β-cyclodextrine and Water: Semiempirical Calculations

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AM1 and PM3 calculations were carried out on β-cyclodextrine (β-CD) undecahydrate in the experimental conformation at 120 K. The calculated β-CD/water interaction energies are very small and indicative for each water molecule of an unfavorable condition in respect to that of pure water. The conformationally optimized system was also studied: β-CD appears highly symmetrical with negligible dipole moment, mainly because of the circular arrangement of the single vectors. Primary hydroxyls can easily rotate, while the secondary ones are stabilized by heteroannular hydrogen bonds and homoannular electrostatic interactions due to the consequent increase of the atomic charges. The β-CD/water interaction energies in the optimized hydrated system are not significantly different from the experimental ones. This almost hydrophobic character is also shown by MM equilibrated solutions: all water molecules are rejected beyond 2.4 Å; between 2.4 and 2.9 Å highly structured water is present.

From a purely enthalpic standpoint the molecule hydration appears highly improbable, thus the formation of β-CD · 11 H_2O must involve a compensation mechanism.

Key words: β-cyclodextrine hydrate, Semiempirical calculations, Water interactions.

Introduction

β-cyclodextrine (β-CD) is a cyclic oligosaccharide composed of seven α,D-glucose units. The toroidal symmetry of the molecule makes it similar to a truncated cone which is 0.78 nm high with an internal cavity diameter of about 0.75 nm.

The marked tendency of the molecule to form inclusion complexes with a variety of substances makes it very relevant in the pharmaceutical and supramolecular fields [1–3].

The role of water in the complexation reaction seems to be of major interest since the inclusion compound is normally formed in aqueous medium: according to Saenger and Steiner [4] this case could be an excellent model for the thermodynamics of the water-biomolecule interaction which is still a widely felt problem [5–8].

Literature theoretical calculations on CD molecules are very rare [9] and mainly based on the molecular mechanics force fields. Thus a computational approach to the problem seemed very useful.

Semiempirical calculations (with intermediate neglect of differential overlaps) were carried out using the "package" MOPAC 6 of J. P. Stewart [10] as distributed for the computer IBM RISC/6000 by QCPE [11]. The temporal succession of the Hamiltonians within the package would point to PM3 [12] as the most refined method. Unfortunately, the limits of its applicability in this context are still scarcely documented, thus the AM1 method [13] was preferred, integrated when possible with PM3 for a useful comparison: AM1 reproduces fairly well the water heat of formation; moreover, the calculated interaction energy for the water dimer is −5.5 kcal/mole in accordance with experiment.

In particular, AM1 was used for all calculations carried out on a single molecule, including geometry optimization, and to evaluate the interaction energy between a single β-CD molecule and a small number of water molecules.

A picture (although mainly qualitative) of the behavior of β-CD in water solution was also obtained by molecular mechanics and in particular by the modified version of MM2 [14] contained in the commercial programm "Hyperchem" [15].

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1. Results

1.1. Experimental β-CD · 11 H_2O

The literature reports diffraction studies at room temperature [16–18] and at 120 K [19] on β-CD recrystallized from water, showing that part of the water molecules is within the molecular cavity, while the others occupy interstices among the macrocycles. At

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room temperature the water molecules are statistically distributed among 16 sites, only 3 of these being fully occupied; on the contrary at 120 K, with the exception of one site, the molecules occupy fixed positions.

Thus, the neutron diffraction crystallographic data at 120 K were chosen as the starting geometry of the hydrated molecule.

Figure 1 shows the experimental structure [19] with the original numbering scheme and symbols which were retained in the present work, in particular all hydroxyl hydrogens are indicated with the symbol D. As it can be noted, there are 12 water oxygen sites fully occupied (from 1 to 14, the room temperature sites 11 and 12 lacking at 120 K) except W8 which showed an occupation of 0.64. Some conformational details of this structure are reported in Table 1.

AM1 and PM3 were first applied to the isolated β-CD molecule: the main results are reported in Table 2.

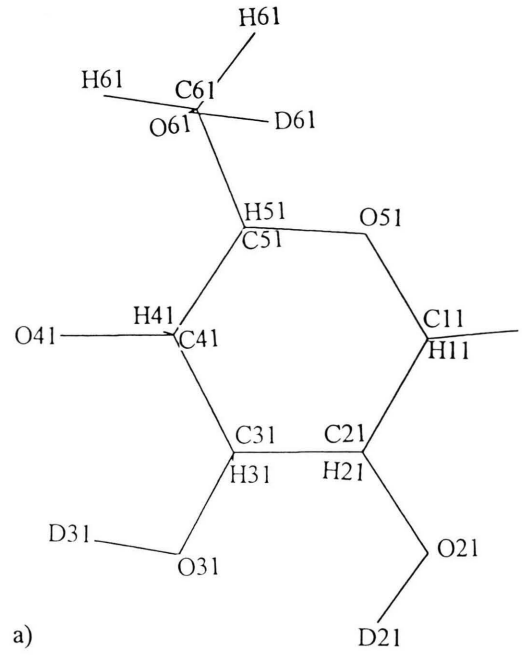
The single water molecules and the dimers (here defined as β-CD + single water) could then be analyzed in order to evaluate the interaction energies (ΔE_{int}) as

$$\Delta E_{int}(AB) = \Delta_f H(AB) - \Delta_f H(A) - \Delta_f H(B) .$$

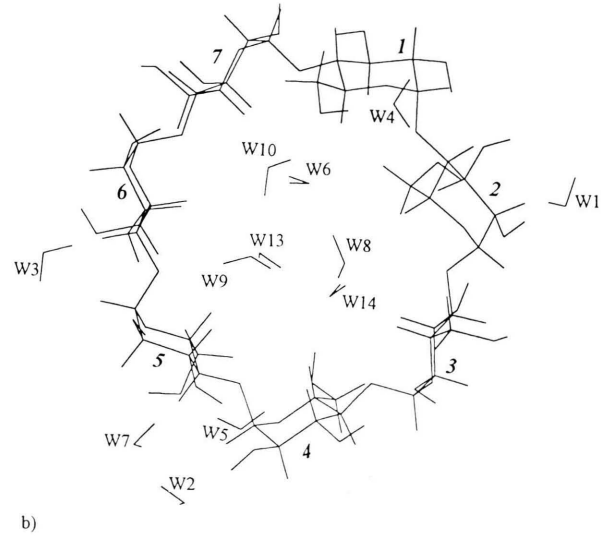
The results are reported in Table 3.

For a single water molecule in optimal conformation the calculated standard heat of formation is -59.2 (AM1) and -53.4 (PM3), to be compared with the experimental value -57.8 kcal mol^{-1}.

The two methods, although yielding different single data, give a common picture of the examined system; some internal waters show $\Delta_f H$ absolute values smaller in respect to the external ones and indicative



a)



b)

Fig. 1. Experimental β-CD hydrated system: a) atomic numbering scheme for glucose unit 1; b) water sites and numbering of the glucose rings.

Table 1. Dihedral angles (degrees) of primary and secondary hydroxyls in the experimental β-CD with: D2 ≡ D2O2C2C1, D3 ≡ D3O3C3C2, H6 ≡ H6C6C5C4, H6′ ≡ H6′C6C5C4, O6 ≡ O6C6C5C4, D6 ≡ D6O6C6C5.

Ring	D2	D3	H6	H6′	O6	D6
1	−40.84	178.97	−51.76	63.71	−171.60	−71.34
2	157.09	53.33	−65.37	177.02	52.09	70.89
3	122.02	152.17	−65.92	175.23	55.45	178.09
4	67.27	177.68	64.04	−54.06	−177.24	79.52
5	−63.96	160.06	−71.74	171.17	46.27	64.19
6	−37.64	170.87	−65.05	177.93	54.88	88.39
7	91.86	62.90	−67.81	176.72	55.67	166.41

Table 2. Calculated standard heat of formation ($\Delta_f H$), dipole moment (μ) and ionization potential (I) for the experimental conformation of β-CD.

β-CD$_{exp}$	AM1	PM3
$\Delta_f H$/kcal mol^{-1}	−1 555.946	−1 360.082
μ/D	11.65	10.97
I/eV	10.09	10.28

Table 3. Calculated standard heats of formation of the internal and external water molecules and their interaction energies (kcal mol^{-1}) with experimental β-CD *.

Water	Shortest contact	Dist.	AM1		PM3		ΔE_m
			$\Delta_f H_w$	ΔE_{int}	$\Delta_f H_w$	ΔE_{int}	
W_1: ext.	OW_1 ... D22	1.938	−58.88	−0.69	−52.36	−3.54	−2.11
W_2: ext.	OW_2 ... D24	1.796	−58.63	−1.20	−53.05	−4.39	−2.80
W_3: ext.	DW_3 ... O36	1.827	−58.70	−0.71	−53.34	−3.20	−1.96
W_4: ext.	OW_4 ... D61	1.850	−58.80	−1.39	−52.08	−5.71	−3.55
W_5: ext.	DW_5 ... O64	1.866	−58.75	−1.09	−52.37	−3.84	−2.47
W_6: int.	DW_6 ... O21	2.070	−51.32	−0.82	−43.21	−1.58	−1.20
W_7: ext.	DW_7 ... O35	1.798	−57.04	−1.10	−50.11	−3.57	−2.33
W_8: int.			−58.73	−2.41	−51.97	−2.27	−2.34
W_9: int.	DW_9 ... O46	2.038	−59.08	−3.70	−52.95	−2.25	−2.98
W_{10}: int.	DW_{10} ... O61	1.960	−57.08	−2.21	−51.93	−1.33	−1.77
W_{13}: int.			−50.91	−0.48	−44.04	−0.89	−0.69
W_{14}: int.	OW_{14} ... D64	1.906	−58.10	−1.51	−52.13	−2.64	−2.08

* Columns 2 and 3 report the dimer atoms in shortest contact and the respective distance (Å). For molecules W_8 and W_{13} no specific interaction contact was found. The last column reports mean values of the calculated interaction energies.

Table 4. Standard heat of formation of WINT and interaction energies (kcal mol^{-1}) among the waters in WINT ($\Delta E_{W\text{-}W}$) and in the dimer β-CD + WINT.

	$\Delta_f H_{WINT}$	$\Delta E_{W\text{-}W}$	$\Delta E_{\beta\text{-}CD\text{-}WINT}$
AM1	−349.31	−14.09	−11.51
PM3	−313.19	−16.96	−12.26

of a conformational instability, but no significative difference, between internal and external waters, stems from the interaction energies of the dimers: the calculated absolute values are very small and point for each water molecule to an unfavourable state in respect to that of pure water.

The β-CD tendency to hydration appears negligible and in apparent contrast with the experimental evidence of 6–7 waters within the molecular cavity, i.e. a number very close to the maximum allowed by the cavity volume (about 260 $Å^3$).

On the basis of the experimental evidence [19], the internal waters appear in tight contact with themselves (although not with β-CD) and so arranged to suggest the presence of hydrogen bonded homodromic pentagonal ring.

The attention was thus focused on the 6 internal waters (≡ WINT). The results obtained, reported in Table 4, seem to rule out the hypothesis that the hydration process is stabilized by strong water-water interactions.

Since the experimental partially occupied water sites at room temperature include those fully occupied at 120 K, the room temperature cell parameters were used to artificially expand the 120 K hydrated structure: the results obtained are not significantly different from the previous ones and in particular for the β-CD + WINT dimer give an interaction energy of −12 kcal mol^{-1}.

1.2. The Conformationally Optimized System

The experimental conformation was optimized by a multiple-step process: initial steps were partially suggested by literature information (i.e. fairly rigid ring geometry), then the global structure was gradually refined through increasingly stringent optimization criteria.

The AM1 optimized molecule (≡ CDOPT) is partially shown in Fig. 2, where the intramoleculare hydrogen bond visualized by "Hyperchem" (with bond distance ≤ 2.2 Å, bond angle ≥ 150°) is reported by a dotted line.

The optimized structure appears highly symmetrical with practically identical glucose rings; the O4 heptagon is almost unchanged, but more regular than the experimental one as shown by Table 5 where mean values and standard deviations of adjacent O4 angles are reported (in a regular planar heptagon the angle is 128.57° while all dihedrals are null).

In order to validate the potential energy minimum thus found, the same minimization procedure was carried out by PM3 till a final conformation named OPTPM3.

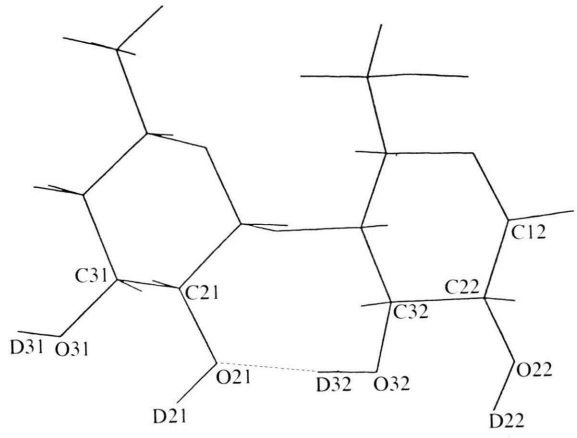


Fig. 2. Glucose rings 1 and 2 in CDOPT. The heteroannular hydrogen bond is indicated by a dotted line.

Table 5. Mean values and standard deviations of bridge oxygen angles (degrees) in CDOPT and experimental conformations.

	CDOPT	EXP
Angle mean values	128.33 ± 2.37	128.24 ± 4.06
Dihedral mean values	0.15 ± 7.93	0.27 ± 9.17

Table 6. Standard heat of formation ($\Delta_f H$), dipole moment (μ) and ionization potential (I) of optimized β-CD by AM1 (CDOPT) and PM3 (OPTPM3).

	CDOPT	OPTPM3
$\Delta_f H$/kcal mol^{-1}	−1656.37	−1459.65
μ/D	0.86	1.65
I/eV	10.61	10.77

Table 6 reports the main values of the two final structures.

It is interesting to observe that PM3 gives for CDOPT the following values:

$$\Delta_f H = -1427.07 \text{ kcal mol}^{-1}, \quad \mu = 1.9 \text{ D}.$$

The two methods essentially localize the same conformational minimum with unique orientation of the hydroxyls as shown in Table 7 where the most relevant dihedral angles are reported. Essentially, the primary hydroxyls point outward with a staggered conformation about the C6-C5 axis and with the O6-D6 bond parallel to the C5-O5 one. The O-D bond of the secondary hydroxyls in position 2 is parallel to the C2-C1 bond, that of position 3 to the C3-C2 one.

According to many authors, the molecule hydroxyls are involved in hydrogen bonds: those concerning the secondary hydroxyls would constitute a belt around the molecular cone responsible of its relative stiffness. For a more detailed analysis in view of the relevance in the β-CD/H_2O interaction, atomic distances and charges are now taken into account.

The relevant distances and net charges are reported in Tables 8 and 9 respectively.

These results seem to confirm the presence of hydrogen bonds involving secondary hydroxyls on adjacent rings, but not those between primary hydroxyls and O5.

In order to evaluate the bond energy, many specific rotations were imposed on the secondary hydroxyls (see Fig. 2) type:

R32) a 120° decrease of the D3 dihedral angle in ring 2, thus breaking the O21-D32 hydrogen bond, but leaving the O32 and D22 geometry unchanged;

R21) a 120° decrease of the D2 dihedral angle in ring 1: this should leave the hydrogen bond essentially unchanged, while the O31-D21 distance is increased from 2.39 to 5.14 Å.

Table 7. Mean values and standard deviations of the primary and secondary hydroxyl dihedral angles (degrees) in CDOPT and OPTPM3. Symbols are the same as in Table 1.

Dihedral	CDOPT	OPTPM3
D2	−169.98 ± 2.10	−170.55 ± 3.05
D3	−172.46 ± 7.35	−170.96 ± 5.79
H6	−175.35 ± 3.94	−178.94 ± 1.45
H6′	−54.10 ± 3.92	−59.82 ± 1.49
O6	61.98 ± 3.97	56.04 ± 1.45
D6	60.64 ± 1.26	64.02 ± 0.54

Table 8. D−O mean distances (Å) and standard deviations in CDOPT.

Same ring		Adjacent rings	
D2−O3	2.39 ± 0.01	O2−D3	2.13 ± 0.02
D6−O5	2.37 ± 0.05		
D6−O4	4.28 ± 0.06		

Table 9. Molecular mean values of electric charges (e) in CDOPT (all standard deviations are null).

Atom	O2	D2	O3	D3	O6	D6	O5	O4
Charge	−0.33	0.24	−0.35	0.24	−0.33	0.21	−0.30	−0.30

The increase (ΔE) of the molecules heat of formation in respect to CDOPT and the atomic charge variation could then be taken into account.

For example, for the cited rotations the observed ΔE values were

R32: 6.83 and R21: 5.17 kcal mol^{-1}.

The energy increase of the conformation R21 seems large in respect to that of R32, which is justified by the breaking of the hydrogen bond. Similar results were obtained for the numerous rotations imposed.

It can be observed that in CDOPT, the charge absolute values of O3 and D2 are also increased due to the hydrogen bond formation; it is then feasible that the electrostatic interaction of these two atomic positions enhances the molecules stabilization.

When affected by the imposed rotations, a decrease of the net charges was always observed, as shown in Table 10.

Table 10. Atomic charge percent decrease for the secondary hydroxyls after the rotations imposed on CDOPT.

Atom	O31	D31	O21	D21	O32	D32	O22	D22
R32	0	0	8.8	4.2	14.3	16.7	0	0
R21	8.6	4.2	8.8	12.5	0	0	0	0

A crude model was applied to all the rotations imposed, namely the observed energy variations were expressed as a linear combination of two contributions: a) O21-D32 hydrogen bond; b) electrostatic interaction between homoannular O3 and D2, with coefficients evaluated on the basis of the observed charge variations.

The secondary hydroxyl groups pictured by this model, on the basis of AM1 atomic charges, would contribute to molecular stabilization by heteroannular hydrogen bonds with typical energy of -5.5 kcal mol^{-1} and a subsequent increase of the atomic charges that gives rise to homoannular electrostatic interactions that can be altered up to 60% by an energy of about 5 kcal mol^{-1}.

Similar rotations, in respect to the optimized structure, were imposed on the primary hydroxyls: the observed energy variations were normally small, in particular the conformation having one or two glucose rings with the dihedrals O6 and D6 set at 180° and $-60°$ (≡ R6), respectively shows an average increment of 0.1 kcal mol^{-1} per ring; moreover, no significant variation of the atomic charges was observed. The primary hydroxyls thus appear fully free to rotate.

Finally, attention was given to the molecular dipole moment which appears largely affected by the geometry of the hydroxyl groups.

The results on many different conformations show that the almost negligible dipole moment of CDOPT arises from compensation mainly due to the circular alignment of the single moments. The only significant contribution seems due to the cone axis component. By changing the orientation of only one hydroxyl, the moment is raised to 1.5–2 D, the largest increments being connected with the O-D bond aligned with the cone axis. Conformations with large dipole moments (up to 10.2) could generally be obtained only at high energy cost (40–50 kcal mol^{-1}) through rotation of both primary and secondary groups.

Particularly significant in this context are the conformations type R6: when the primary hydroxyl of only one or two non adjacent glucose rings have this conformation, the molecular energy is almost unvaried, but the dipole moment is 2.1 and 3.6 D, respectively; when all of the glucose rings are in this conformation, the energy increase is 7.7 kcal mol^{-1}, and the dipole moment is 5.1 D.

The hydrated system (β-CD · 11 H_2O) conformation was also optimized, starting with water molecules in the experimental conformation: no significant variation was observed for CDOPT. The interaction energies of the dimers (β-CD + single water molecule) were in the range found for the experimental ones.

Particularly significant appeared the results obtained for the optimized system CDOPT + 6 internal waters (≡ WINOPT):

$$\Delta_f H_{\text{WINOPT}} = -381.63 \text{ kcal mol}^{-1},$$
$$\Delta E = -8.96 \text{ kcal mol}^{-1}.$$

In respect to the experimental conformation (see Table 4), the internal waters appear more stable (but still less than in pure water), whilst the interaction energy (ΔE) with β-CD is lowered (absolute value) by ≈ 2.5 kcal mol^{-1}.

The OW_{14}-D64 and DW_{10}-O61 distances are raised to 4.89 and 4.86 Å, respectively (see Table 3).

Thus, the β-CD behavior pictured by AM1 appears almost hydrophobic.

1.3. The β-CD Water Solution

A molecular mechanics analysis of β-CD water solution was obtained by using the default MM+ force field in "Hyperchem" [15].

In particular, CDOPT was placed in a $25 \times 25 \times 18$ Å box containing equilibrated [20] water molecules, at room temperature density.

After elimination of the water molecules with contact distance ≤ 2.2 Å, the periodic boundary conditions were applied to the elementary box containing CDOPT + 302 water molecules.

The system was then conformationally optimized using the minimum image restriction.

The initial and final solutions were compared by counting the number of water molecules vs. distance from the solute: 0.1 Å interval steps were used.

Analogous systems were generated and analyzed, in particular by applying a different initial cut (i.e. contact distance ≤ 1.9 Å) or by starting from different β-CD conformations. Very similar results were obtained with an identical water distribution in respect to β-CD.

Typical results are shown in Figure 3. Particularly, in the final solution no water molecule was found at a distance ≤ 2.4 Å, i.e. the ≈ 20 molecules found within that distance in the initial solution (generated with a 1.9 Å contact cut) were globally rejected; the interval 2.4–2.9 Å is strongly characterized by a large increase of the water density, regardless the value of the initial cut; beyond 2.9 Å the final solution is very similar to the initial one up to ≈ 5 Å were a second water shell appears.

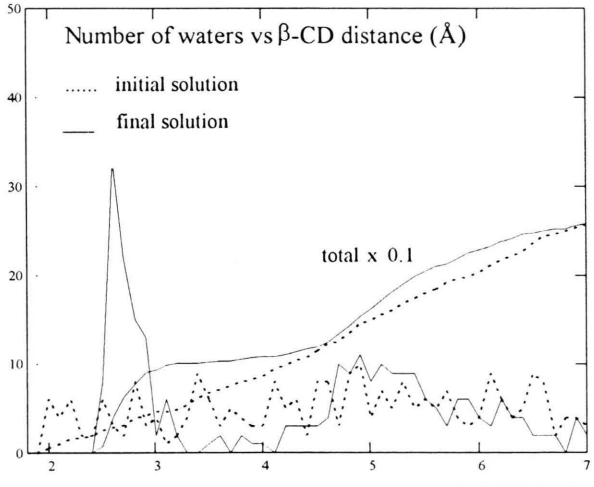


Fig. 3. Distribution of water molecules around β-CD in the initial and MM equilibrated solutions. The total number (10^{-1}) of waters vs. distance is also reported.

Moreover, it was found that the number of internal waters in the final solution (8–9) is always larger than the initial one (4–5) and at a minimum distance from β-CD in the range 2.4–2.7 Å. The interaction energies with β-CD, calculated by MM+ and AM1, appear negligible (≈ -0.5 kcal mol^{-1} per water molecule). Thus, the increase of internal waters seems to follow the β-CD water repulsion and to be mainly due to it.

2. Conclusions

The β-CD/water molecule interaction energies of the hydrated system in the experimental conformation calculated by AM1 and PM3 are very small and such to picture for each water molecule an unfavorable condition in respect to pure water. No differentiation can be made in this respect between internal and external waters.

On the contrary, the $\Delta_f H$ values indicate an unstable conformation for some internal waters but not for the external ones.

The conformationally optimized β-CD is highly symmetrical, particularly in the orientation of primary and secondary hydroxyls. In this conformation the molecule dipole moment is negligible. The value of this quantity is affected by the reciprocal hydroxyl symmetry, the almost circular orientation being responsible of the resultant negligible moment.

The primary hydroxyls can easily rotate, while the secondary ones appear stabilized by interannular hydrogen bonds and electrostatic interactions due to the consequent increase of the atomic charges.

The β-CD/water interaction energies in the conformationally optimized hydrated system are not significantly different from the experimental ones.

In the MM water solution all water molecules are rejected beyond 2.4 Å; between 2.4 and 2.9 Å highly structured water is present.

From a purely enthalpic standpoint, the β-CD hydration appears highly improbable. The β-CD · 11 H_2O formation must thus involve a compensation mechanism for this unfavorable enthalpic picture.

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