JOC Article

Structure and Thermodynamics of α-, *β*-, and *γ*-Cyclodextrin Dimers. Molecular Dynamics Studies of the Solvent Effect and Free Binding Energies[†]

Pascal Bonnet,[‡,§] Carlos Jaime,*[,‡] and Luc Morin-Allory[§]

Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain, and Institut de Chimie Organique et Analytique, UPRES A CNRS 6005, BP 6759, 45067 Orléans, France

carlos.jaime@uab.es

Received July 9, 2002

The α-, *β*-, and *γ*-cyclodextrin (CyDs) dimers were studied by molecular dynamics (MD) simulations in water as an explicit solvent. The relative stability of dimers and the involved molecular interactions were determined. Three possible starting orientations were considered for the dimers: head-to-head, head-to-tail, and tail-to-tail. MD simulations were performed over a period of 5 ns to ensure the stability of the system for both the CyD dimers and monomers. The MM-PBSA methodology was used to obtain the free binding energy of the dimers and to determine the most stable arrangement for each solvated CyD. In a vacuum, MD simulations provided the head-to-head orientation as the most stable orientation for the three CyDs, while in aqueous solution the, the head-to-tail orientation was found to be the most stable for the α-CyD dimer and the tail-to-tail orientation the most stable for the *β*- and *γ*-CyD dimers.

Introduction

Cyclodextrins (CyDs)[1] are interesting natural macromolecules that are cyclic oligomers of glucose. The most common CyDs (native) are composed of 6, 7, or 8 glucose residues linked by α-(1,4) glycosidic bonds,[2,3] and they are called α-, *β*-, and *γ*-CyD, respectively. These CyDs generate a toroidal/hollow truncated cone structure[4] due to the $^4C_1$ chair conformation of the sugar units. Their derivatives have been widely studied[5,6] because of their use as hosts in molecular recognition.[7] The stoichiometries for most of the CyD/substrate inclusion complexes are 1:1[8,9] or 2:1,[10−13] although some cases of 2:2 stoichiometries have also been detected.[14] The inclusion compounds composition critically depends on the types of CyD and on the physicochemical properties of the involved guest. Many studies have investigated either isolated CyDs or their 1:1 inclusion complexes by computational techniques.[15,16]

The formation of 2:1 or 2:2 complexes requires the association of two CyD units. One of the arrangements of the CyD molecules within the crystal lattice is the channel structure.[17] These alignments are defined as "head-to-head" or "head-to-tail" depending on whether two equal or two different rims are facing each other. The head-to-head and head-to-tail dimers of *β*-CyD have recently been studied by ab initio and semiempirical calculations (MNDO/PM3).[18] The results from this study indicate that the head-to-tail dimer is the thermodynamically most stable dimeric arrangement and that the hydrogen bonds between glucose units are formed between the hydrogen of the 2-OH of one glucose and the oxygen of the 3-OH of the next glucose. Molecular dynamics (MD) simulations in a vacuum have previously been used to study the formation of aggregates formed by two CyDs (hereinafter called dimers).[19] The three possible dimers were studied (Figure 1): the two possible head-to-head (that formed by facing the two wider rims,

---

[†] Dedicated to the memory of Peter A. Kollman.

[‡] Universitat Autònoma de Barcelona.

[§] Institut de Chimie Organique et Analytique.

(1) Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743.

(2) Freudenberg, K.; Meyer-Delius, M. *Ber. Dtsch. Chem. Ges.* **1938**, *71*, 1596.

(3) Freudenberg, K.; Plankenhorn, E.; Knauber, H. *Chem. Ind.* **1947**, 731.

(4) Saenger, W. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 344.

(5) A good example of its importance is given in the special issue from *Chem. Rev.* **1998**, *98*, all pages.

(6) Larsen, K. L.; Ueda, H.; Zimmermann W. 8th European Congress on Biotechnology, Budapest, August 17−21, 1997.

(7) Schlenk, W. *Fortschr. Chem. Forsch.* **1951**, *2*, 92.

(8) VanEtten, R. L.; Sebastian, J. F.; Glowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242.

(9) Tabushi, I.; Yamamura, K.; Fujita, K.; Kawakubo, H. *J. Am. Chem. Soc.* **1979**, *101*, 1019.

(10) Cramer, F.; Saenger, W.; Spatz, H. Ch. *J. Am. Chem. Soc.* **1967**, *89*, 14.

(11) Kondo, H.; Nakatani, H.; Hiromi, K. *J. Biochem*. **1976**, *79*, 393.

(12) Ueno, A.; Yoshimura, H.; Saka, R.; Osa, T. *J. Am. Chem. Soc.* **1979**, *101*, 2779.

(13) Odagaki, Y.; Hirotsu, K.; Higuchi, T.; Harada, A.; Takahashi, S. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1230.

(14) (a) Hamai, S. *Recent Res. Devel. Photochem. Photobiol.* **1999**, *3*, 1. (b) Hamai, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2469. (c) Hamai, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 543.

(15) Lipkowitz, K. B. *Chem. Rev.* **1998**, *98*, 1829.

(16) Beà, I.; Jaime, C.; Redondo, J.; Bonnet, P.; Torrens, A.; Frigola, J. *Supram. Chem.* **2002**, *14*, 33.

(17) (a) Szejtli, J. *Cyclodextrin Technology*; Kluwer Academic Publishers: Dordrecht, 1988; pp 6−7. (b) Saenger, W. *Inclusion Compounds II*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Academic Press: London, 1980; p 231.

(18) Avakyan, V. G.; Nazarov, V. B.; Alfimov, M. V.; Bagatur'yants, A. A.; Voronezheva, N. I. *Russian Chem. Bull., Int. Ed.* **2001**, *50*, 206.

(19) Bonnet, P.; Jaime, C.; Morin-Allory, L. *J. Org. Chem.* **2001**, *66*, 689.

10.1021/jo026166v CCC: $22.00

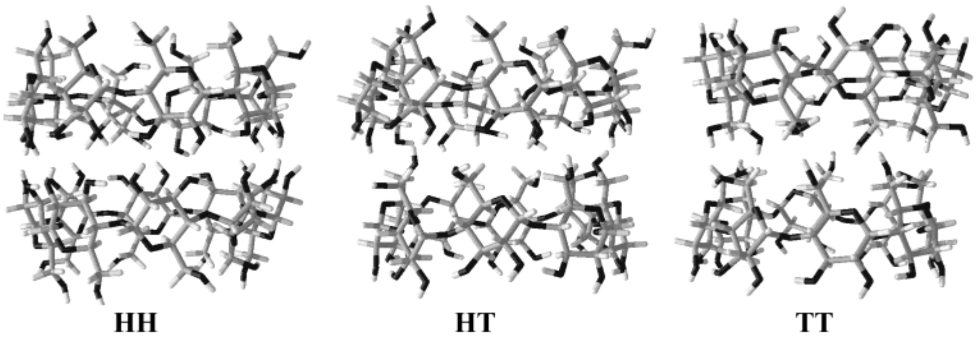

FIGURE 1. Three starting orientations for the γ-CD dimer as an example: head-to-head (HH), head-to-tail (HT), and tail-to-tail (TT).

the head-to-head, and the one facing the two narrower rims, the tail-to-tail) and the head-to-tail. The results showed that head-to-head was the most stable for all α-, β-, and γ-CyD dimers due to the formation of intermolecular hydrogen bonds. A study on the polyrotaxane formed between poly(ethyleneglycol) and 12 α-CyDs indicates that the hydrogen bond formation between CyD units suggest that they assemble in a head-to-head or tail-to-tail sequence.[20] The formation of β-CyD aggregates in aqueous solution has been experimentally detected,[21] but the driving force for this aggregation has never been systematically studied[22] although some studies on 2:1 complexes have been done in very limited cases.[23] Finally, the γ-CyD/(*E*)-stilbene complex crystallizes in a channel-type lattice presenting two distinct inclusion sites, one where two stilbenes are close in space and another where only one stilbene can be accommodated.[24] This can quickly be associated with the head-to-head and head-to-tail orientations for the γ-CyDs.

Most of the experimental works with CyDs are carried out in water. However, it is clear that the effect of water on the interaction between two CyDs might be very important. If hydrogen bonds between the hydroxyl groups of CyDs are the major driving force for holding the two units together, their interaction with the water molecules will compete with the intermolecular CyD−CyD hydrogen bonds, and the stability of the dimers will be reduced.

In this study, the three possible orientations between CyD units were studied by molecular dynamics (MD) simulations: the head-to-head (HH), the head-to-tail (HT), and the tail-to-tail (TT) (Figure 1). It will be shown that the hydrogen bonds between the two CyD units forming the dimer and between each CyD and the solvent are the main driving force for the dimer formation. The results from this work indicate that the most stable orientations are the head-to-tail for the α-CyD dimer and the tail-to-tail for both the β- and γ-CyD dimers.

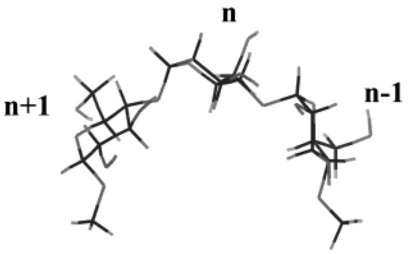

FIGURE 2. One of the possible CyD fragments formed by three glucose units. Only charges of the glucose “n” are kept for the RESP program.

Methods

Atomic Charges. Necessary atomic charges for glucose atoms were obtained by considering the eight lowest energy conformations of a fragment formed by three glucose units (Figure 2) containing methyl groups at each end. These conformers were fully optimized using the Gaussian 94 program[25] at the Hartree−Fock level with the STO-3G basis set. Single-point calculations on each optimized conformer were performed at the RHF/6-31G* level. The eight resulting electrostatic potentials were thus used for the two-stage multiple-conformation RESP[26,27] charge fitting, giving rise to a single set of charges for glucose atoms (Figure 3). The charges obtained for the “n” glucose unit were used to build the CyDs in the MD simulations because they collect the effect of other adjacent glucose units. Parameters complementary to those already described in the parm94[28] force field were added for the glycosidic linkage.[29]

Molecular Dynamics Simulations. Productive MD simulations were run with a 2.0 fs time step under the isothermal−isobaric ensemble (298 K and 1 atm.). The all-atom force field of the AMBER v.5 package,[30] explicit solvation for water

---

(20) Harada, A.; Li, J.; Kamachi, M. *Nature* **1992**, 356, 325.

(21) (a) Coleman, A. W.; Nicolis, I.; Keller, N.; Dalbiez, J. P. *J. Incl. Phenom.* **1992**, *13*, 139. (b) Miyajima, K.; Saweda, M.; Nagakari, M. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3556.

(22) Very recently, a MM study was published for systems with stoichiometries different than 1:1. Pozuelo, J.; Nakamura, A.; Mendicuti, F. *J. Incl. Phenom.* **1999**, *35*, 467.

(23) (a) Ivanov, P.; Salvatierra, D.; Jaime, C. *J. Org. Chem.* **1996**, *61*, 7012. (b) Ivanov, P.; Jaime, C. *J. Mol. Struct.* **1996**, *377*, 137. (c) Dodziuk, H.; Ejchart, A.; Lukin, O.; Vysotsky, M. O *J. Org. Chem.* **1999**, *64*, 1503.

(24) Rao, K. S. S. P.; Hubig, S. M.; Moorthy, J. N.; Kochi, J. K. *J. Org. Chem.* **1999**, *64*, 8098.

(25) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, revision A.1; Gaussian, Inc.: Pittsburgh, PA, 1995.

(26) Bayly, C. I.; Cieplak, P.; Cornell, W. D.; Kollman P. A. *J. Phys. Chem.* **1993,** *97*, 10269.

(27) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Kollman, P. A. *J. Am. Chem. Soc.* **1993**, *115*, 9620.

(28) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M., Jr.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.

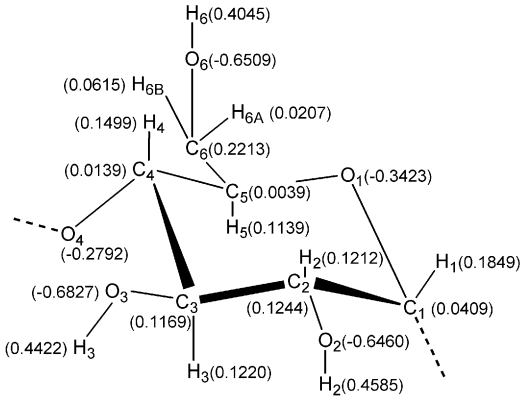

FIGURE 3. Chemical structure, atomic numbering, and charges used for each glucose unit in the MD simulations.

(TIP3P[31] model), and periodic boundary conditions were used.[32] Two cutoffs were used for evaluating the Lennard−Jones interactions (8 and 13 Å for the primary and secondary, respectively). The SHAKE[33] algorithm was applied over all covalent bonds involving hydrogen atoms. For all the studied systems, the energy was initially minimized and the temperature was increased to 300 K at three 50 ps intervals. Once the systems were equilibrated, 5 ns data collection runs were performed. Trajectories were saved every 5 ps (1000 snapshots were collected).

Calculation of Binding Energies. The MM-PBSA methodology[34] was applied to estimate the free energies of binding from the absolute energies in the gas phase (E_{gas}), and the solvation free energies ($\Delta G_{PB} + \Delta G_{nonpolar}$) for the CyD dimers and CyD monomers. This procedure can be summarized as follows:

$$\Delta G_{binding} = \Delta G_{water}(\text{dimer}) - 2^* [\Delta G_{water}(\text{monomer})]$$

The free energies, ΔG_{water}, for each species were evaluated by the following scheme:

$$\Delta G_{water} = E_{gas} + \Delta G_{solvation} - T\Delta S$$

$$\Delta G_{solvation} = \Delta G_{PB} + \Delta G_{nonpolar}$$

$$E_{gas} = E_{internal}\,(\text{bond, angle, torsion}) + E_{electrostatic} + E_{vdW}$$

The term E_{gas} was calculated with the CARNAL and ANAL modules in the AMBER 5.0 software suite. The solvation energy, $\Delta G_{solvation}$, was divided into two terms, the electrostatic contributions, ΔG_{PB}, and all other contributions, $\Delta G_{nonpolar}$. The ΔG_{PB} term was calculated using the Delphi software package,[35] which numerically solves Poisson−Boltzmann equations and calculates the electrostatic energy according to the electrostatic potential. The $\Delta G_{nonpolar}$ was calculated using the MSMS program,[36] which calculates the solvent accessible surface area (SASA), and the nonpolar contribution was calculated as 0.00542 * SASA + 0.92 kcal/mol. Solute entropies, $T\Delta S$, were estimated from normal mode calculations[37] using the NMODE module of AMBER. The rotational, translational, and vibrational entropies were calculated on a Newton−Raphson minimization with a distance-dependent dielectric in the abscence of solvent.

Results and Discussion

Average Structures and Hydrogen Bond Analysis. CyD dimers were built using the available crystallographic data[38,39,40] for each monomer as the starting structure. The three relative orientations in Figure 1 were chosen as starting structures. For each orientation, a distance of 8 Å between the molecular centroids (calculated with respect to all the glycosidic oxygen atoms) was initially set. The relative orientation of the CyD dimers changed along the 5 ns simulation runs. The arrangement of the monomers in the dimer can be referred to as perpendicular, parallel, or V geometries, and they are a consequence of the formation of intermolecular hydrogen bonds between solvent and solute.

Figures 4−6 contain the final structures obtained in these long simulations for the α-, β-, and γ-CyD dimers, respectively. These final structures were used as references and compared with the snapshots of all the trajectories. The root-mean-square deviations (rmsd) vary from 0.4 to 0.8 Å. Values were very small for such large molecules (those for the HH, HT, and TT orientations are as follows: 0.58, 0.58, and 0.74 for α; 0.57, 0.67, and 0.81 for β; and 0.45, 0.57, and 0.58 for γ, respectively), indicating that the final structures can be considered excellent representatives of the average structures along the simulation time.

Relative orientations for α- and β-CyD are not stable during the MD simulations. Only the HH orientation clearly keeps the relative position of the two CyD units, while HT and TT present changes (see Figures 4a and 5a). The HT average structures for the α- and β-CyD dimers adopt a perpendicular arrangement, while the TT average structures are adopting a V form (that for α-CyD being more open than that for β-CyD). The HH average structure for the β-CyD dimer also tends to adopt a V form. Contrary to all this, the relative orientations for the γ-CyD dimers seem to be more stable (Figure 6). Their starting orientations do not present substantial changes during the simulation runs, although the CyDs are bent to present more close contacts. The number of hydroxyl groups present in the γ-CyD dimers is larger than that for the two other CyDs. More intermolecular CyD−CyD hydrogen bonds can be formed in the case of γ-CyD, and thus the starting geometry can be more easily

---

(29) (a) Simmerling, C.; Fox, T.; Kollman, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 5771. (b) Woods, R. J.; Dwek, R. A.; Edge, C. J.; Fraser-Reid, B. *J. Phys. Chem.* **1995**, *99*, 3832.

(30) Case, D. A.; Pearlman, D. A.; Caldwell, J. W.; Cheatham, T. E., III; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Merz, K. M.; Stanton, R. V.; Cheng, A. L.; Vincent, J. J.; Crowley, M.; Ferguson, D. M.; Radmer, R. J.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. *AMBER 5*; University of California−San Francisco: San Francisco, 1997.

(31) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926.

(32) Water boxes contained different numbers of water molecules depending on the orientation and CyD. The number of water molecules used will be given in the HH, HT, and TT order and were as follows: for the α-CyD = 906, 929, and 901; for the β-CyD = 1009, 1116, and 1141; and for the γ-CyD = 1233, 1319, and 1314.

(33) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327.

(34) Srinivasan, J.; Cheatham, T. E.; Cieplak, P.; Kollman, P. A.; Case, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 9401.

(35) Gilson, M. K.; Sharp, K. A.; Honig, B. H. *J. Comput. Chem.* **1987**, *9*, 327.

(36) Sanner, M. F.; Olson, A. J.; Spehner, J. C. *Biopolymers* **1996**, *38*, 305.

(37) Srinivasan, J.; Miller, J.; Kollman, P. A.; Case, D. A. **1998**, *16*, 671.

(38) α-CyD: Manor, P. C.; Saenger, W. *J. Am. Chem. Soc.* **1974**, *96*, 3630.

(39) β-CyD: Betzel, C.; Saenger, W.; Hingerty, B. E.; Brown, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7545.

(40) γ-CyD: Harata, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2763.

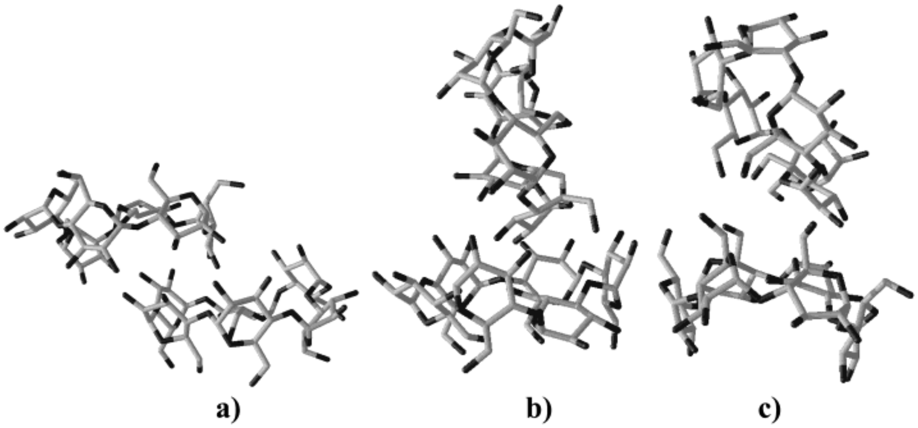

FIGURE 4. Average structures for the three relative orientations of the α-CD dimer (water molecules have been removed for clarity): (a) starting at HH, (b) starting at HT, and (c) Starting at TT.

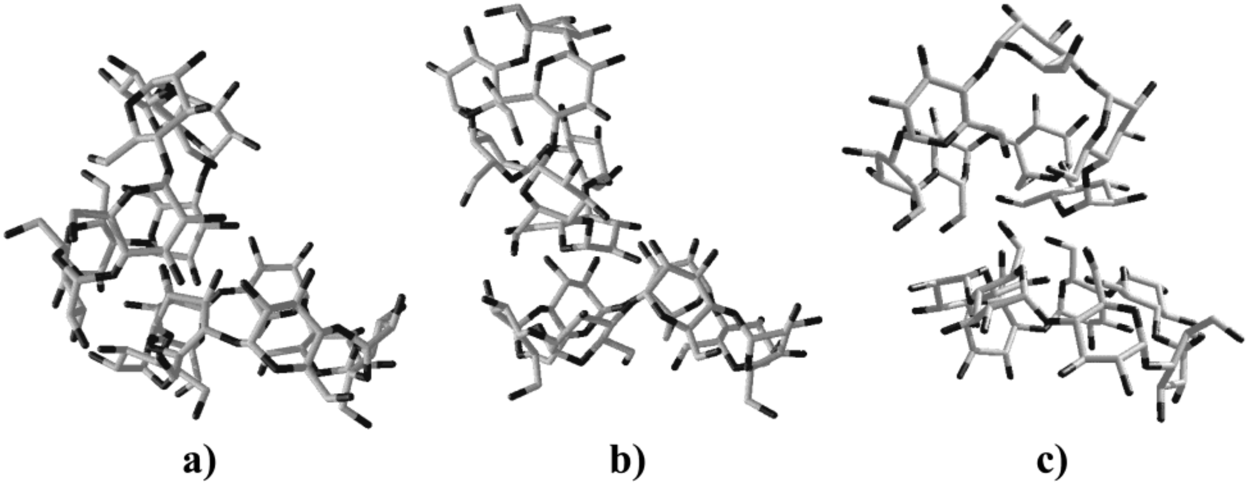

FIGURE 5. Average structures for the three relative orientations of the *β*-CD dimer (water molecules have been removed for clarity): (a) starting at HH, (b) starting at HT, and (c) starting at TT.

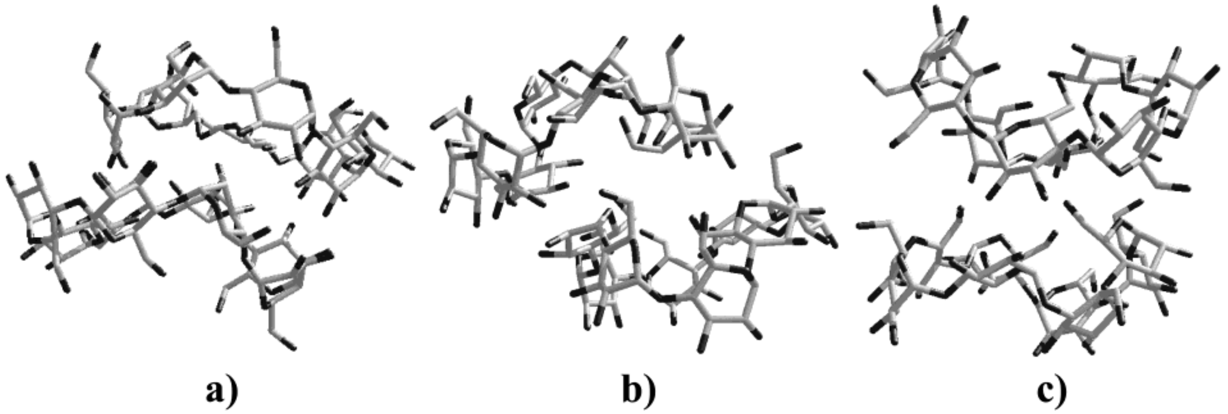

FIGURE 6. Average structures for the three relative orientations of the *γ*-CD dimer (water molecules have been removed for clarity): (a) starting at HH, (b) starting at HT, and (c) starting at TT.

kept. The relative orientation between glucose units within the same CyD is generally well kept. However, the analysis of the average structures for the HT and the TT *γ*-CyD orientations indicates that some glucose units have rotated until positioning the primary hydroxyls in the wider rim (upside down), in agreement with the well-known larger flexibility of *γ*-CyD relative to that of the two other native CyDs.

The hydrogen bond analysis performed along all of the 5 ns simulation runs for the nine studied CyD dimers is gathered in Table 1. HH orientations always make much more solute−solvent hydrogen bonds than HT and TT

TABLE 1. Hydrogen Bond Analysis for the Three Different Dimers (HH, HT, and TT) of the Three Native CyDs (α, *β*, and *γ*)[a]

	α			*β*			*γ*		
	HH	HT	TT	HH	HT	TT	HH	HT	TT
CyD−water	390	175	100	180	50	0	1400	165	170
CyD−CyD	730	600	150	810	290	325	940	580	375
CyD intra	900	1050	900	950	1150	1260	1260	1180	1600
prim−prim	0	0	159	0	0	271	0	42	190
sec−sec	782	396	26	757	362	0	815	149	8
prim−sec	0	1	7	26	66	2	161	136	287

[a] Numbers are sums of the number of close contacts between solute−solvent (CyD−water), solute−solute (CyD−CyD), intramolecular solute (CyD intra), primary−primary hydroxyls (prim−prim), secondary−secondary hydroxyls (sec−sec), and primary−secondary hydroxyls (prim−sec) divided by the number of snapshots of each 5 ns MD simulation (only values >10% were summed).

orientations. This result is in agreement with the larger facility of the primary hydroxyl groups to form hydrogen bonds than secondary hydroxyl groups. The analysis of the CyD−CyD intermolecular hydrogen bonds also confirms this fact since all the dimers present more interactions in the HH orientation.

Table 1 also shows that the *β*-CyD dimers form fewer solute−solvent hydrogen bonds, regardless of the orientation considered. The TT orientation for the *β*-CyD dimer does not even present significant solute−solvent hydrogen bonds (although a large number of interactions with occupancy[41] smaller than 10% were obtained). Interestingly, the *γ*-CyD dimer forms many more solute−solvent hydrogen bonds than α-CyD, while *β*-CyD forms almost no solute−solvent hydrogen bonds. The highest individual values of solute−solvent hydrogen bonds occupancies were 44, 19, and 92% for the α-, *β*-, and *γ*-CyD dimers, respectively. All these results may explain the well-known fact of the lower solubility of the *β*-CyD in aqueous solution in comparison with that of α- and *γ*-CyD.[42] It has also been described that *β*-CyD has a large tendency to form aggregates due to unfavorable interactions with the hydrogen bonded structure of water.[43] On the other hand, it has been experimentally confirmed that aggregation of *γ*-CyD in solution is mainly due to favorable hydrogen bonding between CyDs.[44] The hydrogen bond analysis presented in Table 1 indicates that the *γ*-CyD dimer is computed to have the largest number of intermolecular (CyD−CyD) hydrogen bonds, in perfect agreement with the experiments.

The number of intramolecular hydrogen bonds is different for each CyD dimer, and they increase with the size of the CyD (as expected due to the number of hydroxyl groups). Interestingly, the TT orientation for *γ*-CyD presents the larger number of intramolecular H-bonds due to the large deformation suffered by the parallel arrangement of the glucose units (Figure 6c). The parallel and perfectly arranged initial CyD structures were deformed along the 5 ns simulation. The severity of this deformation depends on the relative orientation. The relative spatial arrangement of the glucose units within each CyD presents considerable changes. This deformation can be clearly seen in the case of *γ*-CyD, where primary hydroxyl groups are mostly rotated toward the interior of the cavity and new O6−H6···O6 intramolecular hydrogen bonds appear (Figure 7).

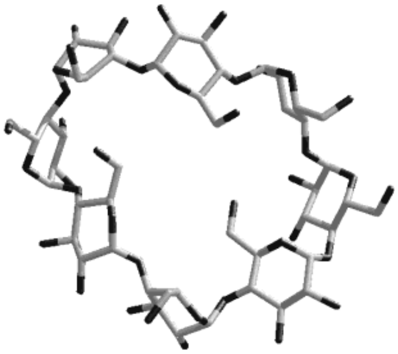

FIGURE 7. Representation of one *γ*-CD structure (water molecules have been removed for clarity) where three glucose units are folded toward the interior of the cavity to form intramolecular O6−H6···O6 hydrogen bonds.

The number of hydrogen bonds, formed between all the possible combinations of hydroxyls on each CyD, was also analyzed (Table 1). The primary−primary, primary−secondary, and secondary−secondary hydrogen bonds were considered.

The HH orientations always present larger numbers of secondary−secondary hydrogen bonds, in clear agreement with the proposed average geometries. Only in the case of *γ*-CyD is the number of primary−secondary hydrogen bonds substantial, although it is much smaller than the number of primary−primary hydrogen bonds. The existence of several glucoses turned upside down in this case exchanges the orientation of primary and secondary hydroxyls and permits the formation of these crossed hydrogen bonds.

The HT orientations should not present secondary−secondary hydrogen bonds if the average orientation was really an HT orientation. However, since in the average orientation both CyDs are perpendicular to each other, the possibility of these secondary−secondary hydrogen bonds increases. Moreover, in the case of *β*-CyD, the primary−secondary hydrogen bonds are larger than in the case of α-CyD, suggesting that the average structure should be slightly less perpendicular and more HT-like (as it is in Figure 5). The case of *γ*-CyD is again noteworthy: the number of hydrogen bonds is well spread among the three possible types, indicating a much larger mobility for this CyD in comparison with the other two.

The TT orientations present a large number of primary−primary hydrogen bonds, in agreement with the proposed average geometries. Only in the case of *γ*-CyD is the number of primary−secondary hydrogen bonds large, even larger than the number of primary−primary hydrogen bonds, and as before this can be attributed to the turning of the glucoses.

No significant differences were detected between the O3−H3···O2 and O2−H2···O3 intramolecular hydrogen bonds in any of the systems studied. These results are

---

[41] Occupancy is defined as the number of snapshots presenting a hydrogen bond divided by the total number of snapshots in an MD simulation multiplied by 100, i.e., a percentage.

[42] French, D.; Lewine, M. L.; Pazur, J. H.; Norberg, E. *J. Am. Chem. Soc.* **1949**, *71*, 353.

[43] (a) Coleman, A. W.; Nicolis, I.; Keller, N.; Dalbiez, J. P. *J. Incl. Phenom. Mol. Recogn. Chem.* **1992**, *13*, 139. (b) Szetjli, J. *Cyclodextrin Technology*; Kluwer: Dordrecht, 1988.

[44] Szente, L.; Szejtli, J.; Kis, G. L. *J. Pharm. Sci.* **1998**, *87*, 778.

TABLE 2. Contribution of Different Energy Terms and Free Binding Energies (kcal/mol) and Standard Deviations (std) for the Three Orientations (HH, HT, and TT) of the α-CyD Dimer as Obtained with the MM/PBSA Methodology

	$\mathrm{dimer}_{\mathrm{complex}}$						$\mathrm{CyD}_{\mathrm{isolated}}$	
	HH (ΔΔ)		HT (ΔΔ)		TT (ΔΔ)		α-CyD (Δ)	
contribution	kcal/mol	std	kcal/mol	std	kcal/mol	std	kcal/mol	std
E_{elect}	350.0	11.5	335.1	14.1	354.5	13.1	184.2	9.5
E_{vdW}	4.7	6.3	6.5	8.0	2.8	5.9	14.5	5.1
E_{int}	366.4	9.6	367.6	9.6	368.9	8.9	188.9	7.5
E_{gas}	721.1	13.5	709.1	17.1	726.2	14.7	387.6	10.3
$\Delta G_{\mathrm{nonpolar}}$	9.1	0.2	9.4	0.3	9.3	0.2	6.3	0.1
ΔG_{PB}	−208.3	8.9	−203.2	11.9	−212.0	11.3	−123.4	6.9
ΔG_{solv}	−199.3	8.8	−193.8	11.7	−202.7	11.2	−117.0	6.9
$E_{(\mathrm{PB}+\mathrm{elect})}$	141.7	7.7	131.8	7.8	142.5	9.0	60.8	5.2
$\Delta G_{\mathrm{water}}$	521.8	10.7	515.3	10.0	523.5	11.4	270.6	7.4
	ΔΔ − 2Δ							
	HH		HT		TT			
$\Delta E_{\mathrm{elect}}$	−18.3	17.7	−33.3	19.4	−13.8	18.8		
ΔE_{vdW}	−24.2	9.6	−22.5	10.7	−26.2	9.3		
ΔE_{int}	−11.6	13.5	−10.3	13.8	−9.0	14.2		
ΔE_{gas}	−54.1	19.9	−66.1	22.5	−49.0	20.1		
$\Delta\Delta G_{\mathrm{nonpolar}}$	−3.6	0.2	−3.3	0.3	−3.3	0.2		
$\Delta\Delta G_{\mathrm{PB}}$	38.4	13.2	43.5	15.4	34.7	15.0		
$\Delta\Delta G_{\mathrm{solv}}$	34.8	13.1	40.3	15.3	31.4	14.9		
$E_{(\mathrm{PB}+\mathrm{elect})}$	20.1	10.7	10.2	10.7	20.9	11.7		
$\Delta\Delta G_{\mathrm{binding}}$	−19.3	14.9	−25.8	14.4	−17.6	15.5		

in contradiction with the molecular orbital studies on maltose and on the β-CyD dimers, which indicate a preference of O2−H2 for acting as a proton donor.[18]

Binding Energies. The binding energies (or dimerization energies) were evaluated with the MM-PBSA methodology.[34] Under this method, binding free energies ($\Delta\Delta G_{\mathrm{water}}$) are estimated using the trajectories of each species from the same simulation run. In the previous section, we have mentioned that in some cases there exist large conformational deformations. It thus seems necessary to take into account the energy involved in these conformational changes occurring during the dimer formation. The binding energies were evaluated using structures coming from simulation runs on isolated CyD monomers solvated with water molecules. Obviously, in our study $\mathrm{CyD1}_{\mathrm{isolated}}$ and $\mathrm{CyD2}_{\mathrm{isolated}}$ represent the same trajectory. Binding energies are thus computed as follows:

$$\mathrm{CyD1}_{\mathrm{isolated}} + \mathrm{CyD2}_{\mathrm{isolated}} \leftrightarrow \mathrm{dimer}_{\mathrm{complex}}$$

Tables 2−4 show all the energy contributions for the different dimers and for isolated CyDs as deduced from the MM-PBSA analysis, as well as the free binding energies for each of the α-, β-, and γ-CyD dimers considered in this work. Negative final free-energy values indicate that a stable dimer is expected to be formed.

Entropy values have been estimated by considering three structures for each MD trajectory. Vibrational entropy values for each CyD do not differ, regardless of the starting orientation, but they always disfavor the free binding energies (Table 5). The $T\Delta S$ term for the dimerization reaction rises slightly with the number of glucose units in a CyD. Normal mode calculations are imperfect estimates for the solute entropies (distorted minimized structures, harmonic approximation, and calculation on few structures);[45] we consider them here exclusively to give us the magnitude of the entropic contribution. Normal mode analyses give only qualitative estimates of the solute entropy.[46] The disorder of water molecules is not considered and will be definitely different for each dimeric structure.[47,48] The number of CyD-water hydrogen bonds in Table 1 suggests that the order of water molecules around the γ-CyD dimer will be very high, and the entropy for the real dimerization process should thus be largely negative.

There is no agreement with the previous study carried out in a vacuum where the HH orientation was the preferred one for the three native CyDs.[19] When the dimers are in aqueous solution, the HT orientation is the most stable for the α-CyD dimer (−25.8 kcal/mol), while the TT orientation is preferred for the β- and γ-CyD dimers (−15.5 and −11.8 kcal/mol, respectively). In general, the ΔE_{gas} contribution always stabilizes the dimer formation by about 50 kcal/mol, while the $\Delta\Delta G_{\mathrm{solv}}$ destabilizes the process by about 30−70 kcal/mol. The energy involved in the solvation is controlling the stability of the dimers, especially in the case of γ-CyD where the $\Delta\Delta G_{\mathrm{solv}}$ is largely positive.

As described before, the most stable structures adopted in aqueous solution are the perperdincular arrangement for the α-CyD dimer and the parallel arrangement for the β- and γ-CyD dimers. In the case of α-CyD in the HT orientation, even having the worst solute−solvent interaction energy ($\Delta\Delta G_{\mathrm{PB}}$), the solute−solute electrostatic interaction ($\Delta E_{\mathrm{elect}}$) is so strong that it finally makes this orientation the most stable. Although, strictly speaking, the β-CyD dimer should adopt the TT orientation in water, differences between all three orientations are small (about 4 kcal/mol), and we can assume that all the orientations will be equally stable. However, the preference for the TT orientation is due to its better solvation (see $\Delta\Delta G_{\mathrm{PB}}$) in Table 3). The case of γ-CyD is slightly

---

(45) Reyes, C. R.; Kollman, P. A. *J. Mol. Biol.* **2000**, *297*, 1145.

(46) Cheatham, T. E.; Srinivasan, J.; Case, D. A.; Kollman, P. A. *J. Biomol. Struct. Dynam.* **1998**, *16*, 265.

TABLE 3. Contribution of the Different Energy Terms and Free Binding Energies (kcal/mol) and Standard Deviations (std) for the Three Orientations (HH, HT, and TT) of the β-CyD Dimer as Obtained with the MM/PBSA Methodology

	dimer$_{\text{complex}}$						CyD$_{\text{isolated}}$	
	HH (ΔΔ)		HT (ΔΔ)		TT (ΔΔ)		β-CyD (Δ)	
contribution	kcal/mol	std	kcal/mol	std	kcal/mol	std	kcal/mol	std
E_{elect}	401.2	14.8	400.3	18.0	400.4	14.9	221.4	12.8
E_{vdW}	5.6	7.7	13.8	9.1	8.4	7.0	12.4	4.5
E_{int}	428.1	10.1	428.6	11.9	432.1	10.1	213.4	7.4
E_{gas}	834.9	16.2	842.7	18.2	840.9	15.9	447.1	13.2
$\Delta G_{\text{nonpolar}}$	10.2	0.2	10.7	0.3	10.6	0.3	6.9	0.1
ΔG_{PB}	−237.1	10.4	−243.7	12.3	−246.0	10.8	−143.5	8.8
ΔG_{solv}	−226.9	10.3	−233.0	12.2	−235.4	10.7	−136.6	8.8
$E_{(\text{PB + elect})}$	164.1	9.0	156.6	13.6	154.4	9.7	77.9	6.8
ΔG_{water}	608.1	10.7	609.7	13.3	605.5	11.4	310.5	8.1
	ΔΔ − 2Δ							
	HH		HT		TT			
ΔE_{elect}	−41.5	23.4	−42.4	25.5	−42.4	23.5		
ΔE_{vdW}	−19.1	10.0	−10.9	11.1	−16.2	9.4		
ΔE_{int}	1.3	15.8	1.8	15.5	5.3	13.8		
ΔE_{gas}	−59.3	24.8	−51.5	26.1	−53.3	24.5		
$\Delta\Delta G_{\text{nonpolar}}$	−3.6	0.2	−3.1	0.4	−3.2	0.3		
$\Delta\Delta G_{\text{PB}}$	49.9	16.2	43.3	17.5	40.9	16.5		
$\Delta\Delta G_{\text{solv}}$	46.3	16.2	40.2	17.4	37.8	16.5		
$E_{(\text{PB + elect})}$	8.4	13.1	0.9	16.6	−1.4	13.6		
$\Delta\Delta G_{\text{binding}}$	−13.0	15.7	−11.3	17.6	−15.5	16.2		

TABLE 4. Contribution of the Different Energy Terms and Free Binding Energies (kcal/mol) and Standard Deviations (std) for the Three Orientations (HH, HT and TT) of the γ-CyD Dimer as Obtained with the MM/PBSA Methodology

	dimer$_{\text{complex}}$						CyD$_{\text{isolated}}$	
	HH (ΔΔ)		HT (ΔΔ)		TT (ΔΔ)		γ-CyD (Δ)	
contribution	kcal/mol	std	kcal/mol	std	kcal/mol	std	kcal/mol	std
E_{elect}	457.2	11.7	461.9	14.6	470.8	16.6	243.5	9.2
E_{vdW}	2.2	7.0	9.8	7.6	−0.3	8.3	17.2	5.6
E_{int}	478.3	11.0	480.5	11.4	474.6	11.0	240.4	7.9
E_{gas}	937.7	15.9	952.2	16.0	945.1	15.0	501.1	10.9
$\Delta G_{\text{nonpolar}}$	11.0	0.1	11.2	0.2	11.1	0.1	7.7	0.1
ΔG_{PB}	−250.5	9.9	−267.3	10.5	−273.3	11.9	−161.5	6.7
ΔG_{solv}	−239.6	9.9	−256.2	10.4	−262.3	11.9	−153.8	6.6
$E_{(\text{PB + elect})}$	206.7	8.4	194.6	10.5	197.5	7.7	82.1	5.9
ΔG_{water}	698.2	12.2	696.0	12.3	682.9	9.5	347.3	8.1
	ΔΔ − 2Δ							
	HH		HT		TT			
ΔE_{elect}	−29.8	17.5	−25.2	19.5	−16.3	21.1		
ΔE_{vdW}	−32.1	10.6	−24.5	11.0	−34.6	11.5		
ΔE_{int}	−2.6	15.2	−0.3	15.9	−6.2	19.9		
ΔE_{gas}	−64.5	22.1	−50.0	22.2	−57.1	21.5		
$\Delta\Delta G_{\text{nonpolar}}$	−4.4	0.2	−4.2	0.2	−4.3	0.2		
$\Delta\Delta G_{\text{PB}}$	72.4	13.7	55.6	14.1	49.6	15.2		
$\Delta\Delta G_{\text{solv}}$	68.0	13.6	51.4	14.0	45.3	15.2		
$E_{(\text{PB + elect})}$	42.6	11.9	30.4	13.4	33.3	11.4		
$\Delta\Delta G_{\text{binding}}$	3.5	16.8	1.4	16.8	−11.8	14.9		

TABLE 5. Entropy Contributions ($T\Delta S$ in kcal/mol) for the Dimerization Process of the Three Native CyDs Estimated from Vibrational Analysis Using Three Structures Distributed along the Trajectory

CyD	HH dimer	HT dimer	TT dimer
α	−16.4	−16.3	−16.0
β	−19.6	−19.8	−19.2
γ	−21.6	−22.0	−20.5

different; the worst solvation energy ($\Delta\Delta G_{\text{PB}}$) for the HH and HT orientations almost compensates the stabilization coming from the ΔE_{gas}. In contrast, its TT orientation presents an intermediate ΔE_{gas} value and a relatively low $\Delta\Delta G_{\text{solv}}$, which finally leads to the lowest binding energy of −11.8 kcal/mol (Table 4).

The free binding energy values largely decrease when increasing the CyD size (Tables 2−4) within each considered orientation. The electrostatic interactions are important for the dimerization process, while solvation does not favor this process. The estimated free energy for the dimerization process suggests that it is favorable except for HH and HT orientations of γ-CyD (for these two systems, such a conclusion may not be drawn). The HH orientations are those presenting the larger number of hydrogen bond interactions. These interactions are of the secondary−secondary type, indicating the larger capability of these secondary hydroxyls to form hydrogen bonds in comparison with the primary ones. Moreover, only *γ*-CyDs present exceptions to this behavior due to the larger mobility of the glucose units within the macrocycle. Larger CyDs having more glucose units, such as 14 or 26 as those recently discovered,[49] will very likely prefer the monomers in aqueous solution.

Conclusions

Results from MD simulations on the three most probable orientations (HH, HT, and TT) of α-, *β*- and *γ*-CyD dimers using the AMBER package with parm94 force field in the presence of explicit water molecules, as a model for aqueous solution, show a behavior completely different from that previously described in a vacuum.[19] When solvated, CyD dimers do not maintain the parallel arrangement along the 5 ns simulations and are not as stable as they are in a vacuum. Obviously, solute−solvent interactions are responsible for these differences and also for the conformational changes observed on the CyD structures. Average arrangements can be classified as parallel, perpendicular, and V forms. The results from this work are in agreement with those obtained in a molecular orbital study on *β*-CyD dimers but only in the sense of predicting which orientation is the most stable, the HT.[18] However, the MO calculations suggest a very different geometry for the dimer (parallel).

According to the MM-PBSA analysis, the binding free energies suggest the HT perpendicular arrangement as the most stable for the α-CyD dimer and the TT V form for the *β*- and *γ*-CyD dimers. Binding free energies decrease (and quickly reach positive values) when increasing numbers of glucose units within the same orientation, suggesting that large CyDs will likely exist in the form of monomers. These results forecast difficulties in the preparation of 2:1 host−guest complexes with large CyDs as hosts.

Acknowledgment. Intensive computations were performed in CESCA-C^4 (Catalonia, Spain). Grants from the Training and Mobility of Researchers (TMR) and the Improving Human Potential (IHP) programs from European Community are gratefully thanked by P.B.. This work was supported in part by the University of Orleans (France). “Ministerio de Ciencia y Tecnología” (Spain) is acknowledged for financial support through Grant PPQ2000-0369.

JO026166V

---

[47] Beà, I.; Jaime, C.; Kollman, P. *Theor. Chem. Acc.* **2002**, in press.

[48] Beà, I.; Cervelló, E.; Kollman, P. A.; Jaime, C. *Comb. Chem. High Throughput Screening* **2001**, *4*, 605.

[49] (a) Jacob, J.; Gessler, K.; Hoffmann, D.; Sanbe, H.; Koizumi, K.; Smith, S. M.; Takaha, T.; Saenger, W. *Angew. Chem., Int. Ed.* **1998**, *37*, 606. (b) Saenger, W.; Jacob, J.; Gessler, K.; Steiner, T.; Hoffmann, D.; Sanbe, H.; Koizumi, K.; Smith, S. M.; Takaha, T. *Chem. Rev.* **1998**, *98*, 1787.