

# A look inside the cavity of hydrated $\alpha$ -cyclodextrin: A computer simulation study

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## Abstract

Monte Carlo simulations of hydrated  $\alpha$ -cyclodextrin are made to analyze the properties of the water molecules inside the hydrophobic cavity. An average of five water molecules is found in the cavity, where only 2.4 are hydrogen bonded to the  $\alpha$ -CD. The hydrogen-bond interactions between the  $\alpha$ -CD and the inner water molecules are reduced when compared with those outside the cavity. The free energy of binding one water molecule inside the hydrated  $\alpha$ -CD is obtained using the thermodynamic perturbation theory. From these the entropy contribution is obtained to ascertain the hydrophobic strength of the cavity.

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## 1. Introduction

Considerable interest is devoted to the chemistry and technology of cyclodextrins (CD) [1,2]. CDs are starch-derived molecules possessing a hydrophobic cavity with an enormous host potential that make them very important for molecular encapsulation and for drug carriers [3]. Different cavity sizes are possible depending on the number of elementary glucose units. In spite of the great number of investigations made on CDs, the knowledge of the cavity properties is still limited. In this direction, it is useful to obtain additional information that could be helpful in understanding the hydrophobic activity of their cavities and the driving forces for the inclusion mechanism. There are several studies concerned with the properties of CDs in water [4–13]. Experimental information has been obtained in the crystalline form [4–7] but that does not emphasize the role of liquid water, although it provides information regarding the hydration and possible number of surrounding water molecules. This subject has been pursued intensely by Saenger et al. [14]. Early computer simulations have

compared different CDs and stressed the effects of the solvent on their properties [8]. There are many previous theoretical studies of CDs, both isolated and in water, as described in a review article [9]. A recent theoretical study [10], at the semi-empirical level, analyzed the formation of the observed crystalline hexahydrate that includes two water molecules inside the cavity [4,11]. Recent spectroscopic studies [15,16] have probed the influence of the hydrophobic cavity in the orientation of the encapsulated guest molecule.

In this Letter, we study the cavity of the six-glucose  $\alpha$ -CD (Fig. 1) in water at room temperature. We use Monte Carlo computer simulation to analyze the behavior of the water molecules inside the hydrophobic cavity of  $\alpha$ -CD. We determine the average number of water molecules and their interaction strength. We analyze the effect of the confinement on the water molecules inside the cavity comparing the simulation of hydrated  $\alpha$ -CD with another simulation where the atoms of the  $\alpha$ -CD are considered as simple hard spheres. In addition, we obtain the Gibbs free energy of binding a water molecule inside the cavity of  $\alpha$ -CD using the thermodynamic perturbation theory [17,18]. Using the calculated values of the free energy and the enthalpy of binding, we estimate the entropy contribution to stabilize the process

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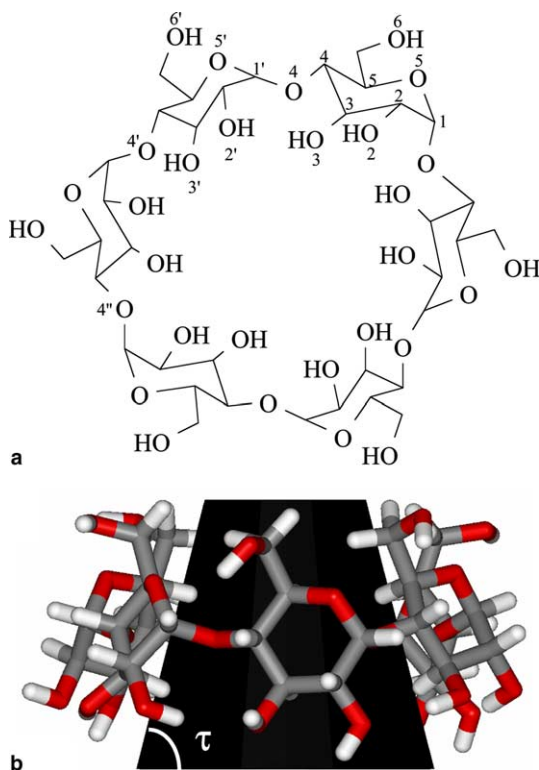


Fig. 1. (a) Six-glucose  $\alpha$ -cyclodextrin and definition of the atomic labels. (b) The side view of the molecule (the dark cone is just for better visualization).

of bringing one water molecule from the bulk to the inside the cavity of the  $\alpha$ -CD.

## 2. The structure of isolated $\alpha$ -CD

Experimental structural parameters of crystalline  $\alpha$ -CD are available from X-ray studies [5–7]. Theoretical geometries have also been obtained using semi-empirical models [9,19,20]. Because of the large number of local minima we found convenient to first perform a detailed analysis of the structures of  $\alpha$ -CD. We used the AM-

BER force field [21] to obtain a sample of optimized geometries (35 different minima were found at this level). We selected the seven geometries that were lowest in energy to start full geometry optimizations at the Hartree–Fock (HF) level using the 6-21G basis set (a total of 714 contracted Gaussian-type basis functions). All ab initio calculations are made using GAUSSIAN-98 [22]. The two geometries, obtained with AMBER and the HF/6-21G, lowest in energy are given in Table 1. The geometry notation is conventional [9] and the atomic labels are presented in Fig. 1. There is generally good agreement between our two theoretical results, the experimental values [5–7] and previous AM1 calculations [5,19]. The theoretical deviation is related to the six glucose values. The AMBER geometry is thus found to be in good agreement with geometries inferred from X-ray data and is henceforth be used. A simplifying assumption is that the geometry of  $\alpha$ -CD will be kept fixed in the simulations. It is known that  $\alpha$ -CD is not rigid [13,23] but we assume that this is not severe [8] in the properties reported here. In fact, the average geometry is very similar to the one used here.

## 3. Monte Carlo simulation of $\alpha$ -CD in water

### 3.1. Simulation details

We first performed two Monte Carlo (MC) simulations of  $\alpha$ -CD in aqueous solution. In one, we considered the interaction between the  $\alpha$ -CD and the water molecules described by the Lennard-Jones plus Coulomb (LJC) pair-wise potential, using the AMBER force field [24,25] for the  $\alpha$ -CD and the SPC model [26] for water. To analyze separately the role of the intermolecular interaction between water and  $\alpha$ -CD, another equivalent simulation was performed considering the atoms of  $\alpha$ -CD as simple hard spheres (HS) with a radius given by the  $\sigma$  parameter of the Lennard-Jones potential. The MC simulations are

Table 1  
Calculated and experimental structures of  $\alpha$ -cyclodextrin

Property	Definition	AMBER	HF/6-21G	Exp. <sup>a</sup>	Exp. [7]
$R_{\text{hex}}$	O4–O4'	$4.30 \pm 0.03 \text{ \AA}$	$4.28 \pm 0.04 \text{ \AA}$	$4.24 \pm 0.09 \text{ \AA}$	$4.235 \pm 0.033 \text{ \AA}$
$R_{\text{HB}}$	O3–O2'	$3.22 \pm 0.03 \text{ \AA}$	$2.86 \pm 0.02 \text{ \AA}$	$3.0 \pm 0.4 \text{ \AA}$	$2.981 \pm 0.066 \text{ \AA}$
$\delta$	O4–O4'–O4''	$120 \pm 1^\circ$	$120 \pm 2^\circ$	$120 \pm 5^\circ$	$119.9 \pm 1.6 \text{ \AA}$
$\varphi$	C4–O4–C1'	$117.8 \pm 0.3^\circ$	$121.2 \pm 0.8^\circ$	$119 \pm 1^\circ$	$118.4 \pm 0.5 \text{ \AA}$
$\tau$	Torus	$76 \pm 7^\circ$	$83 \pm 5^\circ$	$80 \pm 9^\circ$	
$\Phi$	C4–O4–C1'–O5'	$106 \pm 6^\circ$	$113 \pm 4^\circ$	$109 \pm 7^\circ$	$109.2 \pm 4.6 \text{ \AA}$
$\Psi$	C3–C4–O4–C1'	$130 \pm 8^\circ$	$128 \pm 2^\circ$	$128 \pm 14^\circ$	$128.1 \pm 1.4 \text{ \AA}$
$\mu$	Dipole moment	13.1 D	14.8 D		14.3 D [19] 13.3 D [15]

Atomic labels are presented in Fig. 1.

<sup>a</sup> Geometrical parameters are calculated and averaged from X-ray data in [5,6].

performed using standard procedures [27] for the Metropolis sampling technique in the isothermal–isobaric  $NpT$  ensemble, with periodic boundary conditions, image method and long-range correction for the energy beyond the cut-off radius of 16 Å. All simulations were performed using the program DICE [28]. In both simulations, the system is composed of one  $\alpha$ -CD surrounded by 1000 water molecules, at the pressure of 1 atm and temperature of 298 K. After a long equilibration of  $2 \times 10^7$  steps, the MC simulations were performed with  $4 \times 10^7$  MC steps.

To better understand the hydrophobic character of the  $\alpha$ -CD cavity, we performed other MC simulations to calculate the Gibbs free energy of binding one water molecule inside the cavity. This was made using a hypothetical process [18] in which one water molecule is made to vanish in two locations: (i) in the bulk water, thus computing the  $\Delta G_{W \rightarrow 0}(\text{aq.})$ ; (ii) inside the cavity of a hydrated  $\alpha$ -CD, thus computing the  $\Delta G_{\text{CD-W} \rightarrow \text{CD}}(\text{aq.})$ . Both variations of free energy are calculated using thermodynamic perturbation theory [17,18] and they can be combined [18] to obtain the free energy of binding as

$$\Delta G_{\text{bind}}(\text{aq.}) = \Delta G_{W \rightarrow 0}(\text{aq.}) - \Delta G_{\text{CD-W} \rightarrow \text{CD}}(\text{aq.}). \quad (1)$$

For each term, it was necessary a series of five simulations using the double-wide sampling [29], to make one water molecule gradually disappear from the bulk (system of 1000 water molecules) and inside the hydrated  $\alpha$ -CD (system of one  $\alpha$ -CD and 1000 water molecules), respectively.

We divided this vanishing process in three stages, separately scaling to zero the three parameters of the LJC potential,  $q_i$ ,  $\varepsilon_i$  and  $\sigma_i$ . First, the Coulomb potential is vanishing by scaling to zero the atomic charges,  $q_i$ . The scale factors were  $\lambda = 1.0, 0.8, 0.6, 0.4, 0.2, 0.1$  and 0.0. Therefore, using the double-wide sampling in a simulation performed with  $\lambda_i$ , the atomic charges were perturbed simultaneously to  $\lambda_{i-1}$  and  $\lambda_{i+1}$ . Thus, in this first stage we performed three simulations with  $\lambda_i = 0.8, 0.4$  and 0.1. After that, the attractive well of the LJ potential is made to vanish by scaling to nearly zero the atomic parameter  $\varepsilon_i$ . The scale factors were  $\lambda = 1.0, 0.5$  and 0.01. In this second stage, because of the double-wide sampling, we performed only one simulation with  $\lambda_i = 0.5$ . It is interesting to note that at this point, the interaction potential of the vanishing molecule is described only by soft-spheres with a repulsive behavior of  $(\sigma/r)^{12}$ . At the third stage, this repulsive potential is vanishing by scaling to zero the atomic parameter  $\sigma_i$ . Only one simulation is performed directly from  $\lambda = 1.0$  to 0. Each simulation starts with the last configuration of the previous simulation and it consists of an equilibration phase of  $3 \times 10^6$  MC steps, followed by an average stage of  $6 \times 10^7$  MC steps in the  $NpT$  ensemble at the pressure of 1 atm and temperature of 298 K.

### 3.2. Simulation results

To analyze the hydrogen bonds (HB) formed between  $\alpha$ -CD and water, we have used the geometric and energetic criteria [8,30–32] to identify the HB. A HB was assumed when the distance O–O (between the oxygen atoms of the  $\alpha$ -CD and the oxygen of water molecules) was less than the first minimum in the corresponding radial distribution function (RDF), the angle O–OH was less than  $30^\circ$  and the binding energy was larger than 3.5 kcal/mol. This energy threshold is obtained analyzing the pair-wise energy interaction [30–32]. Table 2 summarizes the number and strengths of these hydrogen bonds. The oxygen atoms of the six inter-saccharides (O4) and six pyranoid rings (O5) are proton acceptors making an average of 0.4 HB in each atom and giving a total of 4.8 HB per ring. The 12 secondary (O2 and O3) and six primary hydroxyls (O6) make 1.3 and 1.7 HB per ring, respectively, giving a total of 15.8 and 10.4 HB. Thus, the total number of HB formed between  $\alpha$ -CD and water is 31, giving an average of ca. 5 HB per ring. It is possible to distinguish the inner and outer HB. The inter-saccharide O4 is the only proton acceptor inside the cavity and makes 2.4 HB with water thus remaining a total of 28.6 HB in the outer part of  $\alpha$ -CD. The total strength of the hydrogen bonded water molecules (average of 2.4) inside the cavity is obtained here as  $13.7 \pm 0.8$  kcal/mol, in close agreement with the result of Linert et al. [8]. As it can be seen in Table 2, the HB interactions between the  $\alpha$ -CD and the waters inside the cavity are reduced in average by ca. 18%, when compared with those interactions outside the cavity. This reduction might then be attributed to the hydrophobic effect of the cavity that decreases the strength of the HBs between the  $\alpha$ -CD and the water molecules inside its cavity and plays an important role in the driving forces for including complexes [11].

X-ray results [4,5] for the crystalline state of  $\alpha$ -CD have shown two water molecules inside the cavity that are hydrogen bonded to  $\alpha$ -CD. This is in agreement with our present result. Fig. 2 shows a superposition of 200 MC configurations with water molecules inside the cavity. This gives a representation of the configuration space that is occupied by the water molecules that are

Table 2

Average number (including all six glucose), oxygen–oxygen distance and binding energy of hydrogen bonds between water and  $\alpha$ -cyclodextrin

Binding site	Average number	$R_{\text{OO}}$ (Å)	Energy/bond (kcal/mol)
O2 and O3	$15.8 \pm 0.4$	$2.80 \pm 0.02$	$6.3 \pm 0.1$
O4	$2.4 \pm 0.1$	$2.73 \pm 0.01$	$5.7 \pm 0.1$
O5	$2.4 \pm 0.1$	$2.70 \pm 0.01$	$8.0 \pm 0.2$
O6	$10.4 \pm 0.3$	$2.81 \pm 0.02$	$6.8 \pm 0.1$

See Fig. 1 for definition of atomic labels.

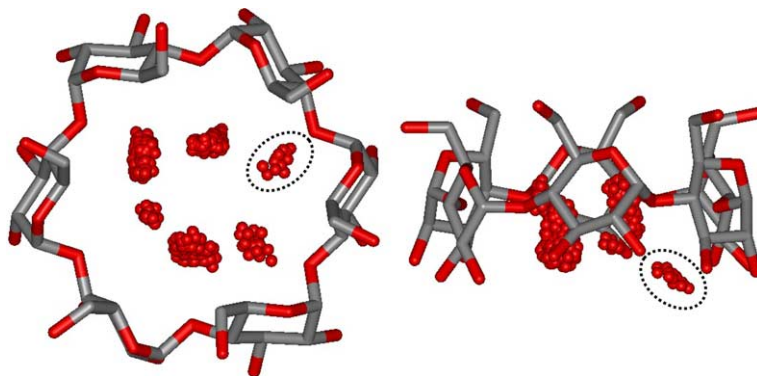


Fig. 2. Configuration space occupied by the water molecules that are hydrogen bonded to the inner wall of  $\alpha$ -cyclodextrin. Top and side views. For simplification the hydrogen atoms are not shown. The side view shows that the marked site may also bind to the secondary hydroxyls (O2 and O3).

inside the cavity and hydrogen-bonded to  $\alpha$ -CD. The side view of Fig. 2 shows that there is one site that is also close to the secondary hydroxyls (O2 and O3), in concordance with observations obtained from the X-ray experiments [4,5].

To obtain the average number of water molecules inside the cavity of the  $\alpha$ -CD, we have first analyzed the RDF between the center of masses of  $\alpha$ -CD and water in the two models used for  $\alpha$ -CD, LJC and HS. The origin of these RDFs lies at the center of the  $\alpha$ -CD cavity. Fig. 3 shows the distribution of water both inside and outside  $\alpha$ -CD. There is a clear internal distribution of water molecules up to 3.75 Å and an external distribution beyond 7.0 Å for the LJC model and 6.0 Å for the HS model. Between these two limits of the internal and external distributions lies the rim of the  $\alpha$ -CD. For distances below 3.75 Å, we obtain an average of five water molecules inside the cavity, for both the LJC and HS models. For comparison, we have performed a simulation of bulk water using the same potential and obtain 7.4 water molecules when integrating a region of the same volume, i.e., a sphere with radius of 3.75 Å. This

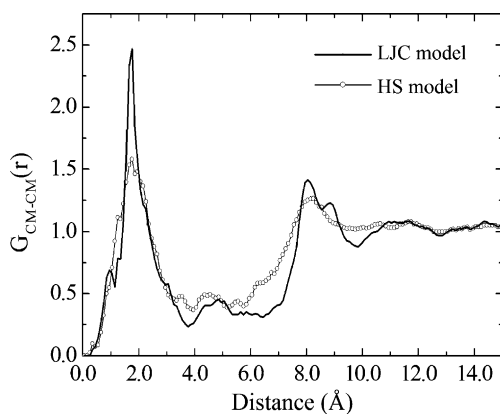


Fig. 3. Radial distribution functions between the center-of-masses of  $\alpha$ -cyclodextrin and water.

reduction of ca. 32% in the water density (from 7.4 to 5 water molecules) is interpreted as originating from the confinement because the number of water molecules in the cavity of  $\alpha$ -CD is the same regardless of whether the interaction between water and  $\alpha$ -CD is operant (LJC model) or inoperant (HS model). In Fig. 4, we present a superposition of 200 MC configurations of five water molecules inside the cavity using both the LJC and HS model for  $\alpha$ -CD, compared to the bulk water. Even though the number of water molecules is the same the configuration space is different when the intermolecular interaction is operative (LJC model).

In the average, our studies show five water molecules inside the cavity making 2.4 hydrogen bonds with  $\alpha$ -CD. As discussed before, X-ray data [4,5] for crystalline  $\alpha$ -CD have seen two water molecules inside the cavity that are hydrogen bonded to  $\alpha$ -CD. It should not be assumed that there are only these two water molecules in the cavity. Our simulation also shows additional 2.6 water molecules bound to one another but unattached from  $\alpha$ -CD. This is in line with the crystal form reported by Chacko and Saenger [7] where precisely 2.6 disordered water molecules were found in the cavity. Puliti et al. [6] have also reported disordered water molecules in the cavity.

Hydrophobic aspects are better understood in thermodynamic terms [33–35]. Hence, we now discuss the thermodynamic aspects, in particular the variations of the free energy and the entropy to bring one water molecule from the bulk to inside the cavity of the hydrated  $\alpha$ -CD. The results of the free energy and enthalpy of binding are summarized in Table 3. The total value obtained to the  $\Delta G_{W \rightarrow 0(aq.)}$  is  $6.27 \pm 0.29$  kcal/mol and  $\Delta G_{CD-W \rightarrow CD(aq.)}$  is  $8.44 \pm 0.30$  kcal/mol. Using Eq. (1), we calculate  $\Delta G_{bind(aq.)}$  as  $-2.17$  kcal/mol. This negative value shows that the inclusion process of water inside the cavity of the  $\alpha$ -CD is spontaneous, what is in agreement with our previous simulation and the experimental observations [4–7]. The differences of enthalpy obtained from the final and initial systems of the vanishing process are also



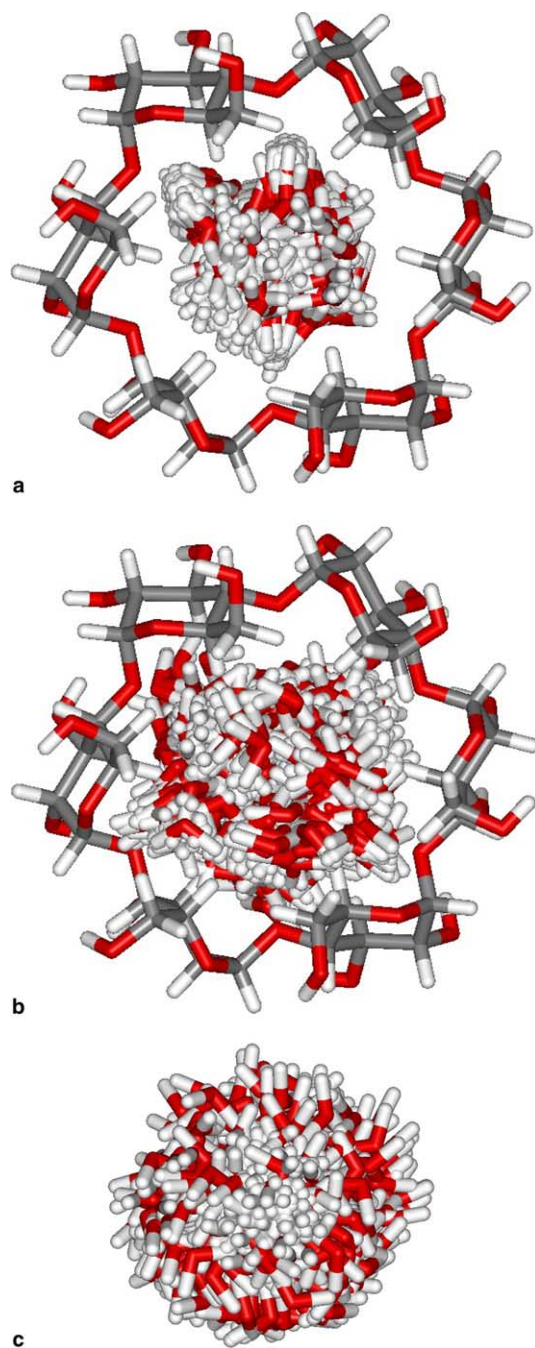


Fig. 4. Illustration of the volume accessible for the water molecules: inside the cavity (a) using the Lennard-Jones plus Coulomb potential, LJC model, (b) using the hard sphere potential, HS model and (c) in the bulk water.

calculated and give  $\Delta H_{W \rightarrow 0}(\text{aq.}) = 10.19 \pm 0.13$  kcal/mol and  $\Delta H_{\text{CD-W} \rightarrow \text{CD}}(\text{aq.}) = 15.16 \pm 0.18$  kcal/mol, giving a  $\Delta H_{\text{bind}}(\text{aq.})$  as  $-4.87$  kcal/mol. Therefore, using the fundamental relation between the variations of free energy, enthalpy and entropy, we obtain that  $T\Delta S_{\text{bind}}(\text{aq.})$  is  $-2.70$  kcal/mol. These results show that to bring one water molecules from the bulk to inside the

Table 3

Gibbs free energy, enthalpy and entropy (in kcal/mol) of a hypothetical process in which one water molecule is made to vanish in two locations: in the bulk water,  $\Delta G_{W \rightarrow 0}(\text{aq.})$ , and inside the cavity of a hydrated  $\alpha$ -CD,  $\Delta G_{\text{CD-W} \rightarrow \text{CD}}(\text{aq.})$ , at 25 °C and 1 atm

$\lambda_i$ in $q$	$\lambda_j$ in $q$	$\Delta G_{\lambda_i W \rightarrow \lambda_j W}(\text{aq.})$	$\Delta G_{\text{CD}-(\lambda_i W) \rightarrow \text{CD}-(\lambda_j W)}(\text{aq.})$
1.0	0.8	3.437	3.165
0.8	0.6	1.562	2.306
0.6	0.4	1.165	1.545
0.4	0.2	0.514	0.864
0.2	0.1	0.144	0.197
0.1	0.0	0.032	0.068
Total of stage 1		6.854	8.144
$\lambda_i$ in $\epsilon$	$\lambda_j$ in $\epsilon$	With $q = 0$	With $q = 0$
1.0	0.5	0.462	0.839
0.5	0.01	0.265	1.464
Total of stage 2		0.727	2.303
$\lambda_i$ in $\sigma$	$\lambda_j$ in $\sigma$	With $q = 0$	With $q = 0$ and $\epsilon = 1\%$
1.0	0.0	$-1.309$	$-2.006$
Total	$\Delta G_{X \rightarrow 0}(\text{aq.})$	$6.27 \pm 0.29$	$8.44 \pm 0.30$
	$\Delta H_{X \rightarrow 0}(\text{aq.})$	$10.19 \pm 0.13$	$15.06 \pm 0.29$
	$T\Delta S_{X \rightarrow 0}(\text{aq.})$	3.92	6.62

cavity of the hydrated  $\alpha$ -CD, the entropy decreases, i.e., the system organizes to make the inclusion process favorable. This gives a direct measure of the hydrophobic strength of the  $\alpha$ -CD cavity.

#### 4. Summary and conclusion

Monte Carlo simulations of  $\alpha$ -CD in aqueous solution are made to analyze the properties of the water molecules inside the hydrophobic cavity. It is found an average of five water molecules inside the cavity, where 2.4 are hydrogen bonded to  $\alpha$ -CD and 2.6 are disordered. Comparing a region of the same volume inside the cavity and in the bulk water, we observe a reduction of ca. 32% of the water density. We interpret this reduction as a confinement effect, because the number of water molecules in the cavity of  $\alpha$ -CD is the same whether the interaction between water and  $\alpha$ -CD is included (LJC model) or excluded (HS model). The hydrophobic effect of the  $\alpha$ -CD cavity was analyzed in two different aspects. One is the hydrogen-bond interaction between the  $\alpha$ -CD and the inner water molecules that are reduced in average by ca. 18%, when compared with those interactions outside the cavity. The second analysis uses thermodynamic calculations and shows that the entropy term at room temperature decreases by 2.7 kcal/mol when bringing one water molecules from the bulk to the inside of the cavity of the hydrated  $\alpha$ -CD. This result ascertains the hydrophobic strength of the cavity.

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