.

The most characteristic features in hydrogen bonding are, for β-cyclodextrin at 293 K, interglucose intramolecular flip-flop hydrogen bonds between all seven glucose units, an infinite flip-flop chain running through the whole crystal structure and disordered water molecules located within the cavity. If the crystal is cooled to 120 K, a neutron diffraction analysis has shown that the hydrogen bonds in the flip-flop chain become static in the homodromic arrangement, the disordered water molecules within the cavity of the β-cyclodextrin become ordered and form a homodromic hydrogen bonded, five-membered cycle, and all of the interglucose flip-flop hydrogen bonds disappear except one which becomes one side of a flip-flop four-membered cycle not observed at room temperature.

In previous contributions (Koehler et al. 1987a, b) molecular dynamics simulations on α- and β-cyclodextrin have been reported. There is good overall agreement between crystal structures and simulated data. The simulations correctly reproduce the distorted, collapsed molecular structure of α-cyclodextrin and the more regular, round structure of β-cyclodextrin as observed crystallographically. For β-cyclodextrin, the flexibility at 293 K is higher than at 120 K, both in experiment (temperature factors) and in simulation, and similar trends are observed for the mobility of the water molecules.

These results suggest that the force field employed in the MD simulations is suitable to describe the cyclodextrin hydrate crystal structures satisfactorily at 293 K and even at 120 K, although the force field was originally developed for room temperature studies. It is the aim of the present paper to summarise the results obtained from the MD simulations and to compare them with the neutron diffraction studies on cyclodextrin hydrates, with emphasis on the hydrogen bonding networks. The central questions are whether the atomic potential function used in the simulations is also able to model the weak hydrogen bonds and their flip-flop dynamics which are much more sensitive towards temperature changes than covalent bonds.

Method and computational details

The molecular dynamics simulations were carried out using the GROMOS (Groningen Molecular Simulation) program package, as previously described (Koehler et al. 1987a, b). The calculations are based on atomic coordinates obtained from the single crystal neutron diffraction studies on α-cyclodextrin at 293 K (Klar et al. 1980) and on β-cyclodextrin at 293 K and at 120 K (Betzel et al. 1984; Zabel et al. 1986), which allowed unambiguous location of all, C, O, H atoms and provided atomic coordinates which served as starting configurations for the MD runs.

In α-cyclodextrin · $6\,H_2O$, all atomic positions are fully occupied and well defined, except hydroxyl group O61, which is twofold disordered (O61*A*, 92% and O61*B*, 8%).[1] The situation is more complex in β-cyclodextrin · $11\,H_2O$, where $11 \cdot 5\,H_2O$ actually were located, and where at 293 K several hydroxyl hydrogen atoms and some of the water molecules are disordered. Thus, for the 11.5 water molecules there are 16 oxygen and 37 hydrogen atomic positions, which reduce at 120 K to 12 oxygen and 26 hydrogen positions.

For α-cyclodextrin at 293 K, four unit cells of the orthorhombic space group $P2_12_12_1$ with four asymmetric units comprising 16 α-cyclodextrin and 96 water molecules were simulated in the periodic "computational box" over a period of 15 ps. Averaging over the 16 asymmetric units and over the last 10 ps led to the MD structure denoted as ⟨*M*1−16; 5−15 ps⟩. For β-cyclodextrin at 293 K and 120 K, again four unit cells were used as the "computational box", but since the space group is monoclinic $P2_1$ with two asymmetric units, the simulation included only 8 β-cyclodextrin plus 96 water molecules. The time period for simulation was 19 ps (at 293 K) and 20 ps (at 120 K), and we averaged over the eight asymmetric units and over the last 15 ps to arrive at the MD structure denoted

[1] In our nomenclature, the first index of an atom name refers to its position in the glucose unit, and the second one to the sequence number of this glucose in the cyclodextrin molecule. Characters A and B refer to alternative experimentally determined sites.

Table 1a. Statistical analysis of hydrogen bonds in the crystal structures of α- and β-cyclodextrin at 293 K and at 120 K

Type of H-bond	*Number of hydrogen bonds*					
	16 α-CD + 96 H_2O structure, 293 K		8 β-CD + 96 H_2O structure, 293 K		8 β-CD + 96 H_2O structure, 120 K	
	exp.	⟨5–15 ps⟩	exp.	⟨4–19 ps⟩	exp.	⟨5–20 ps⟩
Glucose – glucose	192	166	80	67	88	80
Glucose – water	96	84	80	74	80	80
Water – glucose	144	104	96	65	96	93
Water – water	48	37	56	42	88	82
Total	480	391	312	248	352	335
	Geometry of hydrogen bonds					
D – H (nm)	0.096	0.096	0.098	0.095	0.097	0.100
D ··· A (nm)	0.286	0.281	0.287	0.285	0.283	0.275
H ··· A (nm)	0.192	0.189	0.191	0.195	0.189	0.179
DHA (deg.)	165.9	160.8	165.8	159.4	164.4	163.3

CD = cyclodextrin

⟨*M*1–8; 4–19 ps⟩ at 293 K, and ⟨*M*1–8; 5–20 ps⟩ at 120 K.

Constant volume periodic boundary conditions were applied to all three systems. All atoms were allowed to move and were treated explicitly except for the hydrogen atoms attached covalently to the carbon atoms, and all bond distances were held fixed in order to allow for a long MD time step of 2 fs when integrating Newton's equations of motion. The choice of the initial atomic positions has been discussed in (Koehler et al. 1987a, b). During the simulations all molecules follow their own special trajectories depending on their initial velocities, which were chosen from a Maxwellian distribution at the give temperature. Every 0.02 ps a configuration time frame was stored for the analysis of hydrogen bonds as a function of time.

In the analysis of the MD data, the following criterion for the existence of a hydrogen bond between donor (D), hydrogen (H), and acceptor (A) atom was used: distance H ··· A < 0.25 nm and angle D – H ··· A > 135°. When applied to each time frame of the MD trajectories this definition yields the percentage of occurrence of a hydrogen bond. Using this criterion, transitions between different hydrogen bond types can be monitored. Transitions of a hydrogen bond from one to another configuration sometimes show a diffuse pattern: atomic positional fluctuations at the transition state may generate many counted transitions due to a strict application of the hydrogen bond criterion. Therefore we have filtered out the effect of the local fluctuations by counting a transition only when a specific hydrogen bond has ceased to exist during ten time frames (0.2 ps). In this way hydrogen bond lifetimes were calculated, which have a lower bound of 0.2 ps and an upper bound of 10 ps (α-cyclodextrin) or 15 ps (β-cyclodextrin), the time period covered by the MD simulations.

Table 1b. Percentages of occurrence of hydrogen bonds in the MD simulations, listed according to the donor or acceptor function of the specified atom

	Donor	Acceptor						
		O2	O3	O4	O5	O6	OW	Sum
	O2							
α-CD, 293 K		0	5	0	1	2	11	19
β-CD, 293 K		0	5	0	0	1	11	17
β-CD, 120 K		1	9	0	0	0	7	17
	O3							
α-CD, 293 K		11	3	0	0	7	0	21
β-CD, 293 K		11	0	0	0	2	3	16
β-CD, 120 K		5	0	0	0	4	5	14
	O6							
α-CD, 293 K		3	5	0	0	5	9	22
β-CD, 293 K		0	1	0	0	3	13	17
β-CD, 120 K		2	0	0	0	3	12	17
	OW							
α-CD, 293 K		9	5	2	2	9	11	38
β-CD, 293 K		5	6	4	2	11	22	50
β-CD, 120 K		7	3	4	2	12	24	52
	Sum							
α-CD, 293 K		23	18	2	3	23	31	100
β-CD, 293 K		16	12	4	2	17	49	100
β-CD, 120 K		15	12	4	2	19	48	100

CD = cyclodextrin

Results and discussion

Hydrogen bond characteristics

A comparison between the experimentally determined crystal structure of α-cyclodextrin and the MD-simulated structure averaged over time and over the "computational box" (four unit cells) shows (Table 1), that the total number of all hydrogen bonds in the whole system is slightly smaller in the MD averaged

Table 2a–d. Hydrogen bonds of β-cyclodextrin at 293 K from neutron diffraction data (Betzel et al. 1984) and from the MD simulation (eight molecules, 4–19 ps) (Koehler et al. 1987b). Percentage of occurrence in MD and experimental occupancy factors are given together with the average geometries. The hydrogen bond donor is assumed to be an atom of the first asymmetric unit, the acceptor belongs to the asymmetric unit given in the first column. (*T*) indicates an applied symmetry code transfer according to Table 4. As initial positions for hydrogen atoms in the MD simulation, experimental hydrogen A positions have been taken. The hydrogen atoms move in MD, therefore the O37–H37 ··· O26 MD bond with 48.6% also corresponds to the experimental O37–H37B ··· O26 bond, etc. Experimental flip-flop hydrogen bonds are denoted by **

Sym. Code	Donor		Acceptor	MD				Experimental [ND]			
				%	D–A [nm]	H···A [nm]	DHA [deg]	D–A [nm]	H...A [nm]	DHA [deg]	occ. [H]
a) Glucose → glucose > 10%											
1	O32	H32	O21	95.7	0.289	0.194	159.6	0.297	0.206	168.6	0.654**
1	O33	H33	O22	94.3	0.282	0.188	158.8	0.289	0.197	167.6	0.614**
5	O66	H66	O63	88.2	0.282	0.187	158.5	0.282	0.190	164	–
1	O36	H36	O25	76.3	0.280	0.185	159.7	0.280	0.189	163.3	0.417**
1	O31	H31	O27	59.5	0.287	0.192	159.4	0.299	0.199	171.3	0.584**
3	O34	H34	O62	51.4	0.275	0.182	156.6	–	–	–	–
3	O34	HO34B	O62A	–	–	–	–	0.249	0.161	166	–
1	O24	H24	O35	50.9	0.271	0.175	162.3	0.280	0.192	157.7	0.434**
1	O37	H37	O26	48.6	0.275	0.181	157.6	–	–	–	–
1	O37	HO37B	O26	–	–	–	–	0.289	0.199	160.6	0.472**
2	O27	H27	O33	41.2	0.280	0.190	151.2	0.274	0.192	153	– **
3	O35	H35	O61	35.2	0.278	0.185	156.5	–	–	–	–
3	O35	HO35B	O61	–	–	–	–	0.296	0.191	164	–
1	O35	H35	O24	25.1	0.278	0.185	155.3	0.280	0.183	167.4	0.572**
1	O34	H34	O23	24.7	0.284	0.190	157.7	0.287	0.199	172.1	0.463**
1	O26	H26	O37	23.3	0.280	0.186	156.2	–	–	–	–
1	O26	HO26B	O37	–	–	–	–	0.289	0.208	152.7	0.476**
1	O23	H23	O34	20.6	0.278	0.183	161.9	–	–	–	–
1	O23	HO23B	O34	–	–	–	–	0.287	0.192	156.4	0.539**
6	O64	H64	O34	17.7	0.279	0.186	155.0	–	–	–	–
4	O26	H26	O32	17.4	0.292	0.202	149.3	–	–	–	–
1	O25	H25	O36	13.8	0.293	0.200	156.9	0.280	0.191	156.5	0.575**
1	O61	H61	O62	12.3	0.293	0.204	148.3	–	–	–	–
3	O25	H25	O61	11.3	0.298	0.210	148.1	–	–	–	–
6	O63	H63	O54	11.3	0.291	0.201	150.7	–	–	–	–
1	O21	HO21B	O32	–	–	–	–	0.297	0.212	165.8	0.343**
1	O27	HO27B	O31	–	–	–	–	0.294	0.203	163.6	0.344**
3	O62B	HO62B	O23	–	–	–	–	0.299	0.198	170	–
4	O33	HO33B	O27	–	–	–	–	0.274	0.181	163	– **
1	O22	HO22B	O33	–	–	–	–	0.289	0.197	167.6	0.614**
b) Glucose → water > 10%											
2	O22	H22	OW1	94.6	0.274	0.179	159.6	0.282	0.191	173	**
2	O65	H65	OW7	87.5	0.272	0.176	162.6	0.274	0.173	174	
1	O63	H63	OW5	72.3	0.277	0.182	159.8	0.283	0.190	177	
1	O63	HO63	OW5B	–	–	–	–	0.286	0.202	147	
3	O23	H23	OW3	66.5	0.276	0.181	159.7	0.277	0.181	162	**
1	O23	HO23A	OW5B	–	–	–	–	0.267	0.186	136	
4	O62	H62	OW12	58.1	0.272	0.176	161.9	0.293	0.195	164	
3	O67	H67	OW1	46.3	0.277	0.183	157.3	0.304	0.198	169	
4(T)	O64	H64	OW13	46.3	0.271	0.175	163.3	–	–	–	
5	O67	H67	OW3	45.5	0.280	0.185	159.4	–	–	–	
5	O67	HO67B	OW3B	–	–	–	–	0.278	0.202	136	**
5	O67	HO67B	OW3A	–	–	–	–	0.283	0.193	159	
1	O21	H21	OW6	44.7	0.278	0.184	156.9	0.278	0.188	168	
4	O25	H25	OW10	43.7	0.279	0.184	160.8	–	–	–	
1	O26	H26	OW1	38.7	0.281	0.188	156.5	0.274	0.191	164	**
4	O21	H21	OW4	37.7	0.289	0.195	159.6	–	–	–	
1	O61	H61	OW4	36.8	0.275	0.180	160.7	0.271	0.171	175	
4	O27	H27	OW3	32.8	0.291	0.195	162.3	–	–	–	
4(T)	O31	H31	OW4	25.6	0.274	0.180	157.9	–	–	–	
4	O31	HO31B	OW4	–	–	–	–	0.291	0.209	141	**
4(T)	O24	H24	OW2	25.6	0.273	0.176	162.0	–	–	–	

Table 2 (continued)

Sym. Code	Donor		Acceptor	MD				Experimental [ND]			
				%	D–A [nm]	H···A [nm]	DHA [deg]	D–A [nm]	H···A [nm]	DHA [deg]	occ. [H]
4	O24	HO24B	OW2	–	–	–	–	0.286	0.187	168	**
2	O64	H64	OW2	24.4	0.280	0.188	154.0	–	–	–	
2	O25	H25	OW12	22.9	0.287	0.196	153.4	–	–	–	
2	O61	H61	OW6	12.5	0.289	0.195	157.7	–	–	–	
6(T)	O62	H62	OW8	12.3	0.272	0.175	163.3	–	–	–	
7(T)	O36	H36	OW3	12.2	0.293	0.198	159.3	–	–	–	
7	O36	HO36B	OW3A	–	–	–	–	0.290	0.189	160	**
1	O62	H62	OW4	11.1	0.269	0.173	161.5	–	–	–	
4(T)	O61	H61	OW12	10.9	0.275	0.179	161.7	–	–	–	
6(T)	O37	H37	OW1	10.1	0.280	0.188	153.3	0.285	0.188	167	**
c) Water → glucose > 10%											
8(T)	OW1	HW1	O37	59.2	0.277	0.182	159.1	0.285	0.188	177	**
8	OW1	HW2	O26	51.8	0.287	0.194	155.3	–	–	–	
1	OW1	HW1C	O26	–	–	–	–	0.274	0.184	167	**
1	OW2	HW1	O66	46.8	0.281	0.187	158.8	0.275	0.180	176	
1	OW2	HW2	O66	36.4	0.289	0.195	158.4	–	–	–	
6	OW5	HW2	O64	32.3	0.294	0.201	157.3	0.308	0.191	179	
6	OW5	HW1	O65	29.4	0.292	0.197	158.8	0.285	0.177	156	
6	OW5	HW1	O64	29.4	0.289	0.195	158.1	–	–	–	
1	OW7	HW2	O35	27.0	0.288	0.195	156.9	0.292	0.194	179	
2	OW2	HW2	O24	24.6	0.287	0.194	156.0	–	–	–	
	OW2	HW2B	O24	–	–	–	–	0.286	0.199	168	**
6(T)	OW5	HW2	O65	22.9	0.291	0.196	159.7	–	–	–	
8	OW1	HW2	O37	22.0	0.284	0.190	158.9	–	–	–	
3	OW1	HW1	O67	22.0	0.287	0.199	147.2	–	–	–	
7	OW8	HW1	O31	21.3	0.294	0.201	155.5	–	–	–	
1	OW4	HW1	O61	21.2	0.288	0.196	155.6	–	–	–	
7(T)	OW3	HW1	O36	21.0	0.298	0.205	157.1	0.290	0.197	163	**
2	OW12	HW2	O61	18.4	0.290	0.197	156.5	–	–	–	
2	OW4	HW2	O31	16.8	0.282	0.190	153.4	–	–	–	
1	OW3	HW2	O52	15.8	0.294	0.203	152.5	–	–	–	
	OW3B	HW3B2	O52	–	–	–	–	0.314	0.236	152	
1	OW10	HW2	O65	15.5	0.291	0.198	157.4	–	–	–	
3(T)	OW3	HW1	O23	15.0	0.288	0.195	154.7	–	–	–	
	OW3A	HW3A4	O23	–	–	–	–	0.277	0.191	153	**
2	OW6	HW1	O46	12.8	0.304	0.211	155.3	–	–	–	
7	OW3	HW2	O36	12.7	0.301	0.209	156.0	–	–	–	
2(T)	OW10	HW1	O25	12.3	0.296	0.205	152.0	–	–	–	
1	OW10	HW10A	O25	–	–	–	–	0.315	0.233	170	
3	OW1	HW1	O57	12.1	0.290	0.202	146.9	–	–	–	
	OW1	HW1B	O57	–	–	–	–	0.297	0.207	153	
8	OW8	HW2	O43	12.1	0.302	0.211	152.6	–	–	–	
	OW8	HW8A	O43	–	–	–	–	0.310	0.212	174	
4	OW13	HW1	O24	11.4	0.289	0.198	152.5	–	–	–	
7	OW8	HW2	O31	10.8	0.291	0.198	156.6	0.294	0.204	161	
2	OW6	HW2	O47	10.6	0.296	0.203	157.1	–	–	–	
1	OW4	HW2	O62	10.1	0.308	0.218	150.8	–	–	–	
1	OW4	HW4B	O62B	–	–	–	–	0.276	0.188	160	
2	OW4	HW1	O31	10.0	0.289	0.200	150.0	0.291	0.199	164	**
	OW1	HW1H	O22	–	–	–	–	0.282	0.204	160	**
	OW3A	HW3A2	O67	–	–	–	–	0.283	0.194	148	**
	OW5B	HW5E	O25	–	–	–	–	0.287	0.208	153	
	OW5C	HW5H	O23	–	–	–	–	0.313	0.211	171	
	OW14	HW14A	O24	–	–	–	–	0.281	0.203	136	
d) Water → water > 10%											
7	OW12	HW1	OW8	58.4	0.281	0.186	160.0	0.306	0.192	176	
1	OW3	HW2	OW5	38.7	0.287	0.192	159.9	0.282	0.178	169	
4	OW7	HW1	OW2	37.1	0.289	0.195	156.8	0.288	0.198	159	

(continued overleaf)

Table 2 (continued)

Sym. Code	Donor		Acceptor	MD				Experimental [ND]			
				%	D–A [nm]	H···A [nm]	DHA [deg]	D–A [nm]	H···A [nm]	DHA [deg]	occ. [H]
4(T)	OW9	HW2	OW4	25.5	0.292	0.199	155.0	–	–	–	
7	OW8	HW2	OW9	22.4	0.287	0.193	158.6	–	–	–	
1	OW13	HW1	OW12	21.2	0.288	0.195	156.5	–	–	–	
1	OW13	HW2	OW9	20.9	0.288	0.194	158.2	0.285	0.228	120	**
1	OW6	HW2	OW9	20.9	0.287	0.193	159.4	0.307	0.202	146	**
3	OW12	HW2	OW10	20.6	0.299	0.208	152.3	0.272	0.184	148	**
3	OW6	HW1	OW10	19.8	0.288	0.194	157.1	–	–	–	
4	OW7	HW2	OW2	19.1	0.286	0.193	156.9	–	–	–	
1	OW6	HW1	OW9	18.5	0.284	0.191	157.8	–	–	–	
2(T)	OW4	HW2	OW6	18.4	0.295	0.203	154.4	–	–	–	
1	OW9	HW1	OW13	18.3	0.297	0.205	154.7	0.285	0.228	154	**
1	OW13	HW2	OW12	17.7	0.292	0.199	156.5	–	–	–	
4	OW7	HW2	OW10	16.0	0.300	0.209	153.7	–	–	–	
3	OW10	HW2	OW6	15.8	0.296	0.204	155.8	–	–	–	
7	OW8	HW2	OW6	15.0	0.295	0.202	156.0	–	–	–	
2(T)	OW4	HW1	OW9	14.9	0.284	0.191	157.9	–	–	–	
4	OW13	HW1	OW7	14.5	0.299	0.206	156.1	0.276	0.171	157	
1	OW3	HW1	OW5	14.4	0.288	0.194	160.0	–	–	–	
1	OW3A	HW3A3	OW5B	–	–	–	–	0.272	0.174	151	
7	OW12	HW2	OW8	13.4	0.311	0.217	157.6	–	–	–	
1	OW9	HW2	OW13	13.1	0.290	0.197	156.7	–	–	–	
7	OW5	HW2	OW2	12.8	0.295	0.201	158.7	–	–	–	
2(T)	OW7	HW1	OW13	12.7	0.297	0.205	153.7	–	–	–	
7	OW8	HW1	OW6	12.4	0.291	0.198	156.2	–	–	–	
2	OW10	HW2	OW7	12.3	0.298	0.207	153.1	–	–	–	
5	OW10	HW2	OW8	11.7	0.291	0.196	158.6	–	–	–	
4(T)	OW6	HW2	OW4	11.1	0.290	0.199	152.4	–	–	–	
7(T)	OW2	HW1	OW5	10.9	0.290	0.196	159.6	–	–	–	
1	OW2	HW2C	OW5C	–	–	–	–	0.271	0.176	154	
3	OW6	HW2	OW10	10.5	0.290	0.199	153.2	–	–	–	
	OW10	HW10B	OW12	–	–	–	–	0.272	0.225	107	**
1	OW9	HW9B	OW6	–	–	–	–	0.307	0.231	130	**
1	OW14	HW14B	OW13	–	–	–	–	0.255	0.202	111	

structure than in the experimental one (391/480 = 0.81). The same conclusion holds for β-cyclodextrin averaged over four unit cells at 293 K (248/312 = 0.79) and at 120 K (335/352 = 0.95). However, if the number of different hydrogen bonds that occur during the MD trajectory are counted, the simulated structures show a much greater variety of hydrogen bonds than the static experimental structure. In this respect, it is more interesting to analyse the frequencies of hydrogen bond formation in a certain time than to determine whether a hydrogen bond is observed in the time averaged structure.

Are the O2, O3 and O6 hydroxyl groups comparable in their hydrogen bonding properties? Experimental findings based on methylation reactions and on chemical reactions where β-cyclodextrin was used as model enzyme (van Etten et al. 1967; Saenger 1980; D'Souza and Bender 1987), suggested that one of the two secondary hydroxyl groups displayed a high reactivity, and it was concluded that this might be the O3 hydroxyl group (van Etten et al. 1967 and refs. therein). This observation led us to an analysis of the population of hydrogen bonds involving different oxygen atoms in the simulation. Table 1 b displays the results for all possible combinations of the four donor and six acceptor atom types. Hydrogen bonds with O3 atoms as donor (α-cyclodextrin: 21%; β-cyclodextrin, 293 K: 16%; β-cyclodextrin, 120 K: 14%) occur as frequently as hydrogen bonds with an O2 atom as donor (α-cyclodextrin: 19%; β-cyclodextrin, 293 K: 17%; β-cyclodextrin, 120 K: 17%). Therefore, no clear distinction between O3 or O2 hydroxyl groups acting as hydrogen bond donor can be made. The O2 and O6 atoms are slightly better acceptors than the O3 atoms. The glucosidic O4 and O5 atoms as hydrogen bonding acceptors account for only a few percent of the hydrogen bonds in the crystals and in the MD simulations.

The experimentally observed hydrogen bonds in β-cyclodextrin at room temperature and at 120 K are listed in Tables 2 and 3, Figs. 1 a, b and 2 a, b; they are compared to those from the MD simulations. The data were averaged over the eight asymmetric units.

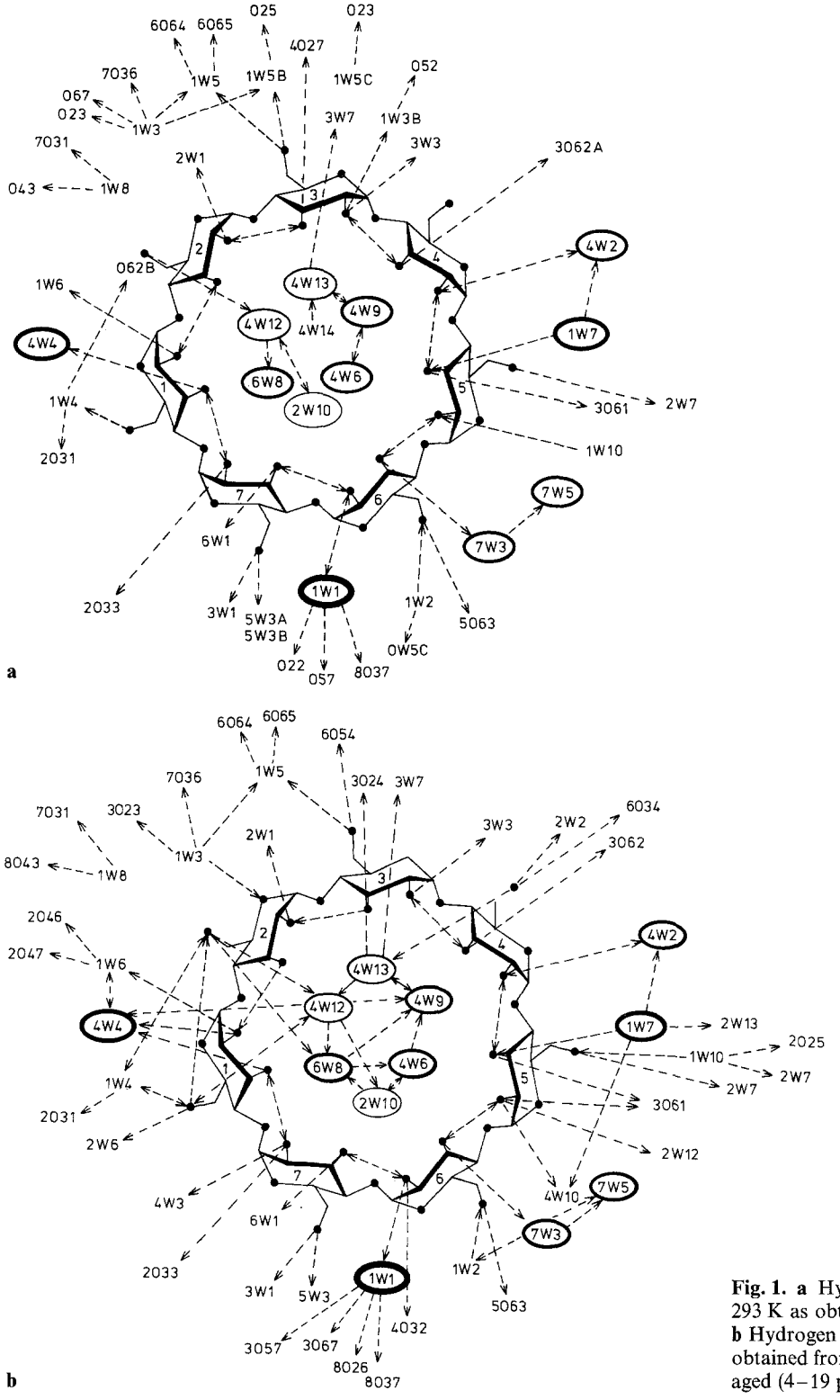


Fig. 1. a Hydrogen bonds of β-cyclodextrin at 293 K as obtained from neutron diffraction data. **b** Hydrogen bonds of β-cyclodextrin at 293 K as obtained from the molecular dynamics time averaged (4–19 ps) structure

Hydrogen bonds in β-cyclodextrin hydrate at 293 K

In the MD simulations, the water molecules can move quite freely in some locations, whereas in others they stay in approximately the same area. From the 96 water molecules in the computational box 77 remained close to the experimental site. 19 waters drifted away and moved into each others original sites (Koehler et al. 1987b). This should be kept in mind when interpreting the data of Table 2 and Fig. 1 a and b concerning hydrogen bonds involving water molecules. The MD simulations show:

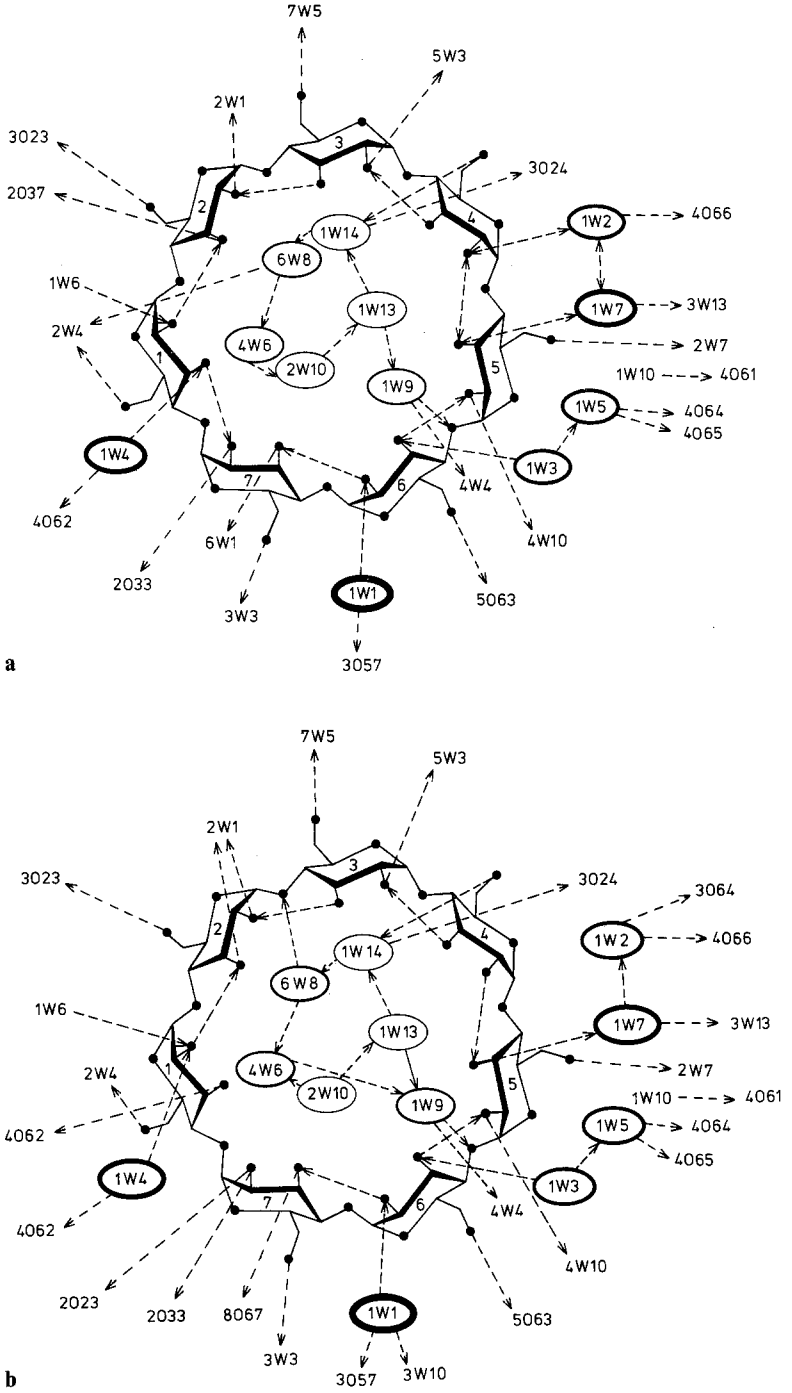


Fig. 2. **a** Hydrogen bonds of β-cyclodextrin at 120 K as obtained from neutron diffraction data **b** Hydrogen bonds of β-cyclodextrin at 120 K as obtained from the molecular dynamics time averaged (5–20 ps) structure

a) The experimentally observed seven intramolecular, interglucose flip-flop hydrogen bonds between O2 and O3 hydroxyl groups in β-cyclodextrin at 293 K are reproduced in the MD simulation. However, the O32–H32 ··· O21 and the O33–H33 ··· O22 bonds dominate with 95.7 and 94.3% occurrence and so their reversed forms O32 ··· H21–O21 and O33 ··· H22–O22 are less frequent (<1%) or do not exist at all. These two preferred hydrogen bonds have the same O3–H ··· O2 orientation as the other six more frequent ones, and in the reverse O2–H ··· O3 direction, only the O24–H24 ··· O35 bond is preferred over O35–H35 ··· O24. This means that there is a general preference in the O3–H ··· O2 orientation.

The average flip-flop bond occurrence is 70%. This value was calculated from the occurrences listed in Table 2, taking the average of all involved hydrogen bonds in both directions, multiplied by factor 2. Close to the most preferred O32–H32 ··· O21 bond the

Table 3a–d. Hydrogen bonds of β-cyclodextrin at 120 K from neutron diffraction data (Zabel et al. 1986), and from the MD simulation (eight molecules, 5–20 ps), (Koehler et al. 1987b)

Sym. Code	Donor		Acceptor	MD				Experimental [ND]			
				%	D–A [nm]	H···A [nm]	DHA [deg]	D–A [nm]	H···A [nm]	DHA [deg]	occ. [H]
a) Glucose → glucose > 1%											
1	O26	H26	O37	100.0	0.266	0.168	164.1	0.294	0.201	158.3	1.0
5	O66	H66	O63	100.0	0.268	0.170	164.6	0.278	0.183	167.2	1.0
3	O62	H62	O23	100.0	0.267	0.169	166.7	0.281	0.185	169.0	1.0
1	O21	H21	O32	99.9	0.276	0.179	164.4	0.296	0.203	158.8	1.0
1	O24	H24	O35	99.9	0.268	0.172	161.1	0.275	0.184	160.0	0.49
1	O36	H36	O25	96.5	0.266	0.173	153.5	0.277	0.184	155.4	1.0
2	O27	H27	O33	95.9	0.300	0.209	150.3	0.276	0.183	160.9	1.0
1	O33	H33	O22	95.4	0.272	0.178	156.7	0.291	0.195	165.2	1.0
4	O31	H31	O62	93.4	0.275	0.183	152.3	–	–	–	–
8	O37	H37	O67	82.2	0.290	0.202	145.5	–	–	–	–
2	O27	H27	O23	21.5	0.303	0.219	140.0	–	–	–	–
1	O34	H34	O23	4.6	0.290	0.193	164.1	0.297	0.200	176.4	1.0
4	O33	H33	O31	1.7	0.266	0.173	153.5	–	–	–	–
1	O31	H31	O27	–	–	–	–	0.299	0.202	171.6	1.0
1	O35	H35A	O24	–	–	–	–	0.275	0.181	159.0	0.49
2	O32	H32	O37	–	–	–	–	0.289	0.193	170.7	1.0
b) Glucose → water > 1%											
1	O64	H64	OW14	100.0	0.267	0.168	170.8	0.285	0.191	164.0	1.0
1	O35	H35	OW7	100.0	0.265	0.168	164.1	0.273	0.177	167.0	0.48
2	O61	H61	OW4	100.0	0.267	0.168	170.3	0.284	0.185	170.9	1.0
2	O65	H65	OW7	100.0	0.267	0.168	170.6	0.273	0.175	173.8	1.0
7	O63	H63	OW5	100.0	0.269	0.171	166.2	0.282	0.186	168.1	1.0
5	O23	H23	OW3	100.0	0.268	0.170	166.9	0.268	0.170	169.7	1.0
2	O22	H22	OW1	100.0	0.262	0.164	165.7	0.288	0.194	163.7	1.0
2	O32	H32	OW1	100.0	0.270	0.171	170.9	–	–	–	–
4	O25	H25	OW10	99.9	0.268	0.172	161.6	0.298	0.205	161.8	1.0
3	O67	H67	OW3	99.6	0.286	0.191	157.9	0.286	0.192	162.4	1.0
1	O24	H24A	OW2	–	–	–	–	0.278	0.179	174.0	0.49
6	O37	H37	OW1	–	–	–	–	0.282	0.184	166.3	1.0
c) Water → glucose > 1%											
4	OW2	HW1	O66	100.0	0.278	0.179	167.8	0.279	0.183	175.5	1.0
4	OW5	HW1	O64	100.0	0.282	0.184	166.8	0.285	0.187	174.2	1.0
4	OW5	HW2	O65	100.0	0.287	0.189	166.9	0.282	0.185	170.4	1.0
3	OW14	HW1	O24	100.0	0.274	0.176	167.4	0.281	0.196	143.0	1.0
1	OW1	HW1	O26	99.9	0.265	0.168	162.1	0.274	0.177	166.4	1.0
1	OW3	HW1	O36	99.9	0.279	0.181	167.0	0.278	0.183	171.4	1.0
1	OW4	HW1	O21	99.9	0.271	0.174	165.4	–	–	–	–
1	OW9	HW1	O46	99.6	0.281	0.184	163.4	0.293	0.204	151.9	1.0
4	OW10	HW1	O61	98.4	0.281	0.186	160.1	0.281	0.196	146.4	1.0
3	OW1	HW2	O57	98.0	0.270	0.178	152.7	0.279	0.186	159.1	1.0
4	OW4	HW2	O62	92.8	0.286	0.193	155.7	0.273	0.179	164.3	1.0
8	OW8	HW2	O43	57.7	0.291	0.200	150.6	–	–	–	–
3	OW2	HW2	O64	14.1	0.320	0.234	143.2	–	–	–	–
1	OW6	HW2	O21	5.9	0.301	0.211	149.0	–	–	–	–
1	OW6	HW1	O21	3.3	0.315	0.225	151.7	0.269	0.207	125.0	1.0
2	OW6	HW2	O41	2.4	0.332	0.241	153.1	–	–	–	–
7	OW8	HW2	O31	2.1	0.329	0.235	158.2	–	–	–	–
1	OW2	HW2A	O24	–	–	–	–	0.278	0.195	164.0	0.47
1	OW4	HW4A	O31	–	–	–	–	0.277	0.183	157.5	1.0
1	OW7	HW7C	O35	–	–	–	–	0.273	0.180	176.6	0.48
d) Water → water > 1%											
1	OW3	HW2	OW5	100.0	0.288	0.190	167.9	0.277	0.179	167.6	1.0
1	OW7	HW2	OW2	100.0	0.279	0.182	163.6	0.283	0.188	156.0	0.49
3	OW7	HW1	OW13	100.0	0.274	0.177	162.8	0.272	0.181	156.2	1.0
7	OW8	HW1	OW6	100.0	0.280	0.182	167.3	0.291	0.194	170.0	1.0

(continued overleaf)

Table 3 (continued)

Sym. Code	Donor		Acceptor	MD				Experimental [ND]			
				%	D–A [nm]	H···A [nm]	DHA [deg]	D–A [nm]	H···A [nm]	DHA [deg]	occ. [H]
4	OW9	HW2	OW4	100.0	0.274	0.176	166.3	0.277	0.180	174.5	1.0
6	OW14	HW2	OW8	100.0	0.273	0.176	166.1	0.280	0.186	163.0	1.0
1	OW13	HW1	OW9	99.9	0.276	0.177	169.2	0.268	0.175	166.0	1.0
1	OW13	HW2	OW14	96.1	0.286	0.190	164.1	0.285	0.200	171.0	1.0
2	OW6	HW1	OW9	90.4	0.281	0.185	162.1	–	–	–	–
4	OW10	HW2	OW13	78.8	0.292	0.194	166.7	0.275	0.187	165.0	1.0
3	OW1	HW2	OW10	22.1	0.290	0.202	147.0	–	–	–	–
3	OW10	HW2	OW6	19.2	0.286	0.190	162.9	–	–	–	–
6	OW13	HW2	OW8	5.4	0.300	0.211	147.5	–	–	–	–
2(T)	OW6	HW2	OW9	4.0	0.275	0.178	165.3	–	–	–	–
1	OW2	HW2C	OW7	–	–	–	–	0.283	0.191	158.0	0.49
3	OW6	HW6B	OW10	–	–	–	–	0.306	0.205	160.0	1.0
5	OW8	HW6B	OW4	–	–	–	–	0.317	0.225	155.0	1.0

Table 4. Corresponding asymmetric unit number combinations for hydrogen bonds. Example: Donor atom in unit 1, acceptor in unit 5 is equivalent to donor in unit 2 and acceptor in unit 6, or to donor in unit 3 and acceptor in unit 7 and so on

1	2	3	4	5	6	7	8
2	3	4	1	6	7	8	5
3	4	1	2	7	8	5	6
4	1	2	3	8	5	6	7
5	6	7	8	1	2	3	4
6	7	8	5	2	3	4	1
7	8	5	6	3	4	1	2
8	5	6	7	4	1	2	3

simulation predicts a bond between the O61 and O62 atoms. This contrasts the neutron diffraction experiment where the distance O61 to O62 is 0.38 nm, O61 to H62 is 0.35 nm, and cannot be considered a hydrogen bond.

b) Four intermolecular hydrogen bonds are reproduced by MD, with acceptor atoms O63(5), O62(3), O33(2) and to O61(3).[2] The latter involves the experimental HO35B position, whereas in MD the starting position for the hydrogen atom was HO35A.

Additionally, MD predicts bonds to O34(6), to O32(4), to O61(3) and another one to O54(6) (see Table 2 and Fig. 1 a, b). The experimentally observed hydrogen bonds to O23(3) and to O27(4) are not reproduced.

c) Glucose-to-water hydrogen bonds are similar in crystallographic experiment and MD simulation. Water 4 has a bridging function between the O6 hydroxyls of glucose units 1 and 2. The disordered oxygen positions of waters 3 and 5 and their hydrogen bonds as found in the experiment cannot have a correspondence in MD, because in the MD analysis no distinction is made between various sites. The high percentage of O63–H63···OW5 (72.3%) most probably covers the possible connections in that part of the structure.

d) Hydrogen bonds involving disordered hydrogen positions such as, for example, the O31–H31···OW4 and the O31–H31B···OW4 bond of the neutron diffraction study also have only one counterpart in the MD simulation, the O31–H31···OW4(4) bond with 25.6% occurrence. Thus the intermolecular hydrogen bonds between glucose atoms and water molecules are well reproduced. Again there are some less-frequent additional bonds observed in the simulation, especially in the neighbourhood of glucose units 1 and 2 and to the included water molecules.

e) The two water-to-glucose hydrogen bonds from OW2 to O66 and from OW7 to O35 are both reproduced with relatively high percentages in the simulation. Additionally, OW4 donates to O61 and to O62 in MD, whereas in neutron diffraction a disordered OW4–HW4B···O62B bond was found. OW10 donates to O65 and O25, and OW3 forms a bond to O52. The water-to-glucose hydrogen bonds involve many disordered oxygen and hydrogen sites as has been found experimentally. In the simulation many water-to-glucose hydrogen bonds with only 10%–30% occurrence are found. This expresses clearly the mobility of the water molecules in the β-cyclodextrin hydrate structure at room temperature.

f) Hydrogen bonds between water molecules are well reproduced by the simulation. As mentioned above, the water molecules are rather mobile and therefore numerous hydrogen bonds are found in the simulation, which display occurrences below 30%.

[2] The number in parentheses indicates to which asymmetric unit the acceptor atom belongs. The donors are in asymmetric unit 1. For eight β-cyclodextrin: Molecules 1 to 4 are in the crystallographically defined $P2_1$ positions, molecules 5 to 8 are in the same order but translated in z. Corresponding asymmetric unit number combinations are in Table 4

g) According to the neutron diffraction study, the four-membered cycle O24(1) ↔ O35(1) ← OW7(1) → OW2(4) ↔ O24(1) displays two flip-flop and two normal hydrogen bonds. Although in the MD simulation all four bonds are of flip-flop type, the agreement with experiment is very good. The two experimental flip-flop bonds have much stronger flip-flow contribution in the MD simulation than the two unidirectional bonds which have less than 10% MD calculated occurrence in the reversed orientations.

In summary, the experimentally observed hydrogen bonds are well reproduced by MD. The computational method predicts many hydrogen bonds with low occurrence in the 1% to 30% range, which are found between the glucose and water atoms and among the water molecules. These hydrogen bonds, even if they really occur, cannot be seen in the neutron diffraction experiments. This is because hydrogen atoms with such low occupancy are indistinguishable from background noise in the nuclear density map.

Hydrogen bonds in β-cyclodextrin hydrate at 120 K

a) Four intramolecular hydrogen bonds between O2 and O3 hydroxyl groups (O26 – H26 ··· O37; O21 – H21 ··· O32; O36 – H36 ··· O25; O33 – H33 ··· O22) with experimental hydrogen bond occupancy factors of 1.0 were reproduced in MD simulations with percentages of occurrence above 95%, and they all have the correct orientation. The O34 – H34 ··· O23 bond is much weaker in MD than in experiment (4.6% occurrence, occupancy factor 1.0) and the O31 – H31 ··· O27 bond is not observed in MD (<1%). The O24 – H24 ··· O35 hydrogen bond and its reversed from O35 – H35A ··· O24 each have experimental hydrogen occupancy factors of 0.49. This bond belongs to the four membered flip-flop hydrogen bond cycle at 120 K, involving the O24, O35, OW7 and OW2 atoms. As can be seen from Table 3, only the O24 – H24 ··· O35 bond with 99.9% occurrence is seen in the MD simulation study. This orientation was chosen as the starting configuration, and it obviously did not change during the simulation period of 20 ps.

b) Three intermolecular glucose-glucose hydrogen bonds correspond exactly in neutron diffraction data and MD simulation as far as their orientation and stability are concerned (O66 – H66 ··· O63; O62 – H62 ··· O23; O27 – H27 ··· O33). One experimentally observed hydrogen bond (O32 – H32 ··· O37) is not found in the simulation. Additionally, there are two strong bonds with occurrence above 80% (O31 – H31 ··· O62; O37 – H37 ··· O67), and two less frequent bonds (O27 – H27 ··· O23; O33 – H33 ··· O31).

c) The experimentally observed glucose-to-water bond O64 – H64 ··· OW14 which binds one of the six water molecules inside the cyclodextrin cavity has 100% occurrence in MD. The O35 – H35 ··· OW7 bond which belongs to the four membered flip-flop cycle, has an occupancy of 0.48 in the experiment, whereas in MD it has an occurrence of 100%. This means that the four membered initial hydrogen bonding configuration did not switch during the simulation period. Also, the reversed experimental O24 – H24 ··· OW2 flip-flop bond with an occupancy of 0.49 is not seen in MD. Seven experimentally located glucose to water hydrogen bonds were exactly reproduced in MD, one is not observed in the simulation, and another one only exists in MD.

d) Well defined hydrogen bonds between three water molecules and atoms in glucose unit 6 are found experimentally and in MD: OW1 – H ··· O26, OW3 – H ··· O36 and OW9 – H ··· O46; the latter binds the included water molecules to the β-cyclodextrin cavity. Another bond between OW6 and O21 is of lower occurrence in the MD simulation and split into two configurations, either involving one or the other hydrogen atom. Water W4 binds experimentally to O31, but in MD it prefers O21. The two bonds belonging to the four membered flip-flop cycle OW2 – H ··· O24 and OW7 – H ··· O35 have experimental occupancy factors of 0.47 and 0.48, but they do not occur in the MD simulation. From the analysis of hydrogen bonds to β-cyclodextrin molecule 1 we see that in the simulation, OW2(1) has shifted a little bit further away from O24 and OW14(3) got closer to the O24 hydroxyl group, (MD: OW14 ··· O24 is 0.27 nm; OW2 ··· O24 is 0.33 nm; experimentally OW14 ··· O24 is 0.28 nm; OW2 ··· O24 is 0.28 nm). All seven experimentally observed water to glucose hydrogen bonds have been simulated with more than 90% occurrence. Four additional less frequent hydrogen bonds are found in the simulation, such as water 8 which forms a 58% occurrence bond to glucosidic oxygen atom O43 (the OW8(1) – H ··· O43(8) bond of Table 3, see also Table 2), which stabilises the six water molecules inside the cyclodextrin cavity.

e) Seven hydrogen bonds between water molecules have experimental occupancy factors of 1.0, and are well reproduced in MD with more than 95% occurrence. The two orientations of the flip-flop bond between OW2 and OW7 are not reproduced in MD, only the starting orientation of the OW7 – HW2 ··· OW2 bond exists with 100% occurrence in the simulation. The bond OW10 – H ··· OW13 between two included waters shows about 79% occurrence. Hydrogen bonds between the six waters, which are located within the cyclodextrin cavity are slightly different in MD than in experiment. The OW6(4) – H ··· OW10(2) bond is reversed and less preferred with only about 20% proba-

bility. There is an additional hydrogen bond between OW6(4) and OW9(1) (90% + 4%), and a less frequent one between OW13 and OW8 (5%). Water 8 does not bind to water 4 as it does in the experiment, but, as mentioned above, to O43. In summary, most of the experimentally observed hydrogen bonds are reproduced with high probability in MD simulation, and MD additionally finds many hydrogen bonds which occur with lower frequency.

Flip-flop hydrogen bonding

A comparison of the experimentally determined (Betzel et al. 1984; Zabel et al. 1986), and MD simulated hydrogen bonded flip-flop systems in β-cyclodextrin at 293 K and 120 K is given in Figs. 3–10. In β-cyclodextrin at 293 K there is an infinite flip-flop chain between O37(1) ↔ O26(1) ↔ OW1(1) ↔ O37 etc which is formed through operation of the 2_1 screw axis. The MD calculation shows that in the simulated eight β-cyclodextrin asymmetric units (four unit cells), there are two infinite chains formed by these atoms. The first chain runs through asymmetric units 1, 8, 3, 6 (Fig. 3a) and the second through 2, 5, 4, 7 (see Fig. 3b). Their percentage of occurrence, the lifetime and number of transitions are given separately for both chains. During the 15 ps analysis period of the simulations both chains are independent and display different orientations of hydrogen bonds. A superposition of the data of this system consisting of eight groups with three hydroxyls each leads to an average that shows flip-flop characteristics. This example illustrates that the molecules in the eight different asymmetric units behave independently during the simulation.

The results represented in Figs. 3–10 demonstrate that the GROMOS force field is able to reproduce statisfactorily the experimentally observed flip-flop hydrogen bonds in β-cyclodextrin at 293 K. At low temperature (120 K), the four-membered flip-flop cycle remained in its starting configuration, which was reproduced in MD with three of these bonds, whereas the fourth flip-flop occurred only with very low per-

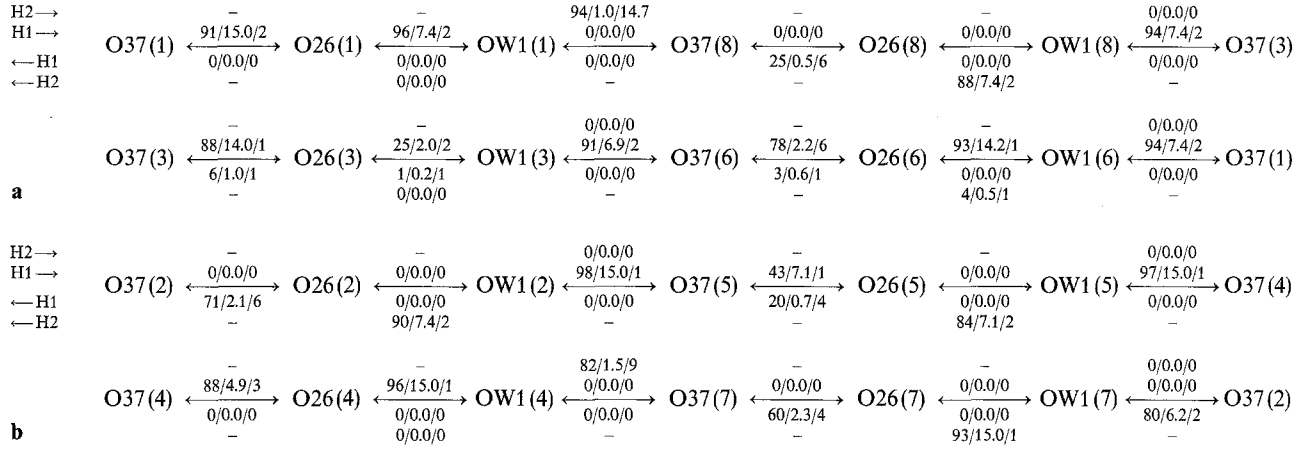


Fig. 3a and b. Hydrogen bond chains for four molecules (**a**) 1-8-3-6 and (**b**) 2-5-4-7 of β-cycodextrin at 293 K. The percentage of hydrogen bond occurrence, the corresponding lifetime (in ps) and the number of transitions as obtained from the MD simulation (eight molecules, 4–19 ps) are given separated by slashes. Hydroxyl groups have only one hydrogen atom, denoted by H1, whereas waters have two, denoted as H1 and H2. These hydrogen atoms can form H ··· Oxygen bonds to the right neighbour oxygen, denoted as H →, or to the left one, Oxygen ··· H, denoted as ← H

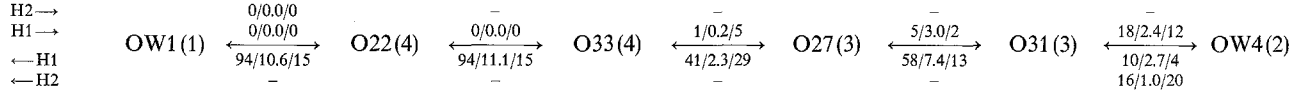


Fig. 4. Hydrogen bond chain involving molecules from units 1-4-3-2 of β-cyclodextrin at 293 K. Summed result of the eight equivalent chains of OW1–O22–O33–O27–O31–OW4 atoms which exist in the computational box of four unit cells. The second chain involves molecules 2-1-4-3, the third 3-2-1-4 and so on. For equivalent symmetry transformations see Table 4

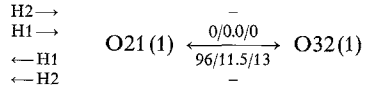


Fig. 5. Hydrogen bond system O21–O32 in β-cyclodextrin at 293 K

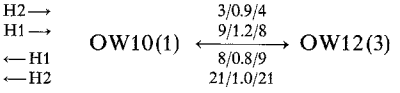


Fig. 6. Hydrogen bond system OW10–OW12 in β-cyclodextrin at 293 K

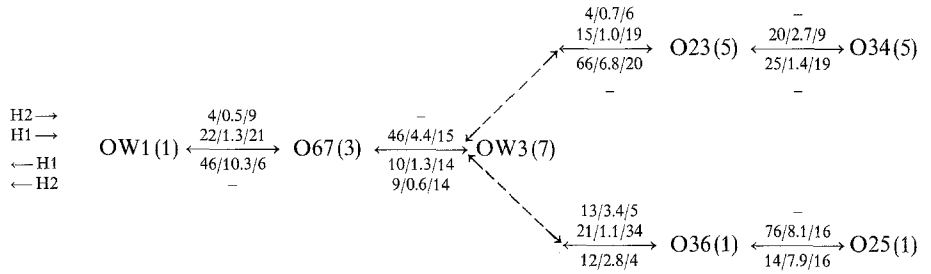


Fig. 7. Branched hydrogen bond chain in β-cyclodextrin at 293 K. There are eight versions of this system in the computational box, which are summed and averaged in this figure. For the crystal symmetry related versions of this branched system use Table 4. We note that the bond between OW1 and O67 is experimentally not of the flip-flop type

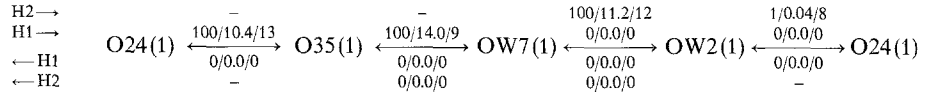
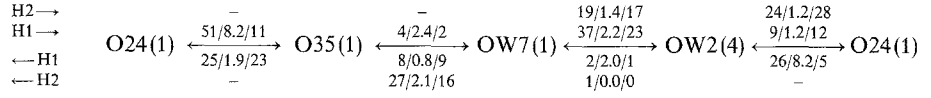
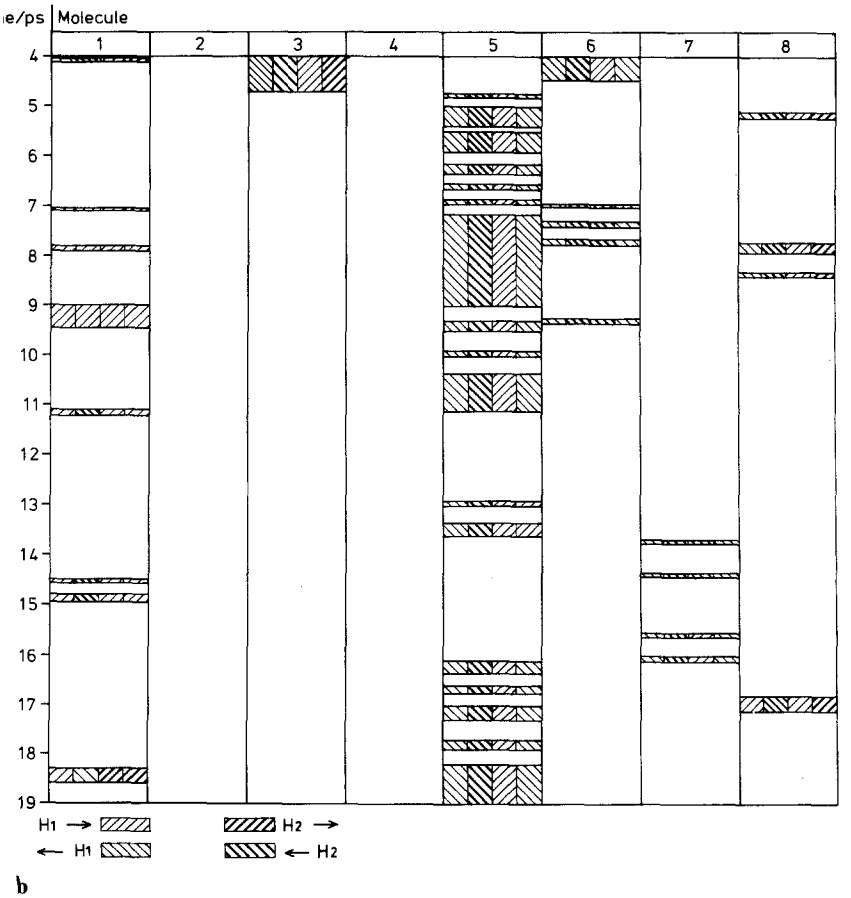


Fig. 8. Hydrogen bonds of the O24(1)–O35(1)–OW7(1)–OW2(1)–O24(1) cycle in β-cyclodextrin at 120 K. Averaged over the eight asymmetric units in the computational box

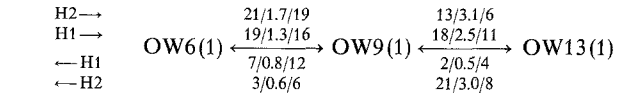


a



b

Fig. 9 a and b. Hydrogen bonds of the O24(1) – O35(1) – OW7(1) – OW2(4) – O24(1) cycle in β-cyclodextrin at 293 K. **a** Percentage of occurrence, lifetime and number of transitions from the MD simulation. **b** Occurrence (larger than 0.05 ps) of hydrogen bonds during the MD simulation.
▨ denotes hydrogen bond O – H1 ··· O
▨ denotes hydrogen bond O – H2 ··· O
▨ denotes hydrogen bond O ··· H1 – O
▨ denotes hydrogen bond O ··· H2 – O



a

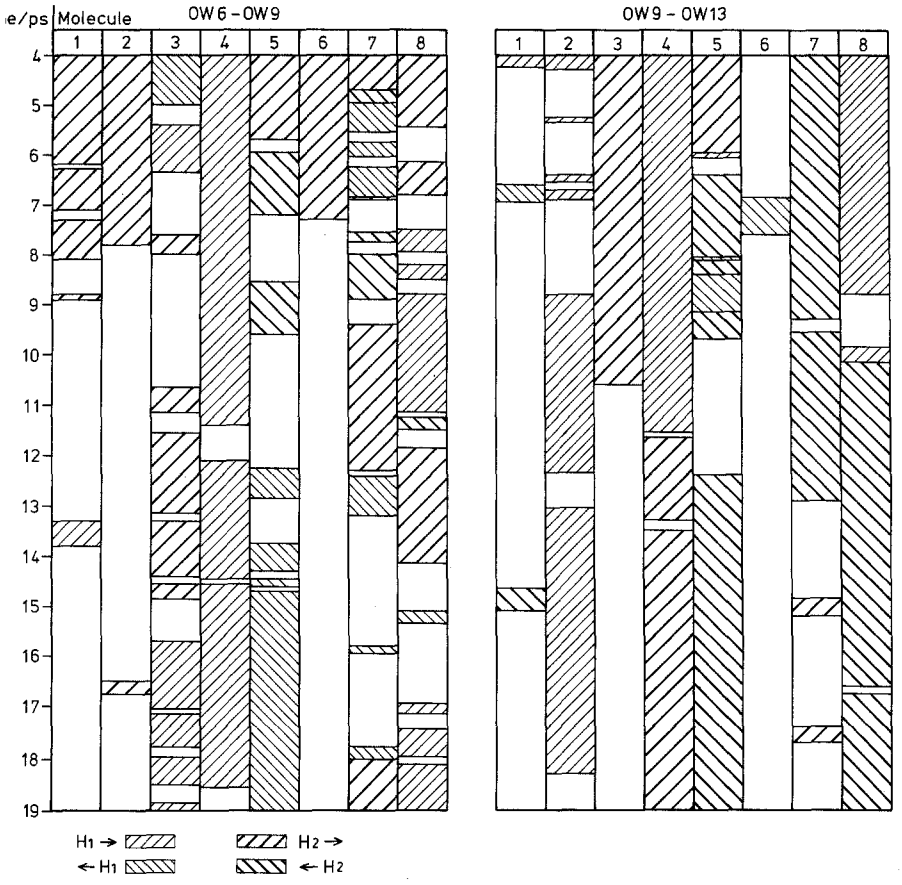
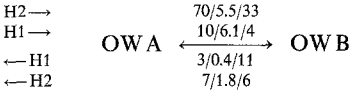
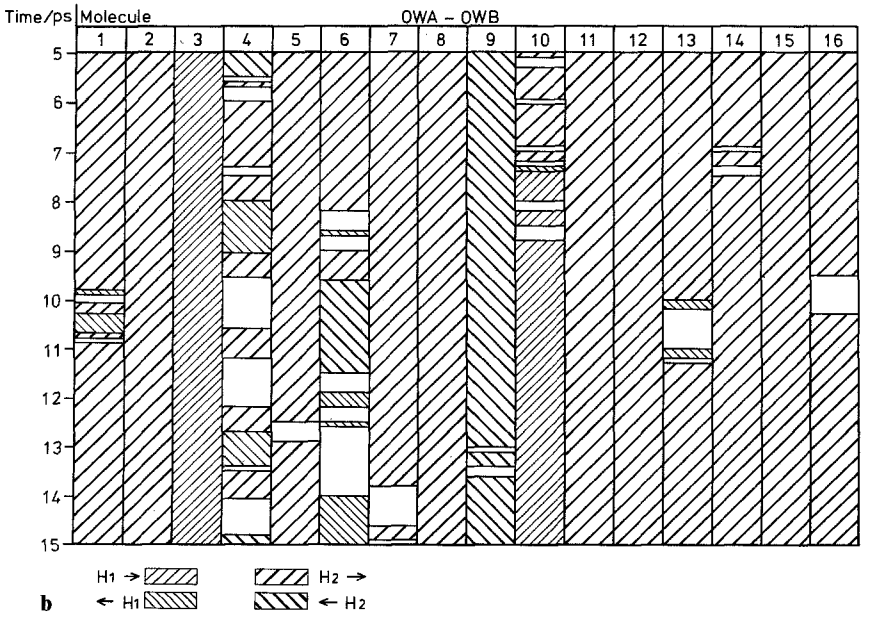


Fig. 10a and b. Hydrogen bonds between three water molecules OW6(1)–OW9(1)–OW13(1) of β-cyclodextrin at 293 K. **a** Percentage of occurrence, lifetime and number of transitions from the MD simulation. **b** Occurrence of hydrogen bonds during the MD simulation. For the notation see Fig. 9



a



b

Fig. 11a and b. Hydrogen bonds between OWA and OWB of the α-cyclodextrin. $6H_2O$ at 293 K. **a** Percentage of occurrence, lifetime and number of transitions from the MD simulation. Results are summarised for the 16 molecules, values are from the MD simulation (5–15 ps). **b** Occurrence of hydrogen bonds during the simulation. For the notation see Fig. 9

centage (1% – 2%), because water 2 has moved slightly in the simulation (Fig. 8). This four-membered cycle was also analysed in the β-cyclodextrin structure at 293 K (Fig. 9).

The main characteristic of the simultaneous occurrence of four hydrogen bonds in the four-membered cycle O24(1) – O35(1) – OW7(1) – OW2(4) – O24(1) in β-cyclodextrin at 293 K is that they have short lifetime in the MD simulation. They change to a different configuration by changing only one bond at the time, and not as expected by a concerted cooperative flip. The time dependent behaviour of the four-membered cycle is shown in more detail in Fig. 9b. During the 15 ps simulation there are eight time periods (longer than 0.05 ps) where the four-membered cycle persists in β-cyclodextrin molecule 1. The lengths of these time periods are all shorter than 0.08 ps with an average length of 0.036 ps. Molecules 2 and 4 do not show the four-membered cycle during the whole 15 ps. In molecule 5 the sum of all 17 time periods where hydrogen bonding occurs is 4.14 ps; this illustrates how large the differences between the trajectories of the molecules can be. The average lifetime of the four-membered cycle is about 0.04 ps. Figure 9b also shows that the directions of the hydrogen bonds in the cycle change during the simulation. The orientation of hydrogen bonds in the first molecule changes, for example, from ← H1 ← H2 H1 → H2 → to H1 → H1 → H1 → H2 → to H1 → H1 → H1 → H1 → to H1 → ← H2 H1 → H1 → and to H1 → ← H1 H2 → H2 →. In the simulation, the four-membered cycle exists only for 5.4% of the time. This low percentage would not allow one to detect the flip-flop cycle experimentally at 293 K. At 120 K, this cycle changes to the flip-flop type O24 ↔ O35(1) ↔ OW7(1) ↔ OW2(1) ↔ O24(1) which is well established experimentally. In the MD simulation, however, it exists only during very short time-intervals. The orientations of its hydrogen bonds are always H1 → H1 → H2 → H2 →. The overall occurrence in all eight asymmetric units is only 0.32 ps during the simulated 15 ps (=0.04 ps per molecule per 15 ps). Most of the time there are only three hydrogen bonds: H1 → H1 → H2 → 0. The MD simulation in this case does not reproduce the experiment to our satisfaction.

The experimentally determined infinite flip-flop chain of OW6 ⇌ OW9 ⇌ OW13 ⇌ OW6 in β-cyclodextrin at 293 K was well reproduced in the MD simulation (Fig. 10a). Figure 10b displays the behaviour of the hydrogen bonds in all eight asymmetric units during the simulated period.

A comparable analysis was carried out for the two enclosed water molecules A and B in α-cyclodextrin · 6 H_2O. The neutron diffraction study showed a clearly defined hydrogen bond OWA – H ··· OWB. As illustrated in Fig. 11a and b, this bond is very well reproduced in the MD simulation with about 80%, and for only 10% of the time shows a flip-flop with a hydrogen bond in the OWB ··· H – OWA direction (Koehler et al. 1987a). This contrasts strikingly the β-cyclodextrin hydrate system where the wider cavity allows the enclosed water molecules to be disordered and to show extensive flip-flop hydrogen bonds.

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