Novel Type of Thermostable Channel Clathrate Hydrate Formed by Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin · 15 H_2O—A Paradigm of the Hydrophobic Effect**

Thammarat Aree, Helga Hoier, Burkhard Schulz, Günter Reck, and Wolfram Saenger*

The α-, β-, γ-cyclodextrins (CDs) are macrocycles composed of six, seven, or eight glucose units linked by $\alpha(1\rightarrow4)$ glycosidic bonds.[1] They adopt the shapes of hollow, truncated cones with both rims covered by OH groups: O6-H at the narrower side and O2-H and O3-H at the wider side (Figure 1; for atom-numbering scheme see Figure 2a). Since all glucose units are oriented *syn*, O2-H and O3-H of adjacent glucose units may form intramolecular O2 ··· O3′ hydrogen bonds which contribute to the conformational stability of the CD macrocycles. If dissolved in water, the CDs show a *positive* solubility coefficient as they are more soluble in hot than in cold water and they crystallize as high hydrates with water molecules located inside/outside the central cavities in ratios 2/4 in α-CD · 6 H_2O,[2] 6.5/5.5 in β-CD · 12 H_2O,[3] and 12/5 in γ-CD · 17 H_2O.[4] In these hydrates, CD hydroxy groups and water molecules form irregular cooperative O–H ··· O hydrogen-bonded networks.[5]

The solubility of the CDs in water is reversed when their glucose units are methylated at the 2- and 6- or the 2-, 3-, and 6-hydroxy groups. (In the following, the methylated CDs are denoted as DIMEA for hexakis(2,6-di-*O*-methyl)-α-CD; TRIMEG for octakis(2,3,6-tri-*O*-methyl)-γ-CD etc.) The solubility coefficients are now *negative*, the methylated CDs being more soluble in cold water (0.2 – 0.6 g mL^{-1}) than in hot water (< 0.05 g mL^{-1})[6] where they crystallize (at 50 – 70 °C) as anhydrous DIMEA,[7] anhydrous DIMEB,[8] anhydrous TRIMEA,[9] TRIMEB · H_2O,[10] and TRIMEG · 2 H_2O.[11] The crystal structures show common features: a) two or three of the O6-CH_3 groups are rotated "inward" to close the cavity from the O6 side and reduce its volume; b) the cavity is either empty or filled by the O6-CH_3 group of an adjacent molecule ("self-inclusion"); c) none, one, or two water of hydration molecules are located in voids between the methylated CDs.

By contrast, TRIMEG and DIMEB crystallize from cold water (18 °C) in heavily hydrated forms, (4TRIMEG) · 19.3 H_2O[12] and DIMEB · 15 H_2O (this work); in one case, DIMEB · 2 H_2O was crystallized,[13] but this could not be reproduced. Since it was of interest to determine the hydration pattern of DIMEB · 15 H_2O and to compare it to (4TRIMEG) · 19.3 H_2O;[12] the X-ray structure analysis described below was carried out.

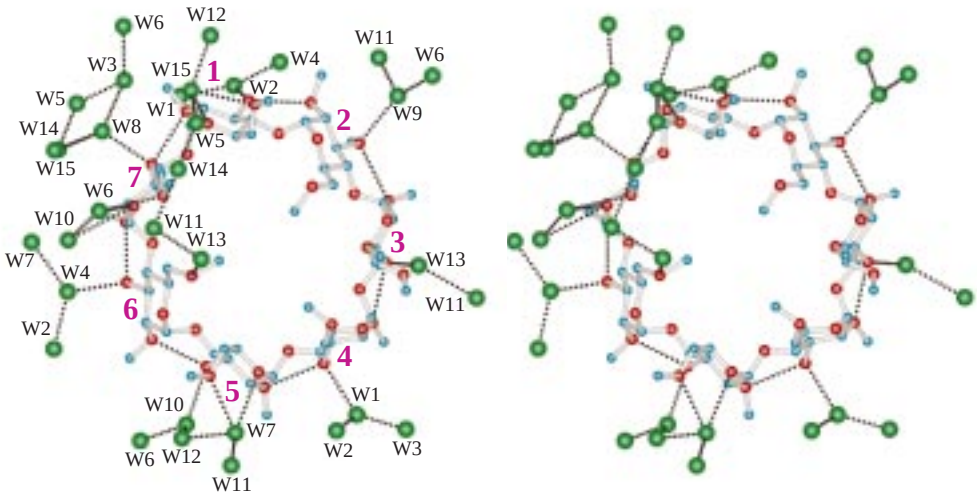

Figure 1. Stereodiagram showing the molecular structure of DIMEB and its hydrogen bonding to water molecules in DIMEB · 15 H_2O. Carbon, oxygen, and water oxygen atoms as cyan, red, and green spheres, respectively; the seven glucose residues are labeled with red numbers, for atom-numbering see Figure 2a. Dotted lines indicate O–H ··· O hydrogen bonds.

In crystalline DIMEB · 15 H_2O,(see Experimental Section), all glucose units of DIMEB adopt the common $^4C_1$ chair conformation and are oriented *syn*; they are stabilized in this conformation by systematic interglucose O3–H ··· O2′ hydrogen bonds with O ··· O distances in the range 2.81(1) – 3.04(1) Å (Figures 1 and 2a). All O2-CH_3 groups point "away" from the cavity, and the O6-CH_3 groups of glucose units 2, 4, and 6 are rotated "toward" the cavity and close it from this side (Figure 1). The cavity is not filled by water but by the O6-CH_3 group of an adjacent DIMEB molecule related by the twofold screw axis parallel to *a*, giving rise to infinite stacks of DIMEB molecules.

The hydration of these stacks by the 15 H_2O molecules is remarkable. The O3-H groups of DIMEB form systematic hydrogen bonds to seven water molecules with O ··· O distances between 2.81(1) and 2.89(1) Å (Figures 1 and 2a). Together with eight additional water molecules, a hydrogen-bonded, regular network of water molecules is constructed that encapsulates the DIMEB molecules in the form categorized as clathrate hydrate channel structure.[14] The network is

[*] Prof. Dr. W. Saenger, Dipl.-Chem. T. Aree, Dr. H. Hoier
Institut für Kristallographie der Freien Universität
Takustrasse 6, 14195 Berlin (Germany)
Fax: (+49) 30-838-6702
E-mail: saenger@chemie.fu-berlin.de
B. Schulz, Dr. G. Reck
Bundesanstalt für Materialforschung und -prüfung
Rudower Chaussee 5, 12489 Berlin (Germany)

[**] Topography of Cyclodextrin Inclusion Complexes, Part 47. This work was financed by a scholarship under the Development and Promotion of Science and Technology Talents Project (DPST) by the Royal Thai Government Agencies and the Institute for the Promotion of Teaching Science and Technology (IPST) to T. Aree, by the Deutsche Forschungsgemeinschaft, and by Fonds der Chemischen Industrie. We are grateful to Prof. Dr. H. Hartl for the DSC measurements. For Part 46, see T. Aree, H. Hoier, B. Schulz, G. Reck, W. Saenger, *Carbohydr. Res.* **1999**, *320*, 120 – 128.

  0570-0833/00/3905-0897 $ 17.50+.50/0

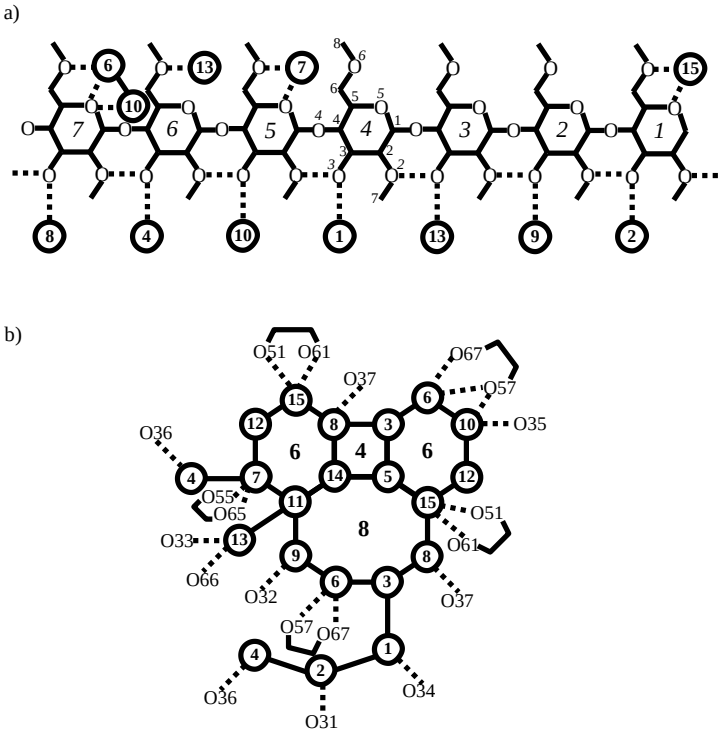

Figure 2. Schematic presentation of a) the hydration of DIMEB, glucose units are numbered sequentially *1* to *7*, atom-numbering scheme is given for glucose unit *4*; methyl-C atoms attached to O2 and O6 are assigned as C7 and C8, respectively. Strong hydrogen bond donors/acceptors O3-H are systematically hydrated, the weak O5, O6 acceptors only partially (glucose units *1*, *5*, *6*, and *7*), and O2 and O4 never. b) The water network showing edge-sharing polygons with one tetra-, two hexa-, and one octagon ($4^1 6^2 8^1$) described by **4**, **6**, **8**; water molecules are denoted as circled numbers; dashed and solid lines indicate hydrogen bonds $O_w \cdots O_{DIMEB}$ and $O_w \cdots O_w$, respectively. The chain W1-W2-W4 and the individual water molecule W13 are not involved in the formation of these polygons.

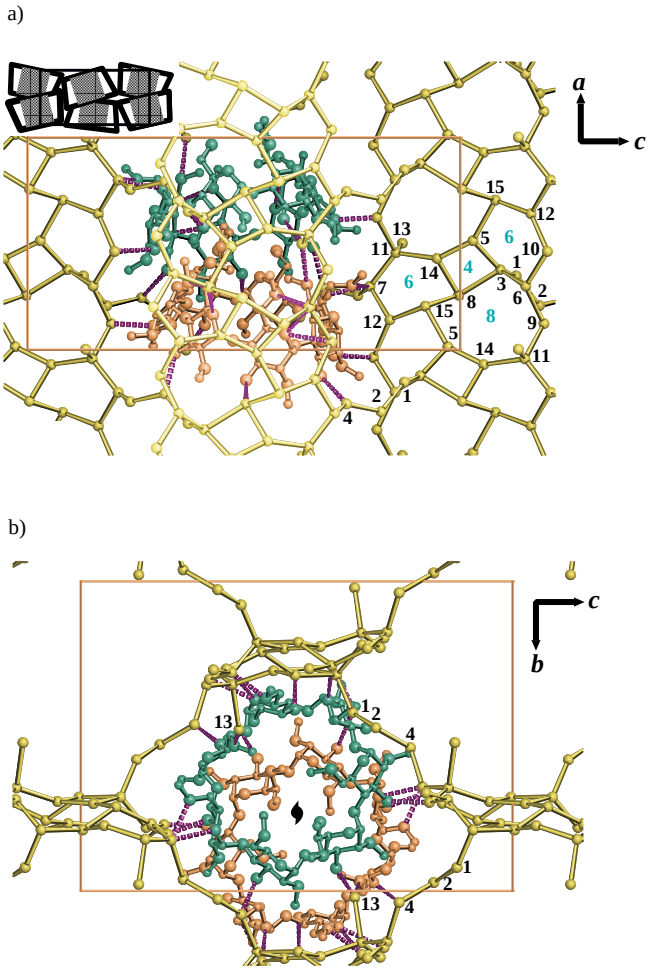

Figure 3. Clathrate hydrate channel structure in the crystal lattice of DIMEB · 15 H_2O in views on *ac* (a) and *bc* planes (b). Two DIMEB molecules related by the twofold screw operation (◆) parallel to *a* are shown in green and orange ball-and-stick representations; larger spheres are oxygen and smaller spheres, carbon atoms. Water molecules (labeled with black numbers) and hydrogen bonds between water molecules are shown as yellow balls and sticks, respectively. Broken pink sticks indicate hydrogen bonds between oxygen atoms of DIMEB and water molecules. The repeating unit ($4^1 6^2 8^1$) of the network formed from water molecules is indicated by cyan numbers ($O_w \cdots O_w$ distances 2.75(1) – 3.26(2) Å). This motif is extended by the 2_1 operation parallel to *a* forming a "ribbon" in the *ac* plane (ribbons closer to the viewer (bright yellow) and further away (dark yellow) (a)). Each "ribbon" is connected by water chains W1-W2-W4 to other ribbons symmetry related by the operation of twofold screw axes parallel to *c*, giving rise to clathrate hydrate channels which accommodate the stacks of DIMEB (b). The inset in a) shows a schematic presentation of crystal packing (water molecules omitted).

formed by a repeating motif described as $4^1 6^2 8^1$ (Figure 2b) which by the operation of the twofold screw axes (Figures 3a and 3b) yields corrugated sheets parallel to the *ac* plane; they are linked by chains of water molecules W1-W2-W4 into channels that harbor the stacks formed by DIMEB molecules.

The clathrate hydrate channel structure of DIMEB · 15 H_2O is highly specific as no other methylated CD has the same constellation of O3-H groups that is required to anchor and stabilize the network of 15 water molecules. This clathrate hydrate is unique in four respects: a) there are no water pentagons that usually dominate clathrate and semiclathrate structures;[14] b) in contrast to all other clathrate hydrates where either no or only few hydrogen bonds are formed between guest and clathrate water molecules, DIMEB · 15H_2O abounds with such interactions (see Figures 2a, 2b, and the broken pink sticks in Figures 3a, 3b). Of the 15 H_2O, only five are *not* hydrogen-bonded to DIMEB, the other ten being in contact with the seven O3-H groups and with the O5 and O6 atoms, (see Figures 1, 2a, and 2b); c) a total of 15 hydrogen bonds anchors DIMEB tightly and specifically to the clathrate hydrate network and gives rise to high thermal stability: upon heating, a crystal-to-crystal phase transition occurs at 110 °C with changes of unit cell constants *b* and *c* of −17.0 % and 30.7 %, respectively, and increase of unit cell volume by 8.5 %, indicating structural changes; at 280 °C the X-ray diffraction pattern breaks down because crystals decompose (see Experimental Section)—by contrast, the most stable known clathrates (of the alkylammonium type) melt in the range −20 to 30 °C;[14] d) DIMEB is the largest guest found thus far hosted by a clathrate hydrate.

Compared with DIMEB, the fully methylated TRIMEG has lost the strong hydrogen bonding donor/acceptor functionality of the O3-H groups; they are replaced by the weak O3-CH_3 acceptors so that no intramolecular O3–H ··· O2′ hydrogen bonds can form. This explains why in (4TRIMEG) ·

  0570-0833/00/3905-0898 $ 17.50+.50/0

19.3 H_2O crystallized from cold water[12] each of the four independent TRIMEG molecules shows two diametrically opposed glucose units flipped from the common *syn* to *anti* orientation; their O6 atoms are now close enough to permit hydrogen bonding to a bridging water molecule. Additional water molecules are located inside and outside the cavities, hydrating the four TRIMEG individually: three to five $O_{TRIMEG} \cdots O_w$ hydrogen bonds connect TRIMEG to water clusters of different sizes and shapes.

It is conceivable that the structures of DIMEB · 15 H_2O and (4TRIMEG) · 19.3 H_2O represent snapshots of hydrated DIMEB and TRIMEG in cold aqueous solutions, similar to those found for trimethylamine decahydrate in crystalline and liquid states.[15] This would correspond to Pauling's clathrate hydrate model of liquid water[16] and to Jeffrey's "liquid analogy" to clathrate hydration.[17] If aqueous solutions of DIMEB or TRIMEG are heated, we envisage that hydration water molecules become more mobile and the macrocycles more flexible so that the hydration networks break down, leading to aggregation and crystallization of DIMEB anhydrate[8] and of TRIMEG · 2 H_2O,[11] the two water molecules being accommodated in intermolecular voids. As this particular behavior can be attributed, in essence, to the hydrophobic effect,[18] the methylated CDs may serve as well-defined, easily obtainable objects to study it in more depth.

Experimental Section

DIMEB (Cyclolab/Budapest) crystallized from aqueous solution (0.3 g mL^{-1}) at 18 °C as pentadecahydrate ($C_{56.25}H_{97.75}O_{35} \cdot 15\,H_2O$), orthorhombic space group $P2_12_12_1$, $a = 14.1632(5)$, $b = 20.8278(7)$, $c = 29.2611(10)$ Å, $M_r = 1604.35$, $V = 8{,}631.7(5)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.214$ g cm^{-3}. A crystal of $0.4 \times 0.5 \times 2.0$ mm^3 was sealed in a quartz capillary, and 37 113 X-ray reflections were collected at room temperature to 0.9 Å resolution on a Bruker-AXS CCD area detector using graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å); semiempirical absorption correction from ψ scans ($\mu = 0.11$ mm^{-1}) and data reduction (with programs SAINT[19] and SHELXTL[20]) yielded 6760 unique reflections. The structure was determined by direct methods (SHELXS-97[21]) and refined by full-matrix least-squares on F^2 (SHELXL-97[22]). All non-hydrogen atoms were treated anisotropically. Most of hydrogen atoms of the β-CD skeleton and some of the water molecules could be located and were refined isotropically—positions of the methyl and hydroxy hydrogen atoms were calculated according to the "riding model";[22] at glucose unit 2, a methyl group was located with an occupancy factor of 0.25 (due to impurity as verified by mass spectrometry, not shown). All 15 water sites were found fully occupied. The refinement of 1110 parameters converged at a final $R = 0.069$, $wR = 0.177$ for 5725 data with $F^2 > 2\sigma(F^2)$,[23] the highest peak and deepest hole are 0.32 and -0.23 e Å^{-3} in the final difference electron density.

For thermal analysis, a DIMEB · 15 H_2O crystal was mounted in an Enraf-Nonius heating device installed on a Mar Research image plate. The same diffraction pattern with 5° oscillation range was taken at 10 °C intervals from 20 to 310 °C using $Cu_{K\alpha}$ radiation from an Enraf Nonius FR571 X-ray generator with rotating anode. Unit cell constants evaluated with DENZO[24] showed a phase transition at 110 °C (changes of unit cell parameters b and c to 17.284 and 38.243 Å), and decomposition (loss of X-ray diffraction) above 280 °C. For differential scanning calorimetry (DSC), powdered DIMEB · 15 H_2O (10 mg) was enclosed in an aluminum pan and heated in a Netsch Instruments apparatus from -100 to 350 °C. Phase transition occurred at 111 °C and decomposition above 280 °C. Figure 1 was drawn with MOLSCRIPT[25] and rendered with RASTER3D,[26] Figures 3a and 3b with SCHAKAL88.[27]

Received: September 13, 1999 [Z14005]

[1] W. Saenger, *Angew. Chem.* **1980**, *92*, 343 – 361; *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 344 – 362; W. Saenger in *Inclusion Compounds*, *Vol. 2* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol), Academic Press, London, **1984**, pp. 231 – 259.

[2] P. C. Manor, W. Saenger, *Nature* **1972**, *237*, 392 – 393.

[3] K. Lindner, W. Saenger, *Carbohydr. Res.* **1982**, *99*, 103 – 115.

[4] J. M. MacLennan, J. J. Stezowski, *Biochem. Biophys. Res. Commun.* **1980**, *92*, 926 – 932.

[5] a) G. A. Jeffrey, W. Saenger, *Hydrogen Bonding in Biological Structures*, Springer, Berlin, **1991**, pp. 309 – 350; b) W. Saenger, J. Jacob, K. Gessler, T. Steiner, D. Hoffmann, H. Sanbe, K. Koizumi, S. M. Smith, T. Takaha, *Chem. Rev.* **1988**, *98*, 1787 – 1802.

[6] K. Uekama, T. Irie in *Cyclodextrins and Their Industrial Uses* (Ed.: D. Duchêne), Editions de Santé, Paris, **1987**, pp. 393 – 439.

[7] a) K. Harata, *Supramol. Chem.* **1995**, *5*, 231 – 236; b) T. Steiner, K. Hirayama, W. Saenger, *Carbohydr. Res.* **1996**, *296*, 69 – 82.

[8] T. Steiner, W. Saenger, *Carbohydr. Res.* **1995**, *275*, 73 – 82.

[9] T. Steiner, W. Saenger, *Carbohydr. Res.* **1996**, *282*, 53 – 63.

[10] M. R. Caira, V. J. Griffith, L. R. Nassimbeni, B. van Oudtshoorn, *J. Chem. Soc. Perkin Trans. 2* **1994**, 2071 – 2072.

[11] T. Steiner, W. Saenger, *Angew. Chem.* **1998**, *110*, 3628 – 3632; *Angew. Chem. Int. Ed.* **1998**, *37*, 3404 – 3407.

[12] T. Aree, I. Usón, B. Schulz, G. Reck, H. Hoier, G. M. Sheldrick, W. Saenger, *J. Am. Chem. Soc.* **1999**, *121*, 3321 – 3327.

[13] T. Aree, W. Saenger, P. Leibnitz, H. Hoier, *Carbohydr. Res.* **1999**, *315*, 199 – 205.

[14] G. A. Jeffrey in *Comprehensive Supramolecular Chemistry*, *Vol. 6* (Eds.: J. L. Atwood, J. E. D. Davies, D. D., MacNicol, F. Vögtle), Pergamon, Oxford, **1996**, pp. 757 – 788.

[15] C. Folzer, R. W. Hendricks, A. H. Narten, *J. Chem. Phys.* **1971**, *54*, 799 – 805.

[16] L. Pauling in *Hydrogen Bonding* (Ed.: D. Hadzi), Pergamon Press, New York, **1959**, pp. 1 – 6.

[17] G. A. Jeffrey, *Acc. Chem. Res.* **1969**, *2*, 344 – 352.

[18] C. Tanford, *Protein Sci.* **1997**, *6*, 1358 – 1366.

[19] Siemens, SAINT, Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA, **1996**.

[20] Siemens, SHELXTL, Version 5.0, Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA, **1996**.

[21] G. M. Sheldrick, SHELXS-97, University of Göttingen, Germany, **1997**.

[22] G. M. Sheldrick, T. R. Schneider in *Methods in Enzymology*, *Vol. 277* (Eds.: C. W. Carter, Jr., R. M. Sweet), Academic Press, San Diego, **1997**, pp. 319 – 343.

[23] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-118694. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[24] Z. Otwinowski, W. Minor in *Methods in Enzymology*, *Vol. 276* (Eds.: C. W. Carter, Jr., R. M. Sweet), Academic Press, San Diego, **1997**, pp. 307 – 326.

[25] P. J. Kraulis, *J. Appl. Crystallogr.* **1991**, *24*, 946 – 950.

[26] D. J. Bacon, W. F. Anderson, *J. Mol. Graphics* **1988**, *6*, 219 – 220; E. A. Merritt, M. E. P. Murphy, *Acta Crystallogr. Sect. D* **1994**, *50*, 869 – 873.

[27] E. Keller, *J. Appl. Crystallogr.* **1989**, *22*, 19 – 22.