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Crystalline β-Cyclodextrin Hydrate at Various Humidities: Fast, Continuous, and Reversible Dehydration Studied by X-ray Diffraction[†]

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Abstract: The crystal structure of β-cyclodextrin hydrate was determined for crystals at four ambient humidities between 15% and 78% and for a crystal in contact with mother liquor. In this humidity range, fast, continuous, and reversible dehydration is observed, which does not affect the ordered crystalline structure. At very low humidities, the crystalline structure irreversibly collapses due to the loss of water. The water content per β-cyclodextrin molecule gradually reduces from 12.3 H_2O at 100% to about 9.4 H_2O at 15% humidity. The dehydration occurs according to a simple law e^{-kt}, and equilibration is reached within a few minutes. The crystal packing arrangement, the pattern of intermolecular hydrogen bonds, and the conformation of the β-cyclodextrin molecules are conserved during dehydration. The loss of crystal water is due to continuous change of the occupanices of partially populated water sites; this affects the water molecules included in the β-cyclodextrin cavity to a larger extent than the interstitial water molecules. These observations imply fast diffusion of water molecules in the solid, although no permanent continuous channels are present in the crystal structure to permit easy exchange of water molecules. The mechanism of water diffusion must therefore involve positional fluctuations of the β-cyclodextrin atoms.

Introduction

In crystalline cyclodextrin (CD) hydrates, which are some of the most important model systems for studying structural and dynamic properties of hydrogen bond networks and of water clusters in biological systems,[1] the cyclodextrin cavities include clusters of water molecules, and additional water molecules are found as space-fillers between neighboring cyclodextrin molecules in the crystal lattice. Crystal structures were determined for hydrates of all four known (underivatized) cyclodextrins: α-CD,[2–5] β-CD,[6–10] γ-CD,[11–13] and δ-CD.[14] These studies showed that the water content per cyclodextrin molecule may depend on the crystallization conditions[4] and also on the subsequent history of the sample.[7] Dehydration phenomena have been described qualitatively from X-ray powder diffraction data.[15]

The best known of the crystalline cyclodextrin hydrates is β-CD dodecahydrate. It was studied under various experimental conditions with calorimetric,[7,16–18] dielectric,[17] incoherent neutron scattering,[19,20] and solid-state NMR[21–24] techniques, and molecular dynamics computer simulations were performed.[25,26] In the first X-ray crystal structure, which was determined on a crystal in contact with mother liquor, about seven disordered water molecules were located in each β-CD cavity, and five more water molecules in interstitial sites between the β-CD macrocycles, resulting in an overall composition β-CD·$(12 \pm 0.5)H_2O$[6] (16 wt % H_2O); this composition will be verified in the present study. In a later neutron diffraction study[8] (in which not all of the weakly populated water sites were located), it was shown that, at room temperature, most water molecules and β-CD hydroxyl groups are orientationally disordered and alternately form hydrogen bonds with different neighbors. It was earlier anticipated[27] and later verified[17,19–22] that this disorder is highly dynamic, i.e. associated with rapid flips of O–H groups between discrete alternative orientations (*"flip–flop" hydrogen bonds*[27]). Very similar disorders of solvent molecules and hydroxyl groups were described for the complex β-CD–ethanol octahydrate.[28]

The orientational and positional disorder resembles hydrogen bond fluctuations in macromolecules,[21] and due to the moderate

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Table 1. Unit Cell Constants[a,b] of β-Cyclodextrin Hydrate at Various Humidities (Space Group $P2_1$)

humidity[c]	salt solution	a (Å)	b (Å)	c (Å)	β (deg)	V (Å^3)	vol. loss	struct. det.
wet crystal[d]		21.283(5)	10.322(1)	15.092(5)	112.41(1)	3065(1)		yes
wet crystal[e]		21.295(3)	10.326(1)	15.109(3)	112.42(7)	3071(1)		
100%	pure H_2O	21.295(11)	10.318(2)	15.108(10)	112.46(3)	3068(3)		
93%	$NH_4H_2PO_4$	21.265(4)	10.314(1)	15.097(4)	112.34(1)	3063(1)	0.2(1)%	
80%	NH_4Cl	21.227(4)	10.288(1)	15.116(4)	112.18(1)	3057(1)	0.4(1)%	
78%	$Na_2S_2O_3{\cdot}5H_2O$	21.233(5)	10.294(1)	15.103(4)	112.22(1)	3056(1)	0.4(1)%	yes
58%	$NaBr{\cdot}2H_2O$	21.161(7)	10.254(1)	15.110(7)	111.91(2)	3042(2)	0.8(1)%	yes
42%	$Zn(NO_3)_2{\cdot}6H_2O$	21.080(7)	10.197(1)	15.131(6)	111.58(2)	3024(2)	1.4(1)%	yes
20%	$KC_2H_3O_2$	20.979(7)	10.146(2)	15.169(6)	111.23(1)	3010(2)	1.9(1)%	
15%	$LiCl{\cdot}H_2O$	20.857(6)	10.158(1)	15.140(6)	110.94(1)	2996(2)	2.3(1)%	yes
~0%	silica gel	crystal	decays					

[a] This unconventional setting of the unit cell is maintained for historical reasons. A conventional setting with smaller β and $a < c$ is obtained by defining the diagonal of the a–c-plane as the new c-axis and the negative c-axis as the new a-axis. This changes the unit cell constants as follows: $a_{new} = c_{old}$, b = unchanged, $c_{new} = [a_{old}^2 + c_{old}^2 - 2a_{old}c_{old}\cos(180° - \beta_{old})]^{1/2}$, $\sin(180° - \beta_{new}) = a_{old}\sin(180° - \beta_{old})/a_{new}$. [b] Estimated standard errors are given in parentheses. [c] For T = 20 °C, taken from ref 30. Slightly different numeric values are in ref 31. [d] Crystal in contact with mother liquor. [e] Crystal from a different sample in contact with mother liquor.

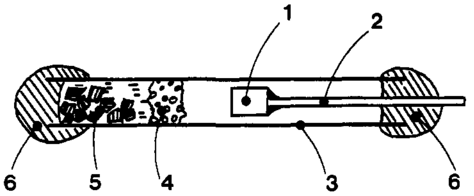

Figure 1. Experimental setup for obtaining defined constant humidities: (1) Crystal glued to (2) glass fiber. (3) Glass capillary, 1-mm diameter. (4) Sponge rubber. (5) Saturated salt solution with excess solid phase; suitable salts were selected from ref 30. (6) Wax seal. This setup is mounted on a goniometer head with the left side down.

size of β-CD hydrates, they can be studied in much greater detail compared to protein or DNA structures. The disordered groups have different local environments; this is most pronounced for the water molecules, of which some are neatly coordinated whereas others exhibit less favorable hydrogen bond geometries.[8,28] Consequently, water positions and hydrogen bond orientations have different individual residence times covering several decades of the time scale. Consistent with computer simulation calculations,[25] it was shown by incoherent neutron scattering that, at room temperature, fast hydrogen bond dynamics in β-CD hydrate occur on a 10^{-11}-s time scale[20] for flipping hydroxyl groups as well as for translational jumps of water molecules. Some of the mobile water molecules and hydroxyl groups move too slowly to be observed in the neutron scattering experiment[20] (residence times > 10^{-10} s), but their reorientation rates are fast on the deuterium NMR time scale, i.e. residence times are $\leq 10^{-6}$ s.[25]

Since many (but not all) crystalline cyclodextrin complexes suffer dehydration in atmosphere with frequent disintegration of the crystals, almost all cyclodextrin crystal structures were determined on crystals sealed in capillaries together with some saturated solvent (in some instances, it can be sufficient to provide an atmosphere of constant moderate humidity[29]). Other physical experiments are usually performed with "dry" material that is not in contact with solvent and potentially is to some degree dehydrated. In a recent crystallographic study, we found that β-CD hydrate reversibly looses some crystal water (from ~12 H_2O to 10.5 H_2O per β-CD or even lower) if exposed to ambient conditions, with the overall crystalline structure remaining intact except for very low humidities.[10] In the present work, the crystallographic study is extended and quantified for several reasons:

(1) The dehydration behavior is of interest if results from different experiments[6–10,16–23] which were not performed under identical conditions are interpreted and compared. Initially, it is not even clear whether fully and partially hydrated crystals have comparable or very different structural and physical properties.

(2) The mechanism and the consequences of dehydration of cyclodextrin complexes, and of hydrated biological compounds in general, are only very poorly understood. In particular, it is of interest to see whether the dehydration takes place stepwise or continuously.

(3) β-CD hydrate is an important example of a solid in which fast diffusion of water molecules through the crystal lattice occurs without the presence of continuous diffusion channels.[10] This effect by itself is of importance and deserves closer investigation.

(4) Many cyclodextrin complexes are stored as dried crystals in contact with the atmosphere and therefore under variable conditions. It is of practical interest to understand the interactions of such materials with the atmosphere.

Experimental Section

Sample Preparation. β-Cyclodextrin hydrate was crystallized by cooling a concentrated aqueous solution from ~70 °C in a Dewar flask.[6,8,10] Crystals were taken from the solvent and exposed to an atmosphere of moderate temperature and humidity to dry for several days (~18 °C, 50–60% humidity). Most of the crystals with dimensions of >0.5 mm cracked upon dehydration, but many of the smaller ones remained intact.

To obtain a wide range of constant and defined humidities, dry crystals were glued to glass fibers and sealed in glass capillaries together with saturated salt solutions (Figure 1 and Table 1); suitable salts were selected from ref 30. During equilibration with the atmosphere inside the capillary, many crystals cracked (often, but not always, within one or a few minutes); this was most pronounced for very high and low final humidities and for specimens of >0.2 mm. Crystals with only a few cracks were still suitable for determining the unit cell constants but not for collection of full X-ray diffraction data sets. Several attempts to collect full data sets with humidities >90% failed due to cracking or even decay of the crystals, and for humidities of ≤20%, only one data set with reduced accuracy could be collected on a very small crystal (Table 2). At zero humidity, crystals almost completely disintegrate.[10]

Crystallographic Experiments. Unit cell constants were determined at T = 18 ± 2 °C for crystals at eight different humidities in the range 15–100% and for a "wet" crystal in contact with mother liquor (Table 1 (Enraf Nonius CAD4 diffractometer, rotating anode generator FR 571, 40 kV, 85 mA, Ni-filtered CuKα radiation with λ = 1.542 Å). Diffraction data sets were collected for crystals at four humidities in the range 15–78% and for a "wet" crystal in contact with mother liquor (Table 2, $\omega/2\theta$-scan mode, $2\theta_{max}$ = 120°, crystallographic resolution $\lambda/2 \sin\theta_{max}$ = 0.89 Å, absorption corrections using the ψ-scan method[32]).

Structure Refinement. Refinement was performed with program[33] SHELX76 [β-CD molecule from ref 6 used as starting model, minimized quantity $\sum w(|F_o| - |F_c|)^2$ with w = 1.0 for reflections with $F_o > \sigma(F_o)$] and controlled by inspection of $2|F_o| - |F_c|$ and $|F_o| - |F_c|$ electron density maps (based on anisotropic displacement parameters) on an Evans & Sutherland

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Table 2. Crystal and X-ray Data for β-Cyclodextrin Hydrate at Various Humidities

humidity[a]	wet	78%	58%	42%	15%
crystal size (mm^3)	0.4 × 0.4 × 0.2	0.5 × 0.4 × 0.2	0.5 × 0.25 × 0.25	0.4 × 0.4 × 0.15	0.2 × 0.2 × 0.1
no. of measured reflns	5039	5011	4996	4938	4928
no. of unique reflns	4560	4516	4580	4401	4073
no. of reflns $F_o > \sigma(F_o)$	4360	4338	4428	4139	3684
refinement R-value[b]	0.061	0.059	0.059	0.060	0.068

[a] For $T = 20$ °C, taken from ref 30. [b] $R = \sum||F_o| - |F_c||/\sum|F_o|$ for reflections with $F_o > \sigma(F_o)$.

PS390 graphics system using FRODO.[34] H atoms bound to C were placed in their theoretical positions. As refinement of very weakly occupied sites seemed unrealistic, minor water sites were only retained in the model if the occupation factors refined to values of ≥0.2. Due to orientational disorder,[8] only a few hydroxyl and water H atoms were recognizable in the difference Fourier maps.

The diffraction data for 15% humidity is of lower quality due to the small crystal size that was necessitated by cracking of larger specimen (Table 2), and consequently, the water sites in the β-CD cavity are less well resolved. For this structure, minor difference electron density peaks suggest the presence of additional weakly populated water sites which did, however, not behave "properly" if tentatively included in the refinement.

Refinements converged with R-valves between 0.059 and 0.061, except for the lowest humidity of 15%, where the final R-value is 0.068 (Table 2).

Bias of Refined Water Occupation Factors. Most water sites are only partially occupied. Refinement of water occupanices is nontrivial and had to be performed with care for two reasons: firstly, due to the coupling of displacement and occupation paramters, refinement had to be very slow and damped, and secondly, the electrons of the two H atoms are partially attributed to the central oxygen atom, which consequently refines to occupation factors which are systematically too high. For a (rough) correction of the latter bias, *all* water occupanices were allowed to vary, leading to values of >1.0 for ordered water molecules. The inverse of the average refined occupancies of five interstitial ordered water molecules and pairs of mutually exclusive sites (Table S-3, deposited as supplementary material) was then used as a weight factor to estimate the true occupancies from the refined ones: $occ_{(\mathrm{true})} \sim occ_{(\mathrm{ref})}/\langle occ_{(\mathrm{ref}),\mathrm{ordered}}\rangle$ (Table S-4, deposited).

Results

(a) General Results. The relevant crystallographic data is shown in Tables 1 and 2. Tables with fractional atomic coordinates (one β-CD molecule and 19 water sites per asymmetric crystal unit) and additional structural details for the five crystal structures are deposited (see end of text).

Atom numbering for the β-CD molecule is as in the previous publications,[8,9,28] with C(1)2 meaning carbon 1 of glucose 2. For easy comparison, the unconventional setting of the unit cell introduced in the first X-ray study[6] is maintained (see footnote *a* of Table 1), and the labeling of the water molecules is also maintained as far as possible: the eight interstitial water sites are labeled as W1, W2, W3A/B, W5A/B/C, and W7. The 11 water sites within the cyclodextrin cavity are labeled as W4, W6, W8A/B, and W9–W15.

(b) Unit Cell Constants as a Function of Humidity. In Table 1, unit cell constants are given for crystals that have experienced the following history: under mother liquor → dry at ~50% humidity → dry at final humidity. The unit cell volume smoothly diminishes with decreasing humidity (Figure 2a) with no sign of discontinuities. This contrasts the discontinuous dehydration behavior observed for a number of protein crystals.[36,37] The relation of volume and humidity is nonlinear, with the slope becoming steeper for lower humidities. The behavior of the individual cell constants upon dehydration is more complicated (Figure 2b), showing that the unit cell does not reduce isotropically.

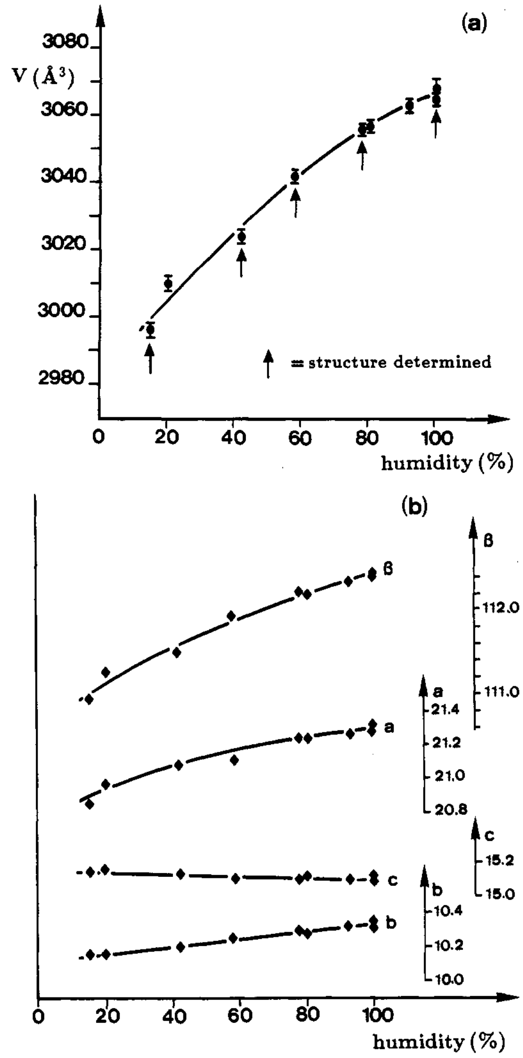

Figure 2. Unit cell parameters of β-cyclodextrin hydrate as a function of ambient humidity at ~18 °C. Numerical data are given in Table 1. (a) Unit cell volume, arrows indicate humidities at which crystal structures were determined. (b) Unit cell axis lengths (Å) and monoclinic angle β (deg). Note that the c-axis increases upon dehydration and the a- and b-axes decrease, indicating a pronounced anisotropic shrinkage of the unit cell.

During the de- and rehydration processes, the water content within the crystal must be inhomogeneously distributed (that close to the surface different from that inside), necessarily leading to mechanical tensions. We assume that this leads to the observed cracking of the crystals which is most pronounced for large crystals and for large humidity changes.

Dry crystals rehydrate if they are put again into saturated mother liquor.[10] Complete rehydration is also obtained through the vapor phase, when dry crystals are exposed to the atmosphere over pure water (100% humidity), without having contact with the bulk liquid (Table 1).

(c) Time Scale of Dehydration. To observe the loss of crystal water with time, the diffraction angle θ of a strong single reflection, which is sensitive to changes in the unit cell dimension, was monitored during the equilibration process (Figure 3a, for

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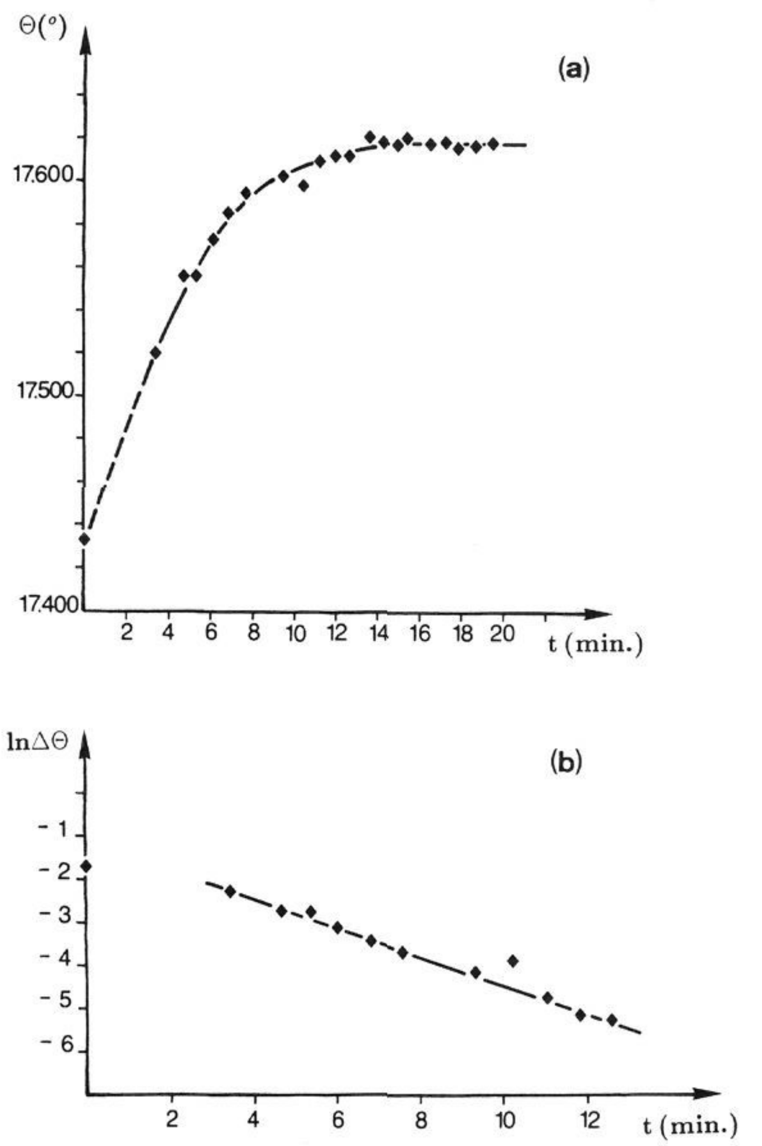

Figure 3. Dehydration of a single crystal as a function of time. The crystal was initially kept at 78% humidity and then exposed to ~50% ambient humidity starting from $t = 0$. (a) Diffraction angle θ of the 040-reflection as a function of time t; equilibration is reached after about 15 min. (b) Logarithm of the angular change $\Delta\theta$ as a function of time $[\Delta\theta(t) = \theta_\infty - \theta(t)]$. The theoretical starting point at $t = 0$ is determined by the known length of the b-axis at the starting humidity; the zero point of the time axis, however, is ill-defined due to the necessary initial mechanical manipulations. The crystal size was 0.4 × 0.3 × 0.2 mm^3, T = 18 °C.

experimental details, see the caption to Figure 3). Equilibration occurs within ~15 min. Drawn on a logarithmic scale, the change of the diffraction angle represents a linear function (Figure 3b), indicating that the dehydration is governed by a simple law of the type e^{-kt}.

The effective rate constant k determining the speed of dehydration (k = 0.33 min^{-t} for the experiment described in Figure 3) must depend on the crystal size and the technical conditions of the experiment. Dehydration will be slower for larger crystals and faster if crystals are ground to powder. Also, pronounced air convection will enhance dehydration.

(d) Crystal Packing Arrangement and β-Cyclodextrin Conformation Remain Unchanged. In crystalline β-CD hydrate, the β-CD molecules are packed in layers of fishbone-type motifs (Figure 4). There is no direct contact between the water clusters included in the individual cyclodextrin cavities (light shading in Figures 4 and 5), but through the cavity opening at the O(6) side, each cluster hydrogen bonds with a finite chain of interstitial water molecules (dark shading in Figures 4 and 5). Only one water molecule, W1, has no hydrogen bond contact with other water molecules.

Upon dehydration, the crystal packing is completely conserved. Judged on the basis of O···O distances, none of the intermolecular hydrogen bonds is broken (Table S-5, deposited). The positions of the interstitial water molecules are conserved as well.

The conformation of the β-CD molecule[6] is not affected by the dehydration: the O···O distances in the ring of intramolecular O(2)···O(3) hydrogen bonds and the interglucose torsion angles are constant within ±0.04 Å and ±3°, respectively, and the ring puckering parameters Θ_2 of the individual glucose residues (as defined by Cremer and Pople[35]) are constant within ±3° (Table S-7, deposited).

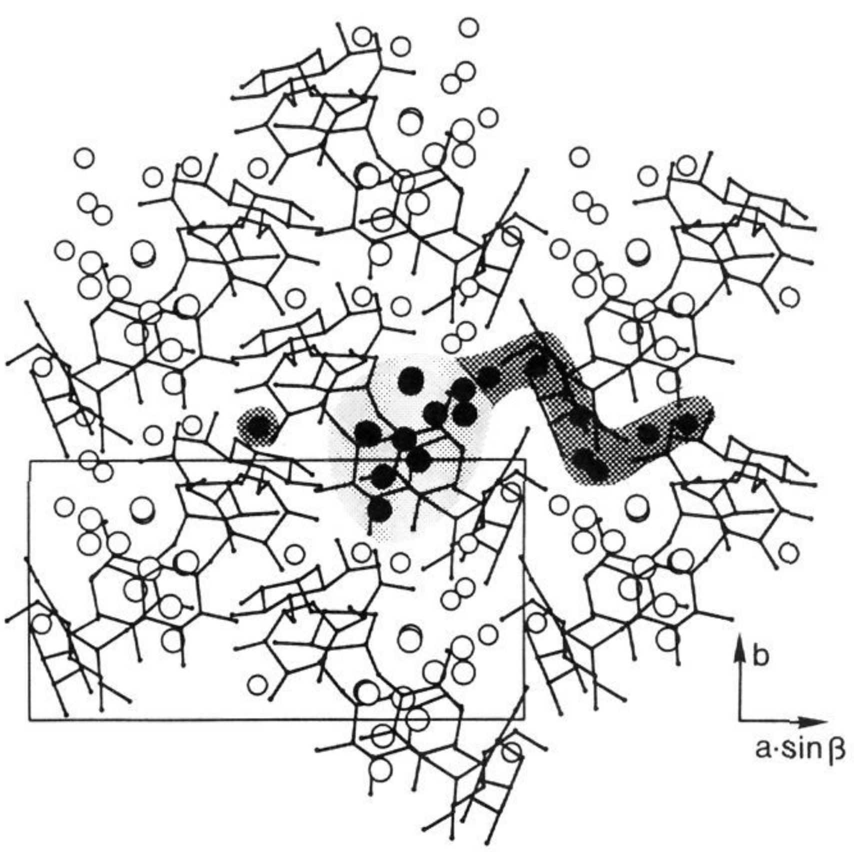

Figure 4. Crystal packing of β-CD hydrate at 78% humidity (the β-CD molecules are shown in side view). At the other humidities, the picture is practically identical. The approximate shape of the volume occupied by the water molecules is shaded for one asymmetric crystal unit. Light shading: the 11 water sites within the β-CD cavity. Dark shading: the eight water sites between the β-CD molecules. Seven of the eight interstitial water sites form a finite chain which is in contact with the water cluster in the β-CD cavity; only one water molecule (W1) is not in hydrogen bonding contact with the others. Labeling of the water sites is given in Figure 5.

As the only exception, there are some changes for the primary hydroxyl groups O(6)1 and O(6)2, which are 2-fold disordered at all humidities (Figure 5). In wet crystals, the orientations A, which point in the direction of the molecular axis, are more strongly populated, whereas at 15% humidity, the orientations B, pointing "outside", exhibit higher occupancies. For the intermediate humidities, a gradual shift of these occupancies is observed (Table S-7 and Figure S-1, deposited.)

(e) Water Content as a Function of Humidity. The sums of occupancies for the 19 water sites are shown as a function of humidity in Figure 6 and Table 3; the subdivision into eight interstitial water sites and 11 water sites in the β-CD cavity is also shown (for T = 18 °C; at higher temperatures, dehydration presumably is enhanced). The total water content per β-CD molecule continuously reduces from ~12.3 at 100% humidity to less than ~11.6 at a "normal" atmospheric humidity of 58% to ~9.4 (or slightly more, see below) at 15% humidity. The dehydration is primarily due to loss of water from the β-CD cavity: at 42% humidity, for example, ~0.2 H_2O are lost from interstitial sites, whereas almost 0.9 H_2O are lost from the cavity, leading to a total loss of ~1.1 H_2O per β-CD.

The water content determined for 15% humidity is probably too low: in structure refinement, some difference electron density suggested the presence of residual weakly occupied water sites. We assume, however, that these residual sites contribute no more than 0.5 H_2O per asymmetric crystal unit, so that the water content at 15% humidity should be around or slightly below 10 H_2O per β-CD molecule.

In remains unclear whether the crystal structure collapses at low humidities (<15%) directly from the form described above or whether there is an intermediate crystalline phase with low water content.

(f) Behavior of Water Networks. The continuous process of dehydration is typically reflected by the well-refined interstitial

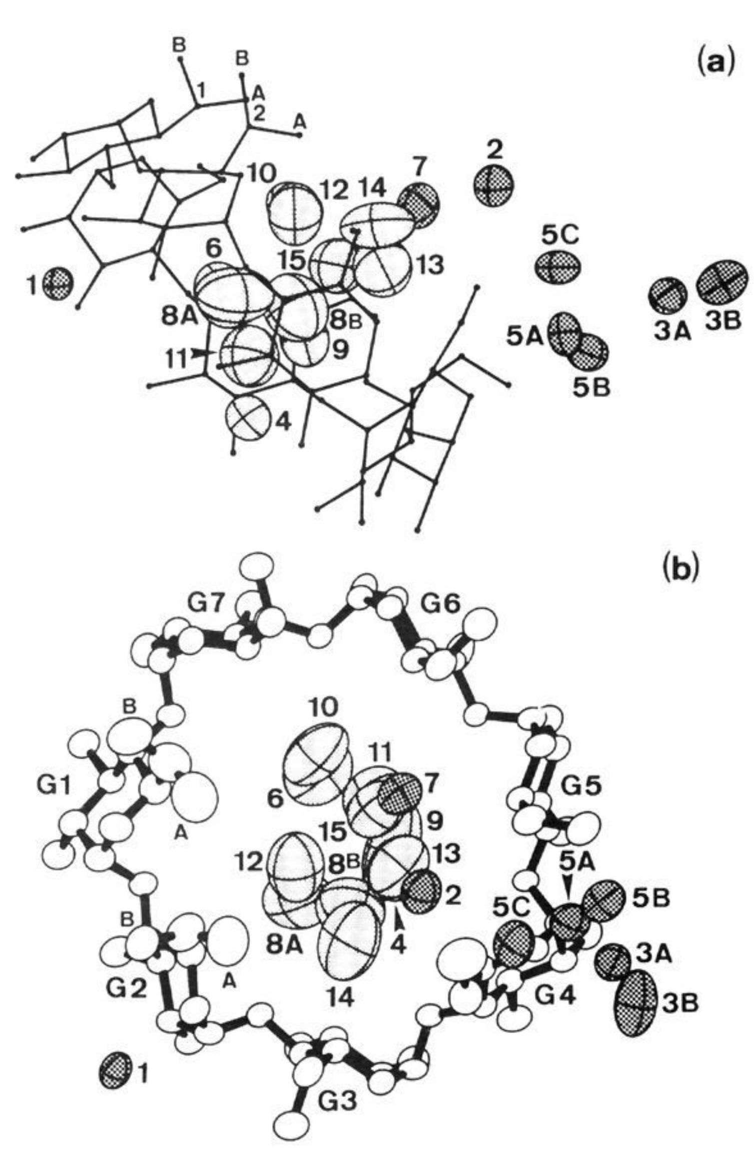

Figure 5. One asymmetric crystal unit of β-CD hydrate at 58% humidity; for water molecules, 50% probability thermal ellipsoids are shown. At the other humidities, the picture is practically identical, with only different water occupancies. Light shading: the 11 water sites within the β-CD cavity. Dark shading: the eight interstitial water sites. (a) Projection as in Figure 4. (b) Projection onto the equatorial plane of the β-CD molecule; the O(6) rim is toward the viewer.

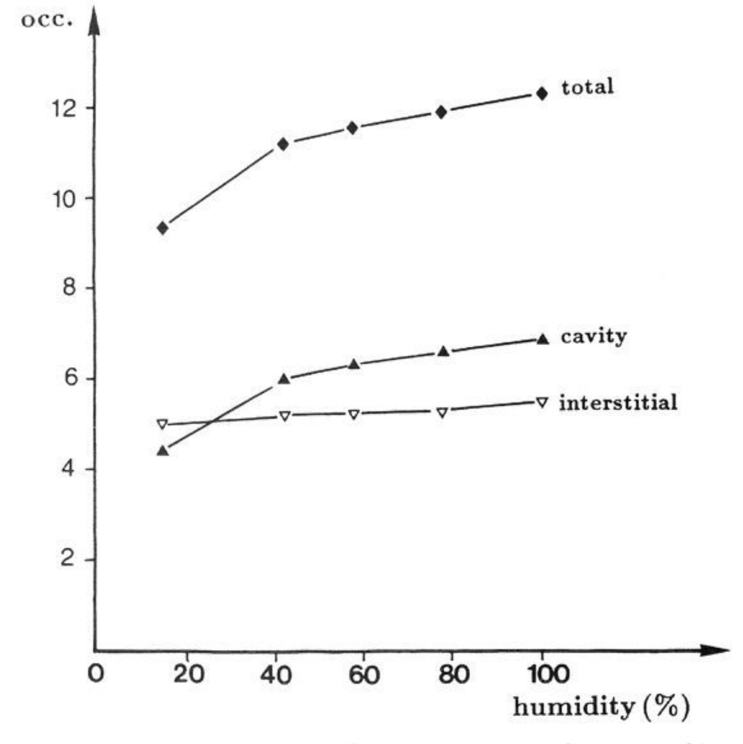

Figure 6. Water content per β-CD molecule as a function of humidity, also shown for the interstitial water sites and the water sites within the β-CD cavity, for T = 18 °C (numerical data in Table 3).

water arrangement W5 A/B/C, which is therefore discussed in greater detail. The electron density distribution of W5 A/B/C is shown for all humidities in Figure 7 together with site separations and occupancies; the intermolecular surrounding is shown in Figure 8a. If a water molecule is located in site W5A, short separations prevent population of sites W5B and W5C. If the site W5A is empty, W5B and W5C, which are at hydrogen bonding distance, are simultaneously occupied. This results in the two possible hydrogen bond networks shown in Figure 8b,c; in the

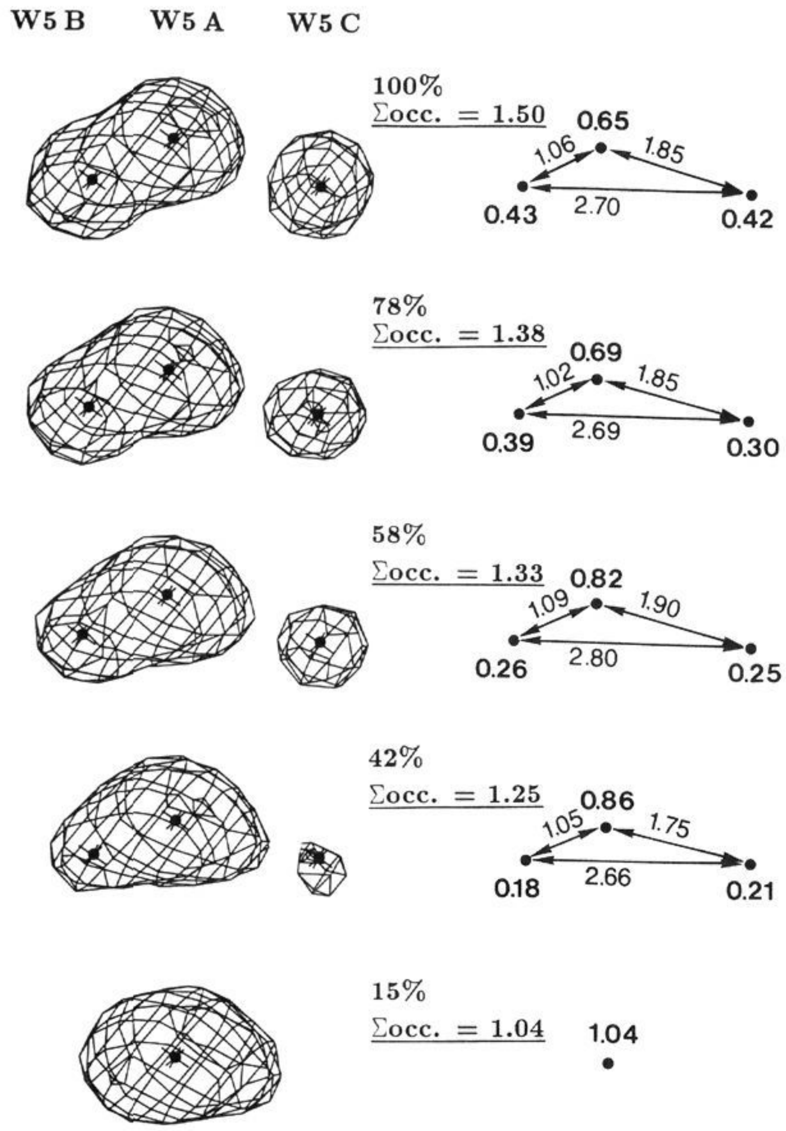

Figure 7. The interstitial water arrangement W5A/B/C at five humidities (100% humidity at the top, 15% humidity at the bottom). Left: $^1/_3$ rms contour of the electron density distribution reflecting the continuous change of occupancies. Right: Site separations (Å) and occupancies, showing that population of site W5A excludes occupancy of sites W5B and W5C, whereas W5B and W5C are in hydrogen bond distance and presumably populated simultaneously. The sum of the water occupancies of the arrangement decreases from ~1.5 at 100% humidity to ~1.0 at 15% humidity.

crystal, some unit cells contain one arrangement, and some the other, and crystallography observes the superimposition of both. During dehydration, the relative occupancy of network B (two water molecules) gradually reduces to the benefit of network A (one water molecule) (Figure 8d). This way, the sum of water occupancies in the arrangement W5 A/B/C gradually reduces from 1.5 at 100% to 1.0 per β-CD molecule at 15% humidity.

Within the β-CD cavity, large anisotropic displacement parameters indicate extensive thermal motions of the water molecules (see Figure 5) and suggest that the positional disorder may be more cmoplex than shown in the refined model. In fact, it is likely that water positions slightly shift depending on whether a neighboring water site is populated or not. In consequence, many of the refined water positions could represent superimpositions of two or more closely neighboring alternative sites with separations significantly shorter than the crystallographic resolution, ~0.9 Å. This circumstance largely obstructs interpreting the water arrangement in the cavity in terms of alternative partially occupied networks.

It becomes clear, however, that the water sites within the β-CD cavity are very unequally affected by dehydration: for two sites (W4 and W6), the occupancy remains practically constant (~1.0); for others, the occupancy reduces with different individual rates, whereas for one it even increases (Figure S-2b, deposited). The situation for the latter site, W8B, is similar to that for the interstitial water W5A discussed above: W8B is placed between the water sites W8A and W9, which are in hydrogen bond distance (Figure 9), and can only be populated if the latter sites are empty. As the occupations of W8A and W9 gradually (and in parallel) reduce during dehydration, the occupancy of the intermediate site W8B increases (Figure 9b–d).

Table 3. Sum of Water Occupation Factors[a] as a Function of Ambient Humidity at $T = 18$ °C

humidity	wet	78%	58%	42%	15%
unit cell volume (Å^3)	3065(1)	3056(1)	3042(2)	3024(2)	2996(2)
sum of occupancies of interstitial water[b]	5.42	5.29	5.25	5.21	5.00
sum of occupanices of water in the cavity[c]	6.84	6.60	6.31	5.98	4.35+[b]
sum of occupancies of all water molecules	12.26	11.89	11.56	11.19	9.35+[b]

[a] Data for the individual water sites in Table S-4, deposited. [b] Water sites W1, W2, W3A/B, W5A/B/C, and W7. [c] Water sites W4, W6, W8A/B, W9, W10, W11, W12, W13, W14, and W15. [d] Minor difference electron density suggests the presence of some residual water sites.

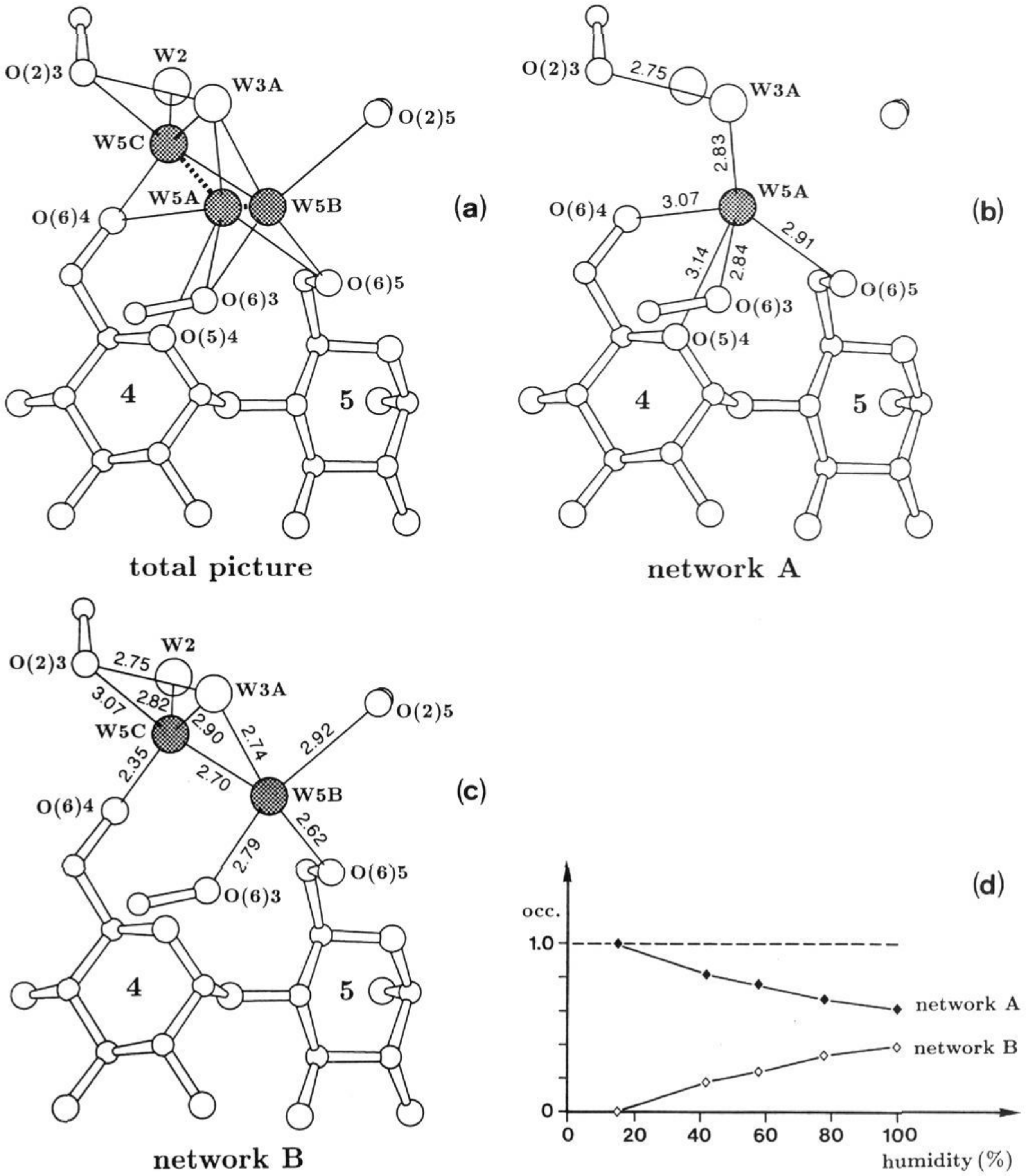

Figure 8. Intermolecular surrounding of the interstitial water arrangement W5A/B/C. (a) Total picture, which represents the superimposition of two alternative networks. (b) Network A, water site W5A occupied. (c) Network B, water sites W5B and W5C occupied. (d) Upon dehydration, the relative occupancy of arrangement A increases at the expense of arrangement B.

(g) The Diffusion Path for Water Molecules Is Not Evident. Dehydration and rehydration as described implies the existence of translation diffusion paths which are accessible for interstitial water molecules as well as for those included in the β-CD cavities. As (due to the fishbone-type packing) the crystal structure contains no wide channels, no such diffusion path is immediately obvious. In search of expected narrow channels, the solvent accessible surface of the β-CD molecules in the crystal packing was inspected with the computer graphics program[38] INSIGHT for all possible mutual orientations of the disordered primary hydroxyl groups O(6)1 and O(6)2. Using probe radii of 1.4 and 1.3 Å for water molecules, *no* translation diffusion paths could be detected. This would exclude the experimentally observed long-range diffusion of water molecules. Probe radii were gradually reduced, and for $r = 1.0$ Å, bottlenecks between adjacent asymmetric units became apparent that could allow diffusion in the direction of the crystallographic *b*-axis.

The above model of the crystal regards the β-CD molecules as completely rigid with atoms fixed at the crystallographically determined positions. In such a crystal, water molecules with radii around 1.4 Å cannot diffuse through the lattice. In fact, however, there are considerable positional fluctuations of the β-CD atoms, which have amplitudes of several 0.1 Å (for example, typical displacement parameters of $U = 0.07$ Å^2 correspond to rms displacements $U^{1/2}$ of over 0.25 Å). In a computer simulation study,[25] rms displacements up to 0.4 Å were predicted for the β-CD hydroxyl O atoms. This is sufficient to temporarily open paths for diffusion, which is presumably further assisted by the dynamical *flip–flop* disorder of hydroxyl orientations. Nevertheless, it remains surprising that an apparently strongly hindered diffusion allows fast dehydration on a minute time scale.

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[38] INSIGHT, Version 2.7; BIOSYM Technologies Inc.: San Diego, CA, 1991.

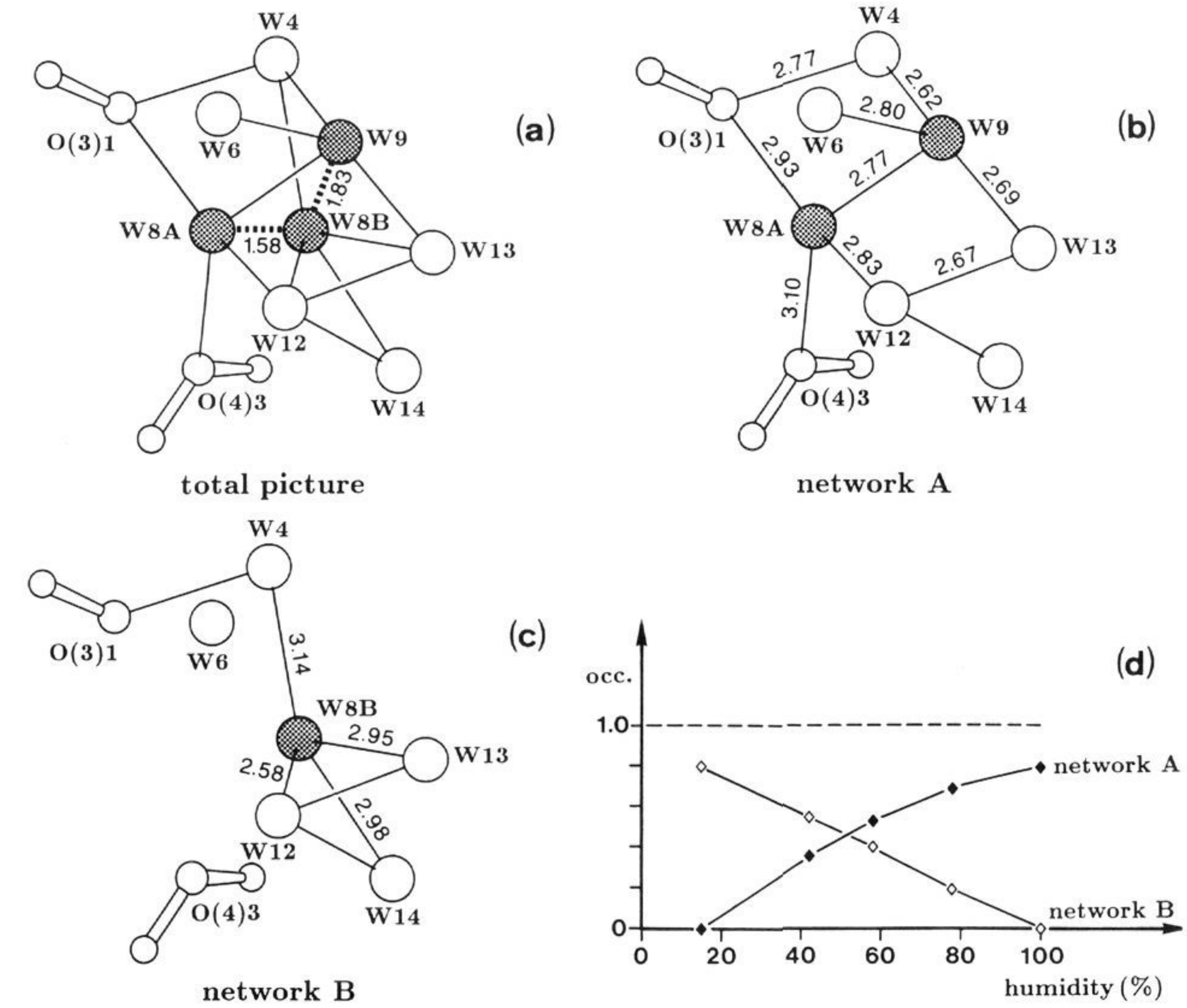

Figure 9. Intermolecular surrounding of the water arrangement W8A/B-W9 within the β-CD cavity. Population of the site W8B excludes occupancy of the sites W8A and W9. (a) Total picture, which represents the superimposition of two alternative networks. (b) Network A, water sites W8A and W9 occupied. (c) Network B, water site W8B occupied. (d) Upon dehydration, the relative occupancy of arrangement B increases at the expense of arrangement A.

Summary and Discussion

(1) The crystal structure of β-cyclodextrin hydrate was determined for crystals at four ambient humidities between 15% and 78% and for a crystal in contact with mother liquor. The unit cell volume gradually diminishes with decreasing humidity, indicating continuous loss of crystal water (Figure 2a). In this humidity range, dehydration is surprisingly fast (equilibration within minutes, Figure 3) and completely reversible. The arrangement and the conformation of the flexible β-CD molecules are conserved. Dehydration is primarily due to loss of water from the β-CD cavity, whereas the interstitial water molecules are affected to a lesser extent (Figure 6 and Table 2b). The character of water disorder in the cavity and in interstices (i.e. positional disorder with discrete sites) remains the same, as well as the disorder of primary hydroxyl groups; this suggests that also the dynamic *flip–flop* disorder of hydrogen bond orientations[8] is present in the whole humidity range.

(2) During the dehydration process, the distribution of the water content within the crystals is necessarily inhomogeneous, causing mechanical tensions which often lead to cracks. Below 15% humidity, the crystalline structure collapses irreversibly.

(3) The water content changes due to a *continuous* change of occupancies of partially populated water sites. Some water sites are fully occupied at all humidities, others change occupancies only slightly, whereas for others, the changes are very pronounced. Alternative networks of partially occupied water sites can be specified to contain different numbers of water molecules. During dehydration, the relative occupanices of the networks with fewer water molecules continuously increase at the expense of the networks with more water sites (Figures 8 and 9). It is assumed that continuous de- and rehydration in this fashion is not unique to β-CD hydrate but also occurs in other hydrated biological systems (in some protein crystals, however, stepwise dehydration was observed[36]).

(4) β-CD hydrate is an impressive example of a solid in which fast diffusion of water molecules is possible without the presence of permanent diffusion channels. If the β-CD molecules are regarded as completely rigid, the crystal packing would be so tight that no water molecules could travel through the lattice; therefore, it must be assumed that the observed long-range mobility is made possible by positional fluctuations of the β-CD atoms, whereby bottlenecks between neighboring asymmetric crystal units are temporarily opened. A quantitative understanding of the diffusion mechanism, which is of more general interest, requires further experimental and computer simulation studies.

(5) The studies reported in refs 15–24 were performed on "dry" β-CD hydrates which were exposed to different local atmospheres and consequently had different hydration states. As in the "normal" humidity range, the main characteristics of the structure are conserved; it can be expected that these studies are basically compatible (except for details). The exchange of water with the atmosphere on a minute time scale must also have consequences for the storage of cyclodextrin complexes, which are often kept as dry powders.

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Supplementary Material Available: Tables listing fractional atomic coordinates, anisotropic and equivalent isotropic displacement parameters, refined and corrected water occupation factors, geometeric parameters describing the conformation of the β-CD molecule, and intermolecular hydrogen bond distances for all five crystal structures and figures showing the humidity dependencies of all relevant occupation factors (33 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.