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Cyclodextrin Inclusion Complexes: Host–Guest Interactions and Hydrogen-Bonding Networks

WOLFRAM SAENGER* AND THOMAS STEINER

Institut für Kristallographie, Freie Universität Berlin, Takustrasse 6, D-14195 Berlin, Germany. E-mail: saenger@chemie.fu-berlin.de

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Abstract

An overview is given on the structural characteristics of α-, β- and γ-cyclodextrins (CDs). These cyclic oligosaccharides consisting of six, seven and eight glucoses form inclusion complexes with guest molecules small enough to fit into their central cavities and serve as good model systems for non-covalent bonding. Depending on the size and the ionic or molecular nature of the guests, the complexes crystallize in cage- or channel-type structures. X-ray and neutron diffraction have been used to study intermolecular interactions; they provide insight into O—H···O and C—H···O hydrogen bonds stabilizing the macrocyclic conformations and the inclusion of the guest molecules. Throughout the crystal lattices of the CD hydrates, which abound with O—H groups, cooperative networks are formed by O—H···O hydrogen bonds; in the β-CD macrocycle, dynamic disorder of the flip-flop type is observed, O—($\frac{1}{2}$H)···($\frac{1}{2}$H)—O, between O(2)—H and O(3)—H groups of adjacent glucoses.

Wolfram Saenger graduated from Technische Hochschule Darmstadt in Chemistry and received his PhD in 1965. After postdoctoral work at Harvard with Professor J. Z. Gougoutas, he joined the Max-Planck-Institut für experimentelle Medezin in Göttingen where he set up an independent research group working on X-ray crystal structure analysis of oligosaccharides, proteins and nucleic acids. In 1972, he received his Habilitation from the Universität Göttingen, and since 1981 he has held the Chair for crystallography at Freie Universität Berlin. In 1987, he received the Leibniz Award and in 1988 the Humboldt Award. Besides structural biology, his current interests are hydrogen bonding and protein aggregation leading to crystallization.

Thomas Steiner graduated in 1986 in experimental Physics at the Technische Universität Graz, Austria. He obtained his PhD at the Freie Universität Berlin in 1990, where he worked with Wolfram Saenger on neutron scattering studies of cyclodextrin complexes. Following a postdoctoral period in the same laboratory, he started independent research work on weak hydrogen bonding, isotropic intermolecular interactions, structure correlation and crystal engineering. During 1998, he is a guest researcher at the Department of Structural Biology of the Weizmann Institute of Science, Israel.

1. Introduction

Amylose, the linear unbranched fraction of starch, consists of glucoses linked exclusively by α(1–4) bonds, and is cleaved by cyclodextrin glucanotransferases into annular-shaped cyclodextrins (CDs), with six, seven, eight and up to 16 glucoses. They are called cyclohexa-, cycloheptа- and cyclooctaamyloses (CA6, CA7, CA8) or α-, β- and γ-CDs, respectively (Szejtli, 1988; Duchêne, 1987; Saenger, 1980). Even larger macrocycles with more than 100 glucoses may be obtained if amylose is treated with disproportionating enzyme (Takaha *et al.*, 1996). The smaller CDs, which are the main topic of this paper, have been investigated thoroughly using MALDI (Bartsch *et al.*, 1996), and spectroscopic, kinetic and crystallographic methods (Szejtli, 1988; Frömming & Szejtli, 1994; Wenz, 1994; Saenger, 1980, 1984; Harata, 1991). In addition, X-ray crystal structure analyses of the hydrates of δ-CD (Fujiwara *et al.*, 1990), ε-CD (CA10) and *i*-CD (CA14) (Jacob *et al.*, 1998; Ueda *et al.*, 1996) have been published, as well as the crystal structure of a cycloamylose containing 26 glucoses, CA26 (Gessler *et al.*, 1998).

The structure of α-CD·6H_2O, shown in Fig. 1, points to the most interesting property of these molecules, namely the formation of inclusion complexes with molecules small enough to fit into the central cavity of the doughnut-shaped macrocycles. The inclusion of guest molecules by the CD is relatively unspecific and occurs even in solution, in contrast to many of the inclusion-forming host lattices which are bound to the crystalline state. Since the inclusion into the CD macrocycle is non-covalent, CDs are frequently used as model compounds to study intermolecular interactions, which are so important in biological structures and processes.

CDs are produced in ton quantities and employed in the pharmaceutical and chemical industries (Frömming & Szejtli, 1994). This is because pharmaceuticals, which are frequently only slightly soluble in body fluids, can be

made more readily available by the formation of inclusion compounds. Other pharmaceuticals that are sensitive to the environment can be encapsulated by CDs to become more stable. In another application, liquid pharmaceuticals can be transformed into powders if included in CDs. In the chemical industry, immobilized CDs are used in column chromatography, and a number of chemical reactions can be made very specific if a reactant or part of it is enclosed in the CD cavity (Szejtli, 1988).

In the following, we focus on the structural properties of the CDs, which have attracted crystallographers since the 1950s. In addition to the structural work, we will discuss hydrogen bonds of the type O—H···O which are abundant in CD crystal structures because each glucose contains three hydroxyl groups.

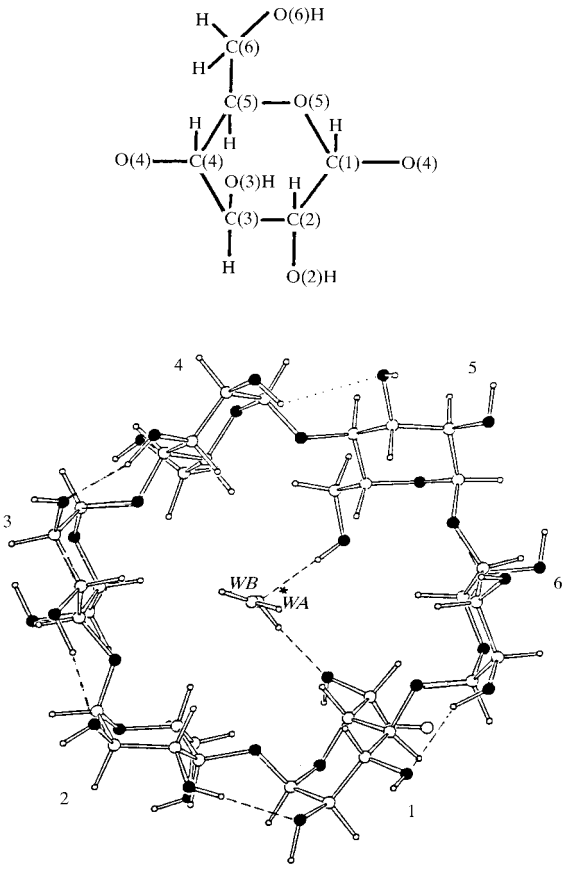

Fig. 1. (Top) Atom numbering in glucose. (Bottom) Structure of α-cyclodextrin hexahydrate (α-CD·6H_2O) showing inclusion of two H_2O molecules in the cavity; four H_2O molecules are located 'outside' the cavity and not drawn. Hydrogen atoms (small spheres) were located by neutron diffraction; hydrogen bonds are indicated by broken lines, oxygen atoms filled. O(6)H groups of glucoses 5 and 1 are in (+)-*gauche* orientation to permit hydrogen bonding to water *WA*, all other O(6)H groups are in the preferred (−)-*gauche* form. Note that interglucose hydrogen bonding may be O(2)—H···O(3′) or O(2)···H—O(3′). The ∗ marks the center of the macrocycle as defined by the nearly coplanar O(4) atoms; *WA* is ∼0.6 Å displaced (Klar *et al.*, 1980).

2. Structural properties of the cyclodextrin macrocycle

In amylose and in the CDs, the glucose units are 'rigid' building blocks adopting a $^4C_1$-chair conformation (Saenger, 1980, 1984; Harata, 1991). Because the glucoses are oriented in register (*cis*) in the CDs, all the secondary O(2) and O(3) hydroxyl groups are on one side of the macrocycles so that intramolecular interglucose hydrogen bonds of the type O(2)···O(3′) can form (the primed atom belongs to the adjacent glucose). The primary O(6) hydroxyl groups are located on the other side of the macrocycle. They may rotate around the C(5)—C(6) bond with the preferred orientation of the O(5)—C(5)—C(6)—O(6) sequence being (−)-*gauche*, which means that the O(6) hydroxyl groups point 'away' from the center of the macrocycle, Fig. 1. In the less preferred (+)-*gauche* conformation, the O(6) hydroxyl group is directed 'towards' the cavity and may hydrogen bond to an enclosed guest molecule. The *trans* conformation has not been observed so far. The shape of the CD is that of a truncated cone rather than of an open cylinder. The wider side of the cone is formed by the secondary O(2) and O(3) atoms, and the narrower side by the primary O(6) atom. The relative orientation of the individual glucoses is best described by the torsion angles about the glucosidic links defined by φ, O(5)—C(1)—O(4′)—C(4′), and ψ, C(1)—O(4′)—C(4′)—C(3′). They are in the ranges $\varphi = 102$–123° and $\psi = 112$–149° for α-, β- and γ-CDs.

The O(4) atoms linking the glucose units in α-, β- and γ-CDs are nearly coplanar and describe roughly regular polygons. As a result of the different curvatures, the mean O(2)···O(3′) hydrogen-bonding distances are 2.98, 2.88 and 2.82 Å in α-, β- and γ-CDs, respectively. The overall shape of the macrocycle can be regular or distorted, depending on the guest molecule and the crystal environment. In particular, α-CDs can have two-, three- or sixfold internal symmetry, or can be quite irregular as in α-CD·6H_2O (Manor & Saenger, 1974).

3. Host–guest interactions

The interactions between the CD host macrocycles and guest molecules included in their cavities have been studied in a number of X-ray analyses (Saenger, 1980, 1984; Harata, 1991). If CD crystallizes from water, it forms hydrates of the form α-CD·6H_2O (Manor & Saenger, 1974; Klar *et al.*, 1980) to α-CD·7.5H_2O (Lindner & Saenger, 1982; Chacko & Saenger, 1981) depending on conditions; β-CD·12H_2O (Betzel *et al.*, 1984); and γ-CD·15.7H_2O (Harata, 1987; Ding *et al.*, 1991). A large variety of other CD inclusion complexes have been crystallized. They range from the inclusion of krypton, alcohols, fatty acids and aromatic molecules

to larger slim molecules like long fatty acids, azobenzenes, styrenes, drug molecules, a number of organic and inorganic ions, and even some organometallic compounds like ferrocene. In some cases, guest molecules are also found cocrystallized between the CD molecules. The extreme case is β-CD·(pyridine)$_8$·3H$_2$O, where only two pyridines are included in the cavity (de Rango *et al.*, 1992).

The involvement of the CD hydroxyl groups in hydrogen bonding to the guest molecules is mostly restricted to the primary O(6)—H groups because they are flexible and can rotate about the C(5)—C(6) bond in contrast to the secondary O(2) and O(3) atoms which are rigid due to the preferred $^4C_1$ form of the glucose units. In addition to direct hydrogen bonding between the guest and CD molecules, there can be water-mediated hydrogen bonds, and water may also be enclosed within the cavity if the guest molecule is too small to fill it properly, Fig. 2. Guest molecules can also be statistically disordered in the cavity volume so that it is sometimes impossible to locate them properly. Another interesting feature of the CD macrocycle is its chirality. This implies that one of the enantiomers of a racemic mixture has a higher affinity for the CD cavity than the other, permitting their separation. This is frequently applied in chromatographic material containing immobilized CDs (Szejtli, 1988; Harata *et al.*, 1987; Caira *et al.*, 1994).

4. Crystal packing types: channels and cages

Depending on the nature and size of a guest molecule, an inclusion complex may crystallize in three different forms: two are of the cage type, where the cavity of each CD is blocked on both sides by adjacent CD molecules in the crystal lattice, Fig. 3. In the channel-type crystal structures, the CD macrocycles are stacked like coins in a roll so that the cavities form infinite channels (Saenger, 1984, 1985).

α-, β- and γ-CDs differ in their properties to form cage or channel types of crystal packing. If crystallized as hydrates, all three CDs form cage-type crystal structures in which the CD macrocycles are arranged crosswise in a herringbone motif. With α-CD, this type of packing is also found with small molecular guests like krypton, iodine, methanol to 1-butanol, and acetic acid to butyric acid. With larger molecular guests or with salts, α-CD prefers channel-type complexes in which the macrocycles may be oriented head to head (with hydrogen bonding between secondary hydroxyl groups on one side and between primary hydroxyl groups on the other side) or head to tail (with hydrogen bonds between secondary and primary groups). Of particular interest is the inclusion of iodine in α-CD: with molecular I_2, a cage-type complex is formed, whereas, with metal triiodides, polyiodide is enclosed in channel-type complexes, and the cations are located between the α-CD molecules (Noltemeyer & Saenger, 1980).

If α-CD forms inclusion complexes with small aromatic guests, the benzene ring enters the cavity head-on and distorts the α-CD macrocycle into an elliptical form, showing that the host has some freedom to adapt to the shape of the guest molecule. The slight distortion is sufficient to produce a unique 'brick'-type pattern where the α-CD inclusion complexes are arranged in layers; adjacent layers are shifted relative to each other so that the cavities are blocked on both sides, forming cage-type packing (Fig. 3). With some other aromatic guests, α-CD forms channel complexes in head-to-tail arrangement.

β-CD forms herringbone-type cage complexes with water and small alcohols. With larger guests, the preferred packing motif is a 'basket' formed between two β-CD molecules hydrogen bonded with their secondary O(2), O(3) hydroxyl groups. The guest molecules are located within these baskets which are stacked either collinearly to form channel-type structures or can be displaced sideways to different degrees. This depends on the guest molecules and on the mode of hydration. There are two types of channels; in one, the 'baskets' are related by lattice translations, and, in the other, by the operation of a 2_1 screw axis. The cage-type structures have been classified into two patterns, both similar to Fig. 3(*b*); in the 'intermediate form', the 'baskets' are slightly displaced laterally, and in the

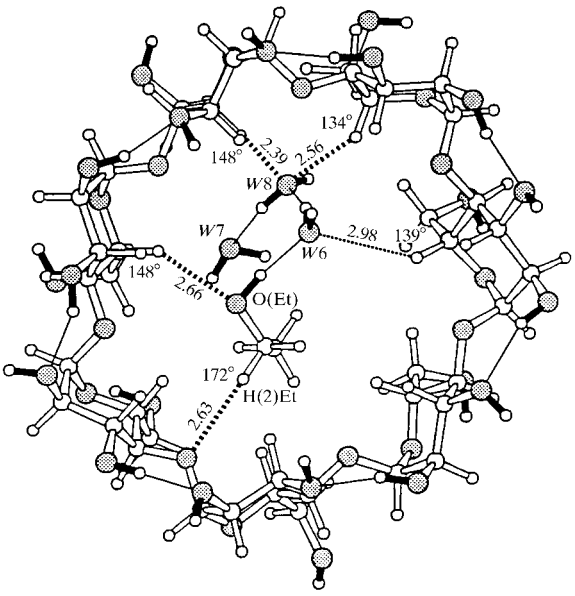

Fig. 2. Neutron diffraction structure of β-CD·EtOD·8D$_2$O at 15 K. O—D bonds are drawn filled, O atoms stippled, and D and H atoms are shown as small spheres. Normal O—D···O hydrogen bonds are shown as thin lines, short and long C—H···O bonds as thick and thin broken lines; C—H···O distances are given in Å, C—H···O angles in ° (Steiner & Saenger, 1992*c*).

'chessboard form' they are displaced so severely that alternate positions of the 'baskets' are filled by hydration water (Mentzafos *et al.*, 1991).

γ-CD forms herringbone-type cage complexes only with water. With other guest molecules, channel-type structures crystallize in a tetragonal space group with the fourfold rotation axis coinciding with the symmetry axis of the eight-membered macrocycle. The γ-CD molecules show a unique stacking with alternating head-to-head *and* head-to-tail hydrogen bonding, which implies that $3 \times \frac{1}{4}\gamma$-CD molecules constitute the asymmetric unit; the guest molecules are disordered along the fourfold symmetry axis if they do not exhibit fourfold symmetry (Steiner & Saenger, 1998*a*).

5. Cyclodextrins as models for hydrogen-bonding studies

There are three main reasons why CDs serve as good model systems for the study of hydrogen bonding: first, they are intermediate in size between small organic and biological macromolecules and exhibit several characteristics of the latter; second, each glucose contains three —OH groups which, together with guest and water of hydration molecules, can form extended networks of O—H···O hydrogen bonds; third, crystals of CD inclusion complexes diffract to a nominal resolution of 0.9 Å or better, and can be grown to sizes of several mm^3 suitable for neutron diffraction studies. This allows H atoms to be located and refined with similar accuracies to those for the other atoms.

5.1. *Cooperative networks formed by O—H···O hydrogen bonds*

Since hydroxyl groups act as hydrogen-bond donors *and* acceptors, they can form chains of the type $O^{\delta-}$—$H^{\delta+}\cdots O^{\delta-}$—$H^{\delta+}\cdots O^{\delta-}$, in which the individual hydrogen bonds enhance the strength of each other by mutual polarization through the σ-bonds. As a result of this cooperative effect (Jeffrey & Saenger, 1991), which typically yields around 20% gain in energy compared with isolated hydrogen bonds (Koehler, Saenger & Lesyng, 1987), hydroxyl groups tend to arrange in chains or rings rather than in more irregular arrangements. To distinguish hydrogen-bonding arrays of different orientational regularity, the terms *homodromic*, *antidromic* and *heterodromic* have been coined, see Fig. 4 (Saenger, 1979). The homodromic arrays (all hydrogen bonds 'run' in the same direction) have the highest degree of cooperativity, and are therefore preferably formed; the irregular heterodromic arrays are only rarely observed, if at all.

In all the CD crystal structures analyzed so far, extended networks of cooperative hydrogen bonds have been observed. Typically, they are composed of four-, five- and six-membered rings, and of infinite and finite homodromic chains. These networks have been characterized from neutron diffraction data for α-CD·$6H_2O$ (Klar *et al.*, 1980), β-CD·$11.6D_2O$ (Zabel *et al.*, 1986) and β-CD·EtOD·$8D_2O$ where all O—H are deuterated, O—D, to reduce incoherent neutron scattering at H atoms (Steiner *et al.*, 1990). Within the arrays, the hydrogen-bond geometry is very flexible, allowing considerable lengthening of H···O distances and large deviations from linearity (Steiner & Saenger, 1992*a*). However, geometries are strictly limited by the shortest possible H···H approach of 2.05 Å (Steiner & Saenger, 1991, 1992*b*).

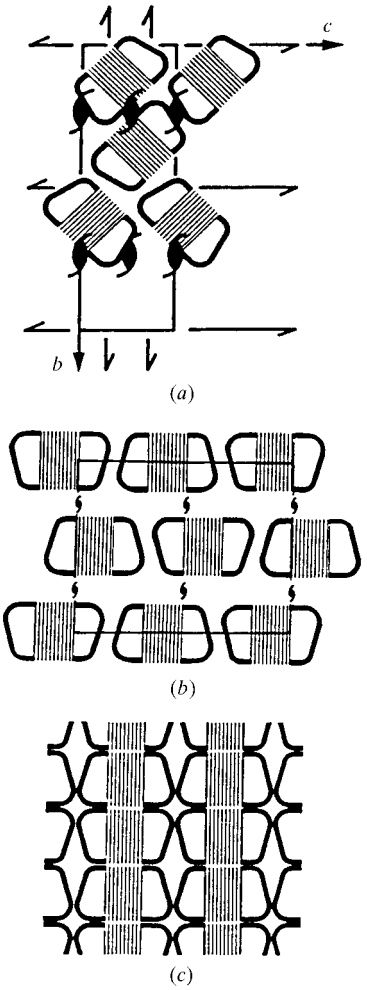

Fig. 3. Packing schemes in crystals of cyclodextrin inclusion complexes, see also §4. (*a*) Herringbone-type cage; (*b*) brick-type cage; (*c*) channel type shown with head-to-head-arranged CD molecules (Saenger, 1984, 1985). In β-CD inclusion complexes, β-CD commonly forms basket-like dimers in which the macrocycles are connected by hydrogen bonds between O(2)H and O(3) groups. These 'baskets' are arranged in cage and channel patterns similar to (*b*) and (*c*), respectively, see text.

5.2. *Three-center hydrogen bonds*

Since the hydrogen-bonding force is primarily electrostatic, it diminishes slowly with increasing distance and is operative at longer distances than the H···O van der Waals separation. Therefore, an O—H donor can interact with a number of close and distant acceptors at the same time (Fig. 4*c*); with two acceptors, this is called a 'three-center hydrogen bond', and, with three acceptors, it is analogously called a 'four-center hydrogen bond' (Jeffrey & Saenger, 1991). These configurations are normally unsymmetric with shorter 'major' and longer 'minor' components of the three-center bond.

Since CDs have a large number of O atoms, their crystal structures abound with three- and four-center hydrogen bonds, as is shown schematically for two examples in Fig. 5. In particular, the interglucose intramolecular O(2)—H···O(3′) and O(3)—H···O(2′) hydrogen bonds systematically have a minor component to O(4) linking the two adjacent glucose units.

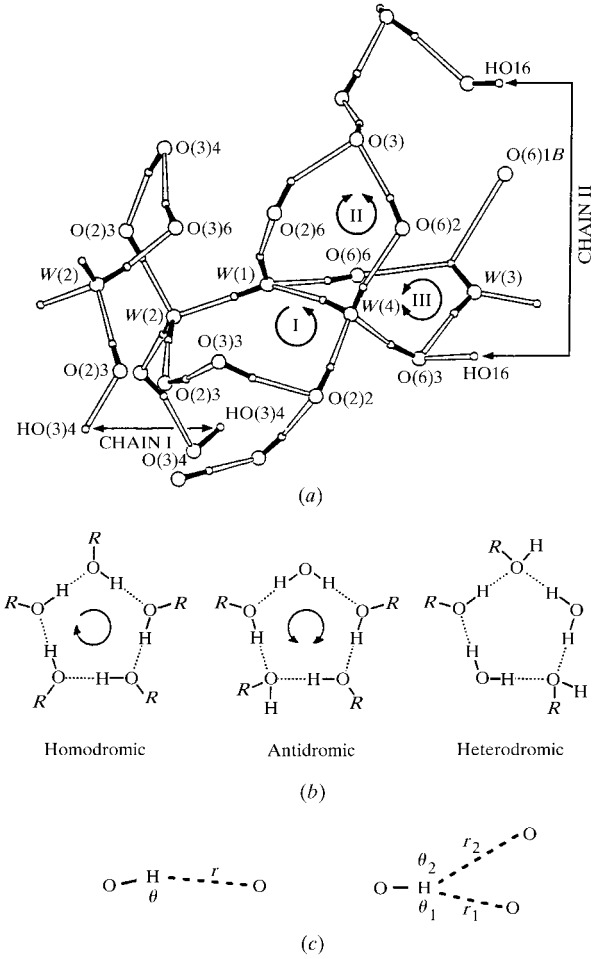

Fig. 4. (*a*) Section of crystal structure of α-CD·6H_2O to show cooperative hydrogen bonding; only O—H groups are drawn. Rings are indicated by curved arrows; I is homodromic, II and III are antidromic, chains are infinite and homodromic (Saenger, 1979). (*b*) Definition of homo-, anti-, heterodromic; the latter is not observed in crystal structures because it is not cooperative. (*c*) Definition of two-center (left) and three-center (right) hydrogen bonds.

5.3. *C—H···O hydrogen bonds*

Although C—H groups are much weaker hydrogen-bond donors than O—H or N—H, their donor potentials should not be neglected. Despite their relative weakness (*ca* 2–8 kJ mol^{-1}), C—H···O hydrogen bonds have been shown to be of importance in many organic and biological structures (Desiraju, 1996; Steiner, 1997, and references therein). Because C—H groups in carbohydrates are 'activated' by electron-withdrawing O atoms linked to the same C atom (see Fig. 1), they are more polarized than C(sp^3)—H in pure hydrocarbons. In this kind of C—H group, the partial charge on H is about +0.13, compared with +0.34 for H of hydroxyl groups (Rasmussen, 1982).

In addition to the intramolecular inter-residue contacts, C(6)—H···O(5′), which occur systematically and stabilize the annular conformation of the CD (Fig. 6; Steiner & Saenger, 1992*c*, 1996; Caira *et al.*, 1994), there are also C—H···O interactions between the host and guest molecules. They are often formed between polar guest molecules and the cavity lining, as in β-CD·EtOD·8D_2O (Fig. 2, Steiner & Saenger, 1992*c*). A number of related crystal structures show that this is the normal situation when hydrophilic guest molecules like water or alcohols are included in relatively hydrophobic cavities: as they cannot satisfy their acceptor potential with conventional partners, they resort to the weaker C—H···O hydrogen bonds involving the cavity wall (Steiner & Saenger, 1993, 1995; Steiner, Moreira da Silva *et al.*, 1995). In quantum-chemical calculations, the energies of these interactions are estimated to be slightly above 4 kJ mol^{-1}, *i.e.* about a quarter to a third of those of conventional hydrogen bonds (Starikov *et al.*, 1998).

5.4. *Flip-flop hydrogen bonds*

In the room-temperature neutron crystal structure of β-CD·11D_2O, 35 of the 53 O—H···O hydrogen bonds including all seven O(2)···O(3′) interglucose interactions are of the type O—($\frac{1}{2}$H)···($\frac{1}{2}$H)—O. This was interpreted as orientational dynamic disorder, O—H···O—H $\rightleftharpoons$ H—O···H—O, called 'flip-flop hydrogen bonds' (Saenger *et al.*, 1982; Betzel *et al.*, 1984). The disorder disappears upon cooling (Hanabata *et al.*, 1987) and gives rise to cooperative homodromic arrangements (Zabel *et al.*, 1986). Related disorder was also observed in β-CD·EtOD·8D_2O, see Fig. 5, but not for the smaller α-CD.

Molecular dynamics simulations on β-CD·11H_2O predict jump rates on the 10^{11} s^{-1} timescale at room temperature (Koehler, Saenger & van Gunsteren, 1987, 1988), supported by incoherent neutron scattering

(Steiner, Saenger & Lechner, 1991) and NMR studies (Usha & Wittebort, 1989, 1992) on β-CD·11H_2O. The dynamics can be described as rotational jumps of hydroxyl groups and water molecules. Jump rates range over several decades from the NMR timescale of $\sim 10^6\ s^{-1}$ for the most stable to about $2 \times 10^{11}\ s^{-1}$ for the least stable hydrogen bonds. This is slower than typical hydrogen-bond dynamics in solution, $10^{12}\ s^{-1}$, but clearly within the 'biological' timescale.

6. Diffusion of water in the crystal lattice of β-CD hydrate

The flip-flop disorder in crystals of β-CD·$n H_2O$ is associated with long-range water diffusion indicated by a non-stoichiometric water content in equilibrium with the atmospheric humidity, ranging from $n = 9.4$ at 15% to $n = 12.3$ at 100% relative humidity. It adjusts to ambient humidity changes within minutes (Steiner & Koellner, 1994), and the observed equilibration rates imply that water molecules travel through the crystal lattice with a diffusion constant $D \simeq 3 \times 10^{-8}\ cm^2\ s^{-1}$ (compared with $2.2 \times 10^{-5}\ cm^2\ s^{-1}$ in pure water). In two subsequent experiments, isotopically pure water D_2O and $H_2{}^{18}O$ were diffused into the crystal lattice and the H_2O/D_2O and $H_2{}^{16}O/H_2{}^{18}O$ exchanges were monitored by Raman spectroscopy and mass spectrometry, respectively (Steiner, Koellner *et al.*, 1995); it occurs in the entire crystal volume on a timescale of minutes. It is remarkable that translational diffusion takes place in β-CD·$n H_2O$ although no permanent channels are present in the cage-type crystal lattice. This implies that

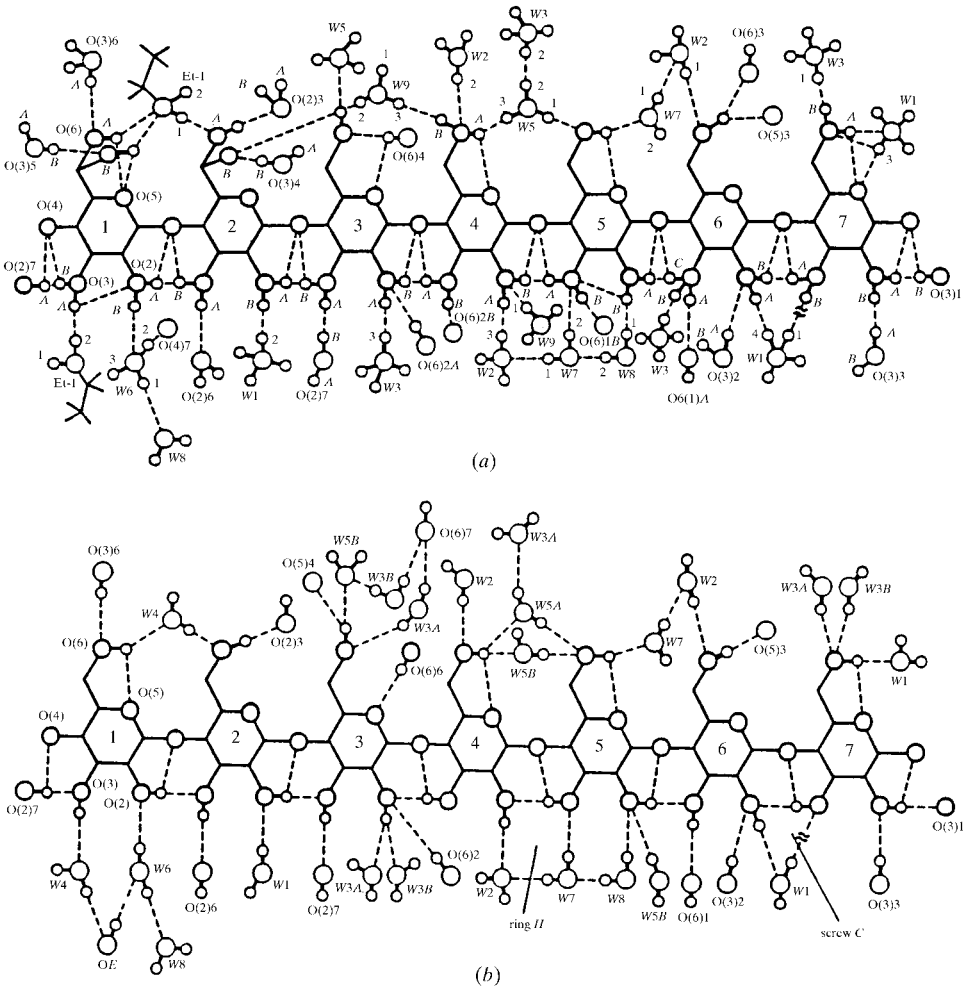

Fig. 5. Schematic representation of two- and three-center hydrogen bonding in neutron diffraction studies of β-CD·EtOD·8D_2O. (*a*) At 295 K, all of the O(2)···O(3′) hydrogen bonds are of the flip-flop type; the disordered half-occupied D sites indicated by *A* and *B* have three-center minor components to the adjacent O(4) atoms. (*b*) At 15 K, all flip-flop hydrogen bonds have disappeared to form cooperative homodromic cycles and chains (not shown). Note the occurrence of intraglucose O(6)—H···O(5) hydrogen bonds stabilizing the conformation about the C(5)—C(6) bond. Several disordered water sites at 295 K have disappeared at 15 K but *W*3, rotationally disordered at 295 K, is site-disordered at 15 K (*W*3*A*, *W*3*B*) (Steiner *et al.*, 1990; Steiner, Mason & Saenger, 1991).

suitable diffusion passages are temporarily opened by positional fluctuations of the CD atoms, a mechanism that is not yet understood in detail.

7. Permethylated cyclodextrins show extreme molecular distortions

If the O—H groups in the 2- and 6- or in the 2-, 3- and 6-positions of a CD are methylated, the structural and physical properties become very different from those of the native CDs. Most surprisingly, the temperature coefficients of the methylated CDs are negative, *i.e.* in cold water they are even more soluble than their native homologs and, at higher temperatures, they crystallize as mono-, di- or anhydrates. The crystal structures of the permethylated α-, β- and γ-CDs show that the cavities are no longer open but closed by 'inward' rotation of O(6)—CH_3 groups so that they are bowl shaped (Steiner & Saenger, 1996, 1998*b*). In permethyl-α-CD, this closure requires that two diametrically opposed glucoses are strongly tilted whereas, in permethyl-β-CD, one of the glucose residues is inverted from the normal $^4C_1$ to a $^1C_4$ chair conformation, which is unique for a CD (Caira *et al.*, 1994; Steiner & Saenger, 1998*b*). Permethyl-γ-CD adopts a highly distorted conformation where, at two diametrically opposed sites, adjacent glucoses are oriented *trans* and not *cis* as usually observed for CD macrocycles. This is analogous to the 'band-flip' motif found in the larger cycloamyloses, where it serves to relieve steric strain (Jacob *et al.*, 1998; Gessler *et al.*, 1998). Permethyl-γ-CD is the smallest CD for which this motif has been found, suggesting that O(2)···O(3′) hydrogen bonding is important for the stabilization of the 'round' form of the CD macrocycles. In fact, CDs with only the O—H groups in positions 2 and 6 methylated again adopt the 'round' form stabilized by O(3)—H···O(2′) hydrogen bonding.

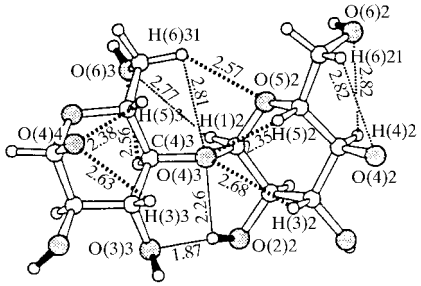

Fig. 6. Intramolecular stabilization of CDs by hydrogen bonds of type O—D···O (thin lines) and C—H···O (thick broken lines for H···O < 2.7 Å and dotted lines for 2.7 ≤ H···O < 3.0 Å). Distances are given in Å. Oxygen atoms are shown stippled, O—D bonds solid; O(4)3 is atom O(4) of glucose number 3 *etc*. The C—H···O interactions of O(4)3 with H(5)2 and H(3)2 are inherent to the $^4C_1$-chair form of glucose; they are not merely 'forced contacts' because related interactions of axial O substituents also occur in substituted cyclohexanes where they could be avoided by chair inversion (Steiner & Saenger, 1998*c*). Glucoses 2 and 3 in β-CD·EtOD·8D_2O are shown at 15 K (Steiner & Saenger, 1992*c*).

8. The band-flip motif releases steric strain in larger cycloamyloses

If the CD macrocycle is enlarged beyond γ-CD, steric strain builds up because the curvature associated with α(1–4) bonded glucoses in the $^4C_1$ chair form has to decrease (Jacob *et al.*, 1998). In δ-CD·15.75H_2O crystallizing in the typical herringbone-type cage form, all glucoses are still *cis* but the macrocycle is elliptically distorted in the form of a boat (Fujiwara *et al.*, 1990).

In the even larger cycloamyloses with ten and more glucoses, the additional strain is relieved by the novel 'band-flip' motif. The crystal structures of CA10·23.3H_2O, CA14·27.3H_2O and $(CA26)_2$·76.8H_2O (Ueda *et al.*, 1996; Jacob *et al.*, 1998; Gessler *et al.*, 1998) show that all glucoses are again *cis* except at two diametrically opposed sites in the macrocycles where adjacent glucoses are flipped ∼180° to be *trans*. This 'band-flip' can be visualized by cutting a band, rotating one end with respect to the other and gluing the two ends together. It is stabilized by a three-center hydrogen bond with O(3)—H as donor and the major component to O(6′) [in (+)-*gauche* orientation], the minor to O(5′). At the flip sites, the glucosidic torsion angles are $\varphi = 86$ to 90° and $\psi = -46$ to −52°.

The molecular shapes of CA10 and CA14 are comparable. The macrocycles are folded like butterflies, with the band-flips at the 'body' and the 'wings' shaped like segments of CDs. The cavities are narrow and it is still questionable whether these CA can form inclusion complexes. By contrast, the macrocycle of CA26 is composed of two halves connected by band-flips, each folded into two left-helical turns (as known for V-amylose) whose central channels are filled by disordered water molecules – analogous to the famous 'iodine's blue'.

9. Outlook

The CDs were discovered in 1903 by Schardinger and it is still not known for what purpose they are produced by enzymes found in a wide variety of plants and microorganisms. The inclusion properties of the CDs were first observed in the late 1940s and have found wide applications in the pharmaceutical and chemical industries during the past 20 years. Besides the 'native' CDs described in this paper, there are a large number of enzymatically and chemically modified CDs, in which all or only some of the hydroxyl groups have been modified or replaced by other non-functional or functional groups. Of special interest are those modifications of CDs where functional groups are introduced that provide novel features like sensors or artificial enzymes.

Because of their unique properties and easy availability, CD molecules constitute some of the best known 'supramolecular compounds' and will continue to be of interest in the future. The now available larger cycloamyloses (CA) open new horizons. Whereas the smaller CA with 10 and 14 glucoses probably do not form inclusion complexes, the larger ones like CA26 are well suited as host molecules. We can look forward to many new studies utilizing these molecules once they are available on a large preparative scale.

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