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Molecular Interactions and Inclusion Phenomena in Substituted β-Cyclodextrins. Simple Inclusion Probes: H_2O, C, CH_4, C_6H_6, NH_4^+, $HCOO^-$

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Abstract. Using a simple molecular mechanics approach interaction energy profiles of simple probes (C, CH_4, C_6H_6, H_2O, NH_4^+, and $HCOO^-$) passing through the center of the β-CD ring cavity along the main molecular symmetry axis were first evaluated. Molecular Electrostatic Potential (MEP) values along the same path were also evaluated. The effect of the flexibility of the host β-CD molecule together with solute-solvent (H_2O) interactions have been represented by averaging structures of MD calculations for β-CD alone and β-CD surrounded by 133 H_2O molecules. The effect of various substitutions of β-CD has also been evaluated. Small symmetric hydrophobic probes (such as C, CH_4, C_6H_6) are predicted to form stable inclusion complexes with non-substituted and substituted β-CDs, the probe position typically being near the cavity center. The stability of the inclusion complexes will increase with increasing size and aliphatic character of the probe. Small polar and charged probes (such as H_2O, NH_4^+, $HCOO^-$) are predicted to prefer the interaction with the solvent (water) in the bulk phase rather than the formation of inclusion complexes with non-substituted and substituted β-CDs. Guest–host interactions in the stable inclusion complexes with hydrophobic probes are almost entirely dominated by dispersion interactions. The MEP reaches magnitudes close to zero in the center of the non-substituted β-CD ring cavity and in most of the studied substituted β-CDs and shows maximum positive or negative values outside of the cavity, near the ring faces. Substitution of β-CD by neutral substituents leads to enhanced binding of hydrophobic probes and significant changes in the MEP profile along the β-CD symmetry axis.

Key words: β-cyclodextrin, substituted β-cyclodextrin, molecular simulation, inclusion complexes, probe interactions, host-guest interaction profiles.

1. Introduction

The cyclic oligosaccharide β-cyclodextrin (β-CD) consists of seven α-D-glucopyranose residues covalently bound by α-1,4-linkages forming thus a ring-shaped structure with a central cavity of an approximately 7 Å diameter [1]. The

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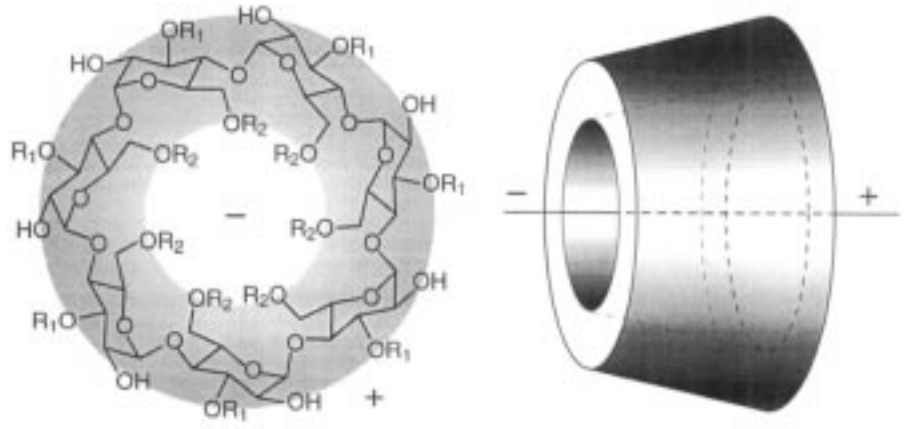
cavity, lined by hydrogen atoms and the glycosidic oxygen bridges, is relatively hydrophobic compared to water. On the other hand, the external surface of the β-CD ring contains a network of intramolecular O2 - O3 hydrogen bonds between adjacent residues, stabilizing the rigid ring conformation, as well as the O6 hydroxyl groups network interacting with the solvent, donating a hydrophilic character to the external surface. Because of these structural properties β-CD can form inclusion complexes with other organic guest molecules residing partially within the ring cavity. The stability of β-CD guest-host complexes has been recently reviewed [2]. Due to this ability β-CD has been studied as a potential catalyst and drug-complexing agent [3, 4]. The inclusion of a guest drug into the non-toxic host β-CD may lead to improved solubility of the drug complex in aqueous medium and increased oral bioavailability. Therefore, experimental and theoretical information on the geometry of inclusion complexes, the guest molecule affinity to β-CD, and the nature of the guest–host interactions are increasingly important.

Previously several theoretical studies on the conformational stability of cyclodextrins and their binding to biologically active molecules have been reported [5–9]. Kohler et al. [5, 6] carried out a molecular dynamics study on the conformation and cooperative effects in hydrogen bonding networks in various cyclodextrin crystals. A molecular modeling study of structural effects, docking and drug binding to cyclcodextrins has been reported by Tong et al. [8] and Amato et al. [9]. Other molecular mechanics studies [10–12] and semiempirical quantum chemistry studies [13, 14] of cyclodextrins and their complexes [15–18] have also been reported. Hydrophobicity potential profiles for cyclodextrins have been described by Lichtenhalter and Immel [19]. Reconsideration of the flexibility of β-CD has been published very recently [20].

The apparent molar volume of cyclodextrins determined by density measurements in aqueous solutions [21] showed close agreement with the volume computed by molecular modeling methods, which indicates that water penetrates the central cavity easily [19]. It is generally assumed that the host molecule binding in the inclusion complexes with cyclodextrins is controlled by (i) the steric requirements, (ii) the hydrophobic effects, (iii) entropic factors related to the release of water from the cyclodextrin cavity into the bulk phase. For more details see the exhaustive review by Connors [2]. However, to the best of our knowledge no detailed study dealing with the nature of interactions in the β-CD cavity interior has been performed so far.

Therefore, in this report we have focused on the analysis of the nature of guest molecule–host β-CD interactions present in the inclusion complexes. For this purpose we have selected a group of small host molecules (probes) such as: H_2O, C, CH_4, C_6H_6, NH_4^+, $HCOO^-$ which includes hydrophilic and hydrophobic, neutral and charged models of the side chains of amino acid residues and/or common functional groups in biologically active compounds. In a computer simulation these probes have been passed through the center of the β-CD ring cavity and the potential energy curves of the guest-host interaction were analyzed in the framework of



Substituent in R_1, R_2:	Substituent Notation:	β-CD Notation:
–H	H	A
$-CH_2-CH(OH)-CH_3$	OH	B, C
$-CH_2-CH(NH_2)-CH_3$	NH	D, E
$-CH_2-COO^-$	CO	F, G
$-CH_2-COO^-\cdots NH_4^+$	CN	G, I

Figure 1. Structures of the considered β-CDs consisting of seven α-D-glucopyranose residues and various substituents H—CN in positions R_1, R_2 distributed over residues $r-1-r_7$ (Table I). Side view of the toroid representation indicating also the directionality of the central axis.

a molecular mechanics energy scheme. The affinity of the probes to form inclusion complexes with the β-CDs was compared to their interaction with the bulk solvent. Interaction of the probes with non-substituted β-CD was compared to that of β-CD with various degrees of O3, O6 substitution and the use of different substituents (Figure 1, Table I). Averaged structures of the non-substituted β-CD from a molecular dynamics simulation in vacuum and in water at 25 °C, that correspond closer to the real structural form of the cyclodextrin encountered by a host molecule during molecular interaction either in the gas phase or in solution, were also considered.

2. Computational Methods

Molecular mechanics simulations on the free non-substituted and substituted β-CDs were carried out with the Discover program (version 2.8.7) [22] using an all-atom model for the β-CDs and the probes. Consistent-valence force field (CVFF) [23] and atomic charges, without non-bonding interaction cutoff were used consistently throughout this study. The crystal structure [24] of non-substituted β-CD was relaxed using an effective dielectric constant of 4 to account for the dielectric shielding. Initial optimization of hydrogen atom positions was followed

Table I. Notation of considered non-substituted and substituted β-cyclodextrins.

Notation		r_1[a]		r_2		r_3		r_4		r_5		r_6		r_7	
		R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
β-CD	**A**[b]	H[c]	H	H	H	H	H	H	H	H	H	H	H	H	H
β-CD$_{7,OH}$	**B**	OH	H	OH	H	OH	H	OH	H	H	OH	H	OH	H	OH
β-CD$_{14,OH}$	**C**	OH	OH	OH	OH	OH	OH	OH	OH	OH	OH	OH	OH	OH	OH
β-CD$_{7,NH}$	**D**	NH	H	NH	H	NH	H	NH	H	H	NH	H	NH	H	NH
β-CD$_{14,NH}$	**E**	NH	NH	NH	NH	NH	NH	NH	NH	NH	NH	NH	NH	NH	NH
β-CD$_{7,CO}$	**F**	CO	H	CO	H	CO	H	CO	H	H	CO	H	CO	H	CO
β-CD$_{7,CN}$	**G**	CN	H	CN	H	CN	H	CN	H	H	CN	H	CN	H	CN
β-CD$_{4,CO}$	**H**	CO	H	H	H	CO	H	H	H	H	CO	H	H	H	CO
β-CD$_{4,CN}$	**I**	CN	H	H	H	CN	H	H	H	H	CN	H	H	H	CN

[a] r_1 denotes individual α-D-glucopyranose residues in the β-CDs, R_1 is the substituent at the O3 oxygen, R_2 is the substituent at the O6 oxygen of α-D-glucopyranose residue.
[b] Notation of the β-CDs in Figure 1. [c] Symbols represent individual substituents in R_1 and R_2 of the β-CDs (Figure 1), H = hydrogen atom, OH = —CH_2—CH(OH)—CH_3, NH = —CH_2—CH(NH)—CH_3, CO = —CH_2—COO^-, CN = —CH_2—$COO^- \cdots NH_4^+$.

by steepest descent and conjugate gradient minimizations of the whole β-CD molecule till convergence at the gradient of 0.04 kJ mol^{-1} Å^{-1} was reached. Substituted β-CDs were built from the minimized structure of non-substituted β-CD by adding molecular fragments of various structure to the O3 and O6 oxygens in the selected α-D-glucopyranose residues of the β-CD ring using the Biopolymer module of the InsightII program [22]. The starting orientation of the substituents was built by assuming the initial conformation with the torsion angles of $\chi(C_3—O_3) = 90°$, $\zeta(O_3—C) = 180°$ and $\chi(C_5—C_6) = 90°$, $\zeta(C_6—O_6) = 180°$. Molecular dynamics simulations of the non-substituted β-CDs were carried out for the isolated β-CD molecule ($\epsilon = 4$) and for β-CD hydrated by 133 water molecules in a periodic box for 100 ps at a temperature of 300 K. Averaged MD structures in vacuo and in water $(\beta\text{-CD})_{av}$ were obtained by averaging the β-CD frames over the last 80 ps of the data collection trajectory.

To analyze in detail the nature of intermolecular interactions between the probe approaching the center of the β-CD ring cavity (defined as the center of mass of the seven glycosidic oxygens) in a perpendicular direction to the plane of the host molecule (i.e., along the seven-fold symmetry axis of the β-CD ring) we have simulated the interaction energy profiles along this path. The profile of interaction energy, E_{int}, calculated for a series of fixed positions of the guest molecule along this path, was composed from electrostatic, E_{coul}, and dispersion-repulsion terms, $E_{d,r}$, as defined in the CVFF forcefield. In this relatively simple approach the probe

orientation was preserved and the molecular geometries of the probe and β-CD were kept fixed at each point of the path. The orientation of the probe was selected to resemble a side chain in a larger molecular structure, e.g. a benzene molecule was placed with its 1,4-C—H bonds onto the path axis. The effect of flexibility and the presence of solvent in the host molecule is considered by calculation of probe profiles along the symmetry axis of averaged $(\beta\text{-CD})_{an}$ and $(\beta\text{-CD})_{as}$ structures. However, adaptation of the host molecule to the guest has not been taken into account in this paper.

The solvation Gibbs free energy, G_{solv}, of the considered probes was calculated in the framework of the Polarizable Continuum Model [25, 26] with the dielectric constant of 80 representing water as the solvent and includes electrostatic, dispersion-repulsion and cavitation terms.

3. Results

3.1. NON-SUBSTITUTED β-CYCLODEXTRIN

The energy minimized structure of the β-CD forms a ring (Figure 2A) with an inner diameter of approximately 10.1 Å (distance measured from the glycosidic bond oxygen connecting r_i and r_i+1 α-D-glucopyranose residues to the glycosidic bond oxygen of r_i + 3 and r_i + 4 or r_i + 4 and r_i + 5 residues) and a height of about 5.5 Å (distance measured from O3 to O6 oxygen in the same residue). The inner ring diameter and the ring height determine the size of the host cavity. Thus β-CD can accommodate smaller molecules with a diameter up to approx. 4.0 to 5.0 Å, assuming that a close contact of the molecular surfaces of the host and guest molecules puts the limit on the guest molecule size. Our series of probes contained small molecules: H_2O, C, CH_4, C_6H_6, NH_4^+, $HCOO^-$ that can easily fit into the β-CD ring cavity, therefore no sterical factors have affected the simulated guest–host interaction energy profiles. During the simulations of the inclusion complex formation probes have penetrated into the empty β-CD cavity, i.e., no water molecules have been displaced by the entering probe. The energy profiles thus reflect the net effect of the guest–host non-bonding interaction forces and are analyzed in terms of coulombic and dispersion–repulsion interaction energy components.

The interaction energy profiles of the probes entering the cavity of non-substituted β-CD (Figure 2A) along the path perpendicular to the β-CD ring plane are shown in Figure 3. The guest–host interaction energy, E_{min}, for the energy minimum point on the path (R_{min}, is the distance of the probe center from the center of the β-CD cavity) are given in Table II together with the coulombic component, E_{coul} and the dispersion–repulsion component, $E_{d,r}$. The interaction energy profile for the water probe (Figure 3), where the symmetry axis of H_2O was parallel to the β-CD axis, is a non-symmetric curve with a single minimum at $R_{min} \approx -0.5$ Å with a depth of $E_{min} = -10.0$ kJ mol^{-1} and two maxima at each side of the β-CD ring, i.e., barriers with the height of E_{bar}^{-} = 2.1 kJ mol^{-1}

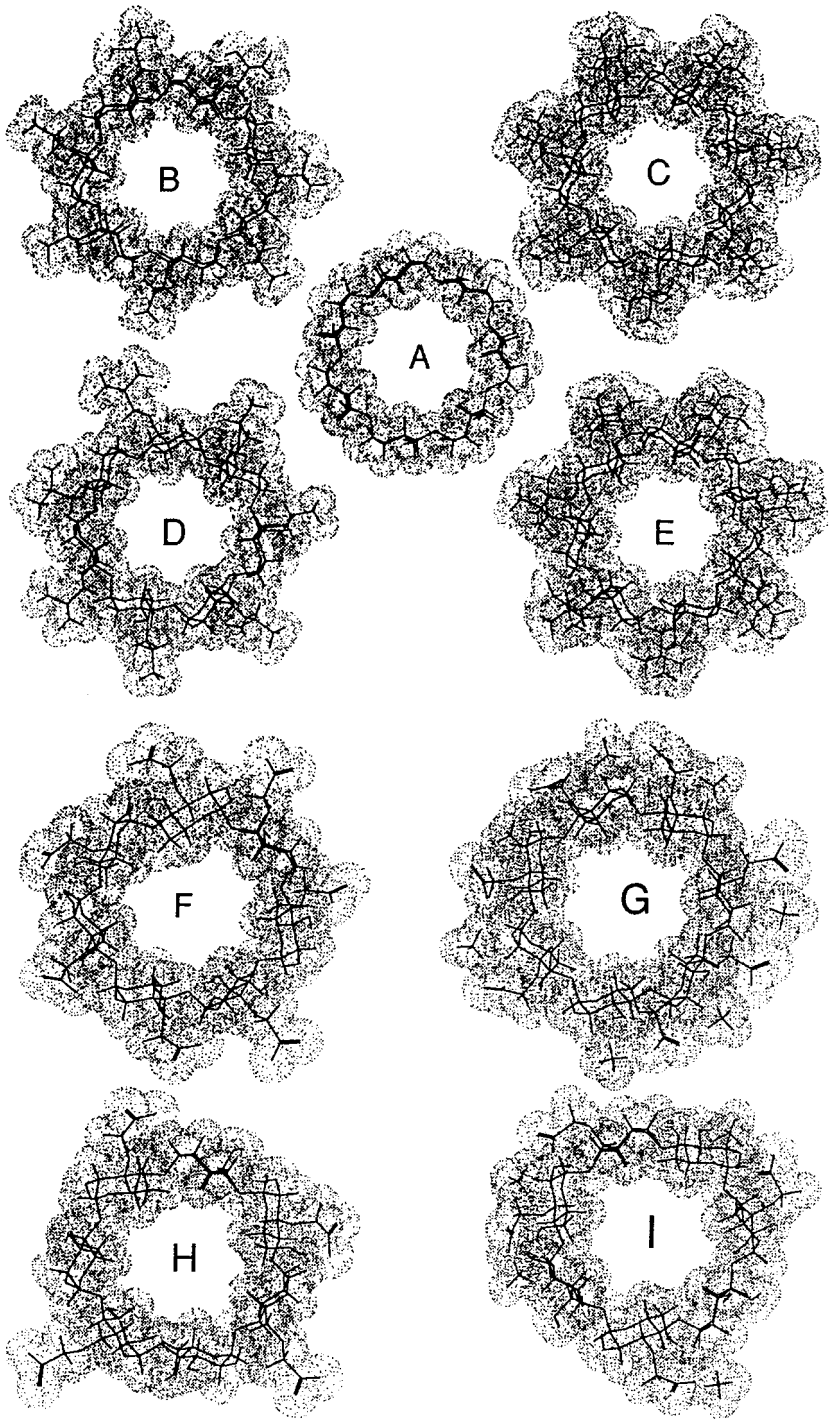


Figure 2. Dotted van der Waals molecular surface of models of substituted β-CDs illustrating the symmetry and size of the ring cavity dependent on the number, type and positions of substituents in positions R_1, R_2 (Table I).

Table II. Interaction energies of probes with non-substituted β-CD and solvation Gibbs free energy in water.

Probe	$R_{\min}$[a] β-CD	E_{coul} β-CD	$E_{d,r}$ β-CD	$E_{\min}$[b] β-CD	G_{solv}[c] β-CD
H_2O	−0.5	−5.0	−5.0	−10.0	−23.4
C[d]	0.0	0.0	−6.7	−6.7	−1.3
CH_4	0.0	0.0	−12.6	−12.6	−9.6
C_6H_6	−0.5	−0.4	−61.1	−61.5	−17.6
NH_4^+	−4.5	−90.0	−2.9	−92.9	−197.1
$HCOO^-$	3.5	−25.9	−10.0	−36.0	−193.3

[a] $R_{\min}$ is the distance of the center of probe to the center of the β-CD cavity, in [Å].
[b] $E_{\min}$ is the total interaction energy, $E_{\text{int}} = E_{\text{coul}} + E_{d,r}$, at the position $R_{\min}$, E_{coul} is the coulombic component, and $E_{d,r}$ is the dispersion–repulsion component, all energies in [kJ mol^{-1}].
[c] Solvation Gibbs free energy of the probes in water estimated by the Polarizable Continuum Model [17, 18] of solvation, in [kJ mol^{-1}].
[d] Neutral carbon atom.

at the O6 ring side at the point $R \approx -7.0$ Å and E_{bar}^{+} = 0.4 kJ mol^{-1} at the O2, O3 ring side, $R \approx 10.0$ Å. It follows from this profile that the inclusion complex of β-CD with one water molecule, located in the very center of the ring cavity, is stabilized by the interaction energy of $E_{\min} = -10.0$ kJ mol^{-1}, but the molecule is trapped in the cavity by the energy of -10.5 kJ mol^{-1}. The coulombic interaction and the dispersion–repulsion interaction both contribute about equally to the total interaction energy and both contributions show a minimum value at $R_{\min}$ = 0.5 Å. The interaction energy of the water–β-CD complex formation and the related barrier heights represent only estimated values since the geometries were kept fixed and no mutual structural adjustment of the guest and host molecule was considered at this stage. The predicted interaction energy profiles and minima locations will obviously depend on the orientation of the probe towards the β-CD axis (especially for polar and non-symmetric probes). The interaction energy profiles of three hydrophobic probes C, CH_4 and C_6H_6 with the non-substituted β-CD show similar features (Figure 3). These profiles are represented by almost symmetric curves with a single minimum close to the cavity center ($R \approx 0.0$ Å) with the interaction energies of the inclusion complex formation for C, CH_4 and C_6H_6 of -6.7 kJ mol^{-1}, -12.6 kJ mol^{-1}, and -61.5 kJ mol^{-1}, respectively, which are approximately proportional to the size of the probe. The interaction energies of these hydrophobic probes with the non-substituted β-CD are composed entirely from dispersion–repulsion interactions in both the short and long range portion

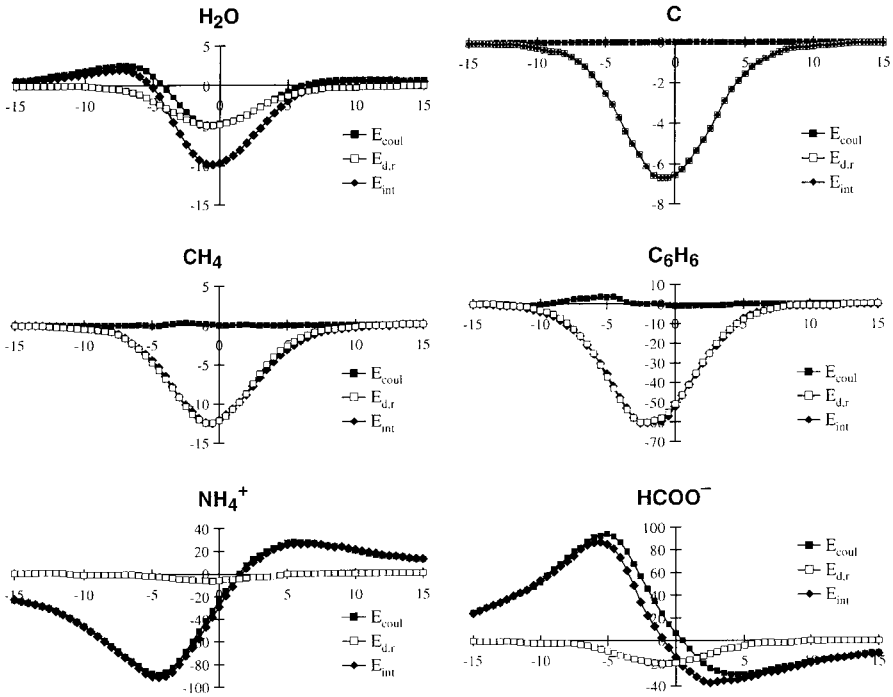


Figure 3. Interaction energy profiles of simple probes entering the cavity of non-substituted β-CD along the symmetry axis perpendicular to the ring plane (energy on y-axis is in [kJ/mol], coordinate on x-axis with the origin placed in the cavity center is in [Å]).

of the reaction path. The coulombic component of the interaction energy cancels out due to the symmetry of the neutral probes (CH_4, C_6H_6) and the β-CD ring (induction effects were completely neglected).

The interaction energy profiles of the charged hydrophilic probes NH_4^+ and $HCOO^-$ show also some degree of similarity. They are both non-symmetric, contain a barrier with a maximum close to the β-CD cavity opening, e.g. for the NH_4^+ probe E_{bar}^+ = 26.4 kJ mol^{-1} at the O6 ring side, $R \approx 6.0$ Å and for the $HCOO^-$ probe E_{bar}^- = 87.9 kJ mol^{-1} at the O2, O3 ring side, $R \approx -5.5$ Å and a minimum at the opposite side of the β-CD ring opening (outside the cavity) e.g. for the NH_4^+ probe $E_{\min} = -92.9$ kJ mol^{-1} at the O6 ring side, $R \approx -4.5$ Å and for the $HCOO^-$ probe $E_{\min} = -36.0$ kJ mol^{-1} at the O2, O3 ring side, $R \approx 3.5$ Å. Both interaction energy curves are dominated by the coulombic component, a minor stabilizing contribution to the total interaction energy from the dispersion–repulsion interaction comes inside the cavity ($R \approx 0.0$ Å), for the NH_4^+ probe $E_{d,r} = -7.1$ kJ mol^{-1} and a somewhat larger contribution for the $HCOO^-$ probe $E_{d,r} = -20.5$ kJ mol^{-1}. This means that the non-substituted β-CD displays a non-symmetric molecular electrostatic potential (MEP) along the pseudo-symmetry axis at the O2, O3 side and on the O6 side of the ring. The interaction energy profile for a simple

ion H^+ (Figure 4 A) reveals that the β-CD maintains a MEP minimum at the O6 side of the ring in its symmetry axis of magnitude $V_{min} = -89.5$ kJ mol^{-1} e^{-1} at a position from the cavity center $R_{min} = -4.5$ Å, while on the O2, O3 side of the ring the β-CD holds a MEP maximum in the symmetry axis of the magnitude V_{max} = 27.6 kJ mol^{-1} e^{-1} at a position from the cavity center R_{max} = 6.0 Å (Table IV). Therefore, in contrast to the neutral small hydrophobic molecules that are attracted towards the β-CD cavity center by dispersion forces the polar or charged molecules tend to adhere to the O2, O3 face (negatively charged species) or to the O6 face (positively charged species) of the β-CD ring captivated by the electrostatic forces. The MEP at the cavity center is relatively low, $V_{cen} = -19.7$ kJ mol^{-1} e^{-1} and the electric field intensity at the cavity center $\mathbf{E}_{cen}$ is oriented fully along the β-CD symmetry axis ($E_{cen}(z) = |\mathbf{E}_{cen}|$, Table IV).

It has to be stressed at this point that small molecules such as the probes considered here posses enough freedom to adjust their position and orientation inside the cavity and at the faces of the β-CD ring, therefore, the interaction energies E_{min} given in this report for the fixed probe orientation and fixed molecular geometries of the guest and host molecules in the inclusion complexes represent only a rough approximation of the complexation energies. On the other hand, in solution most of the hydroxyl groups of β-CD will be engaged in intra- or intermolecular hydrogen bonding with water molecules and therefore will not be entirely exposed towards the approaching probe. Nevertheless, our approach describes the essence of β-CD inclusion complexes formation and enables a consistent comparison between the host molecules of different charge, symmetry and hydrophobicity which can assume a number of positions and orientations in the inclusion complexes.

The formation of an inclusion complex in solution will depend on the competition between the stabilization of the probe inside of the β-CD cavity and hydration. Therefore, in order to predict the existence and estimate the stability of the inclusion complexes, interaction energies at the bound state of the energy profile, E_{min} (which approximately correspond to the energy of inclusion complex formation, neglecting the entropic contribution to the complexation reaction) should be compared to the solvation Gibbs free energies in water, G_{solv}, for the considered probes, Table II. This comparison reveals that polar and charged molecules (such as H_2O, NH_4^+, $HCOO^-$) will prefer the hydrophilic solvent (water) environment to the hydrophobic β-CD cavity and the hydrophobic non-polar probes (such as C, CH_4, C_6H_6) will rather prefer the β-CD cavity interior to the bulk solvent.

3.2. Molecular dynamics simulations of non-substituted β-cyclodextrin

The non-substituted β-CD considered in the previous paragraph was obtained by relaxation of the crystal structure in vacuum, neglecting any deformations by thermal motions of the ring (vibrations, etc.) and represents an ideal structure of the β-CD. We have considered also more realistic structures of the cyclodextrin

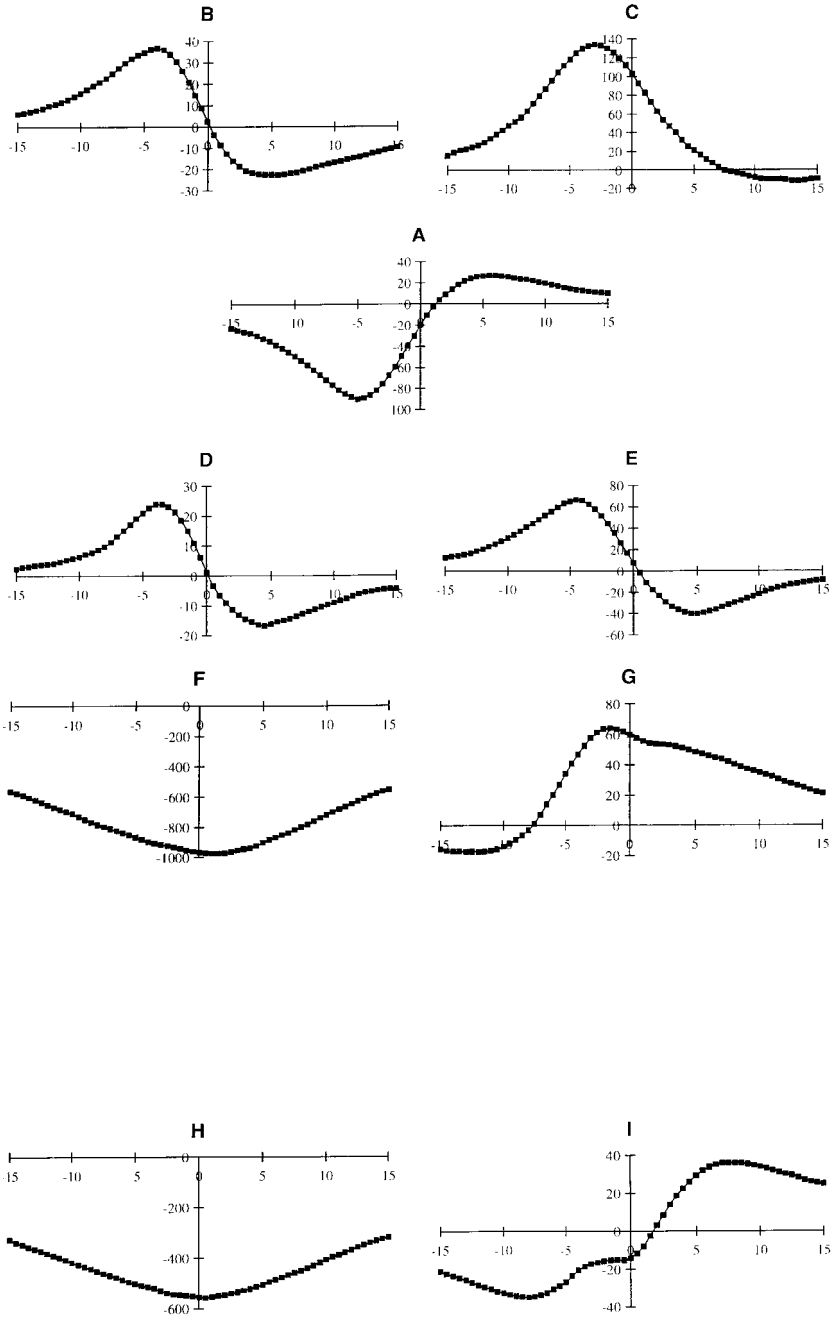


Figure 4. Molecular electrostatic potential profiles along the pseudo-symmetry axis perpendicular to the ring plane of substituted β-CDs (see Table I, Figure 1), (MEP on y-axis is in $-$kJ mol^{-1} e^{-1}, coordinate on x-axis with the origin placed in the cavity center is in [Å]).

Table III. Interaction energies of probes with non-substituted β-CD structure averaged over MD simulation trajectory in vacuum (β-CD_{av}) and with non-substituted β-CD structure averaged over MD simulation trajectory in water (β-CD_{as}).

Probe	R_{min}[a]		E_{coul}		$E_{d,r}$		E_{min}[b]	
	β-CD_{av}	β-CD_{as}	β-CD_{av}	β-CD_{as}	β-CD_{av}	β-CD_{as}	β-CD_{av}	β-CD_{as}
H_2O	−0.5	−1.5	−12.6	0.0	−5.0	−8.8	−6.7	−8.8
C[c]	0.0	0.0	0.0	0.0	−7.1	−11.3	−7.1	−11.3
CH_4	0.0	0.0	0.0	0.0	−13.4	−21.8	−13.4	−21.8
C_6H_6	−0.5	−0.5	0.4	0.8	−64.0	−91.2	−63.6	−90.4
NH_4^+	−3.5	−2.5	−43.5	−47.7	−5.0	−11.7	−48.5	−59.4
$HCOO^-$	4.0	–[d]	−9.2	–[d]	−5.9	–[d]	−15.1	–[d]

[a] R_{min} is the distance of the center of probe to the center of the β-CD cavity, in [Å].

[b] E_{min} is the total interaction energy, $E_{int} = E_{coul} + E_{d,r}$, at the position R_{min}, E_{coul} is the coulombic component, and $E_{d,r}$ is the dispersion–repulsion component, all energies in [kJ mol^{-1}].

[c] Neutral carbon atom.

[d] No minimum found on the interaction energy curve.

Table IV. Molecular electrostatic potential of β-CDs at the extreme points in their symmetry axis.

				O6- Face of β-CD		O2,O3- Face of β-CD	
β-CDs[a]	V_{cen}[b]	$E_{cen}(z)$[c]	$\lvert E_{tot}\rvert$[d]	V_{ext}[e]	R_{ext}[f]	V_{ext}[e]	R_{ext}[f]
β-CD	−19.7	−23.8	23.8	−89.5	−4.5	27.6	6.0
β-$CD_{7,OH}$	2.5	12.6	16.7	37.2	−4.0	−22.2	4.5
β-$CD_{14,OH}$	99.6	19.2	19.2	134.3	−3.0	−11.7	12.0
β-$CD_{7,NH}$	0.8	9.2	14.6	23.8	−3.5	−17.2	4.5
β-$CD_{14,NH}$	20.5	20.1	20.1	66.5	−4.5	−40.6	5.0
β-$CD_{7,CO}$	−962.3	13.0	14.6	–[g]	–[g]	−985.3	1.0
β-$CD_{7,CN}$	59.4	3.8	9.6	63.6	−2.5	–[g]	–[g]
β-$CD_{4,CO}$	−553.5	5.0	13.0	–[g]	–[g]	−560.7	1.0
β-$CD_{4,CN}$	−13.4	−3.8	5.0	−35.6	−7.5	35.6	7.5

[a] For the notation of the substituted β-CDs see Table I.
[b] V_{cen} is MEP of the β-CD molecule in the cavity center in [kJ mol^{-1} e^{-1}].
[c] $E_{cen}(z)$ is the electrostatic field intensity vector component in the direction of the 7-fold symmetry axis of β-CD molecule in the cavity center, in [kJ mol^{-1} e^{-1} $Å^{-1}$].
[d] $|E_{tot}|$ is the magnitude of the electrostatic field intensity vector in the cavity center of β-CD molecule, in [kJ mol^{-1} e^{-1} $Å^{-1}$].
[e] V_{ext} is MEP of the β-CD molecule in its 7-fold symmetry axis at R_{ext}, its maximum or minimum value, in [kJ mol^{-1} e^{-1}].
[f] R_{ext} is the position of the MEP maximum or minimum at the symmetry axis, origin is in the center of the β-CD cavity, in [Å].
[g] No minimum or maximum found within ±15.0 Å from the β-CD.

in the gas phase or in solution obtained from molecular dynamics simulation at 300 K in vacuum and in water, considering 133 solvent molecules. The β-CD structures averaged over the evolution time of an MD simulation, which include implicit effects of the most frequent deformations, as well as deformations caused by interactions with the solvent, represent thus the more realistic alternative. The guest–host interaction energies, E_{min}, for the energy minimum point on the interaction energy profiles of the probes approaching the β-CD structure, averaged in vacuum (β-CD_{av}) and the β-CD structure averaged in the solvent (β-CD_{as}), are given in Table III. Comparison of the interaction energy values in the energy profile minimum, E_{min}, location of the minimum, R_{min}, and the components of the total interaction energy, E_{coul}, $E_{d,r}$, of the fully symmetric static β-CD inclusion complex (β-CD : P) with the averaged complex structures β-CD_{av} : P and β-CD_{as} : P (Tables I, III) leads to the conclusion that both averaged β-CD structures, but especially the β-CD_{as}, enhance the stabilizing interactions with the hydrophobic probes (C, CH_4, C_6H_6) that are caused by fluctuations of the β-CD ring and in the case of the β-CD_{as} structure also by the external pressure of solvent molecules, all leading to more intimate dispersive-repulsive interactions. This significantly decreases also the stabilizing interactions with the charged probes with

respect to the static β-CD structure. The decrease in electrostatic interaction is partially caused by the deviation of the averaged structures from the ideal static symmetry of the β-CD, and to some extent also by the averaged locations of hydrogen atoms. In particular the relatively free rotating hydroxyl groups, and to some extent the different orientation of the -OH groups forming hydrogen bonds with the bulk solvent in the MD averaged structures are the main cause for the observed differences.

3.3. SUBSTITUTED β-CYCLODEXTRINS WITH NEUTRAL SIDE CHAINS

The presence of the substituents on the β-CD ring modifies the affinity of β-CD to accommodate guest molecules in the ring cavity interior in the inclusion complexes. Thus, the structure and properties of the substituent, the number of the substituents, the positions of the α-D-glucopyranose residues to which substituents are attached in the β-CD, and the atomic position within the α-D-glucopyranose ring to which the substituent is bound, etc. will determine the β-CD structure as well as the affinity towards a particular guest molecule.

We have considered two similar substituent structures, namely: —CH_2—CH(OH)—CH_3 (denoted in the following text as the —OH substituent) and —CH_2—CH(NH_2)—CH_3 (denoted as the —NH substituent) and four different substituted β-cyclodextrins. Two β-CDs containing either the —OH or —NH substituent with a total of 7 attached substituents (3 substituents attached to the O6 oxygens of residues $r_1 - r_3$, and 4 substituents attached to O3 oxygens of residues $r_4 - r_7$, β-$CD_{7,OH}$ and β-$CD_{7,NH}$), Figure 2B and D, and two β-CDs containing either the —OH or —NH substituent with the total of 14 attached substituents (2 substituents attached to O3 and O6 oxygens per each residue $r_1 - r_7$, β-$CD_{14,OH}$ and β-$CD_{14,NH}$), Figure 2C and E. Each of the substituted β-CDs contains substituents of only one kind and no mixed substitutions were considered. The type and position of substituents have been selected because of the use in our group of similar β-CD derivatives for biomedical applications [27, 28].

The effect of the substituent structure upon the affinity of β-$CD_{7,OH}$ (Figure 5) and β-$CD_{7,NH}$ towards the probes (Tables V, VI) is only modest for H_2O and the hydrophobic probes (C, CH_4, C_6H_6). As expected, addition of substituents causes for both —OH and —NH type substituents a minor increase in the interaction energy with the hydrophobic probes which are controlled almost entirely by the dispersion–repulsion interactions. Obviously, since the dispersion interaction is attractive for any pair of atoms, any substitution of the β-CDs will lead to higher stability of the inclusion complexes with hydrophobic hosts. On the other hand, the substitution has a rather dramatic effect on the interaction energy with the charged probes. It follows from the MEP profiles along the pseudo-symmetry axis that a significant dependence exists on the number and type of substituent in the β-CD molecule, (Figure 4, Table IV). MEP curves of the 7-times substituted β-cyclodextrins β-$CD_{7,OH}$ and β-$CD_{7,NH}$ (Figure 4B, D) show a reversed profile

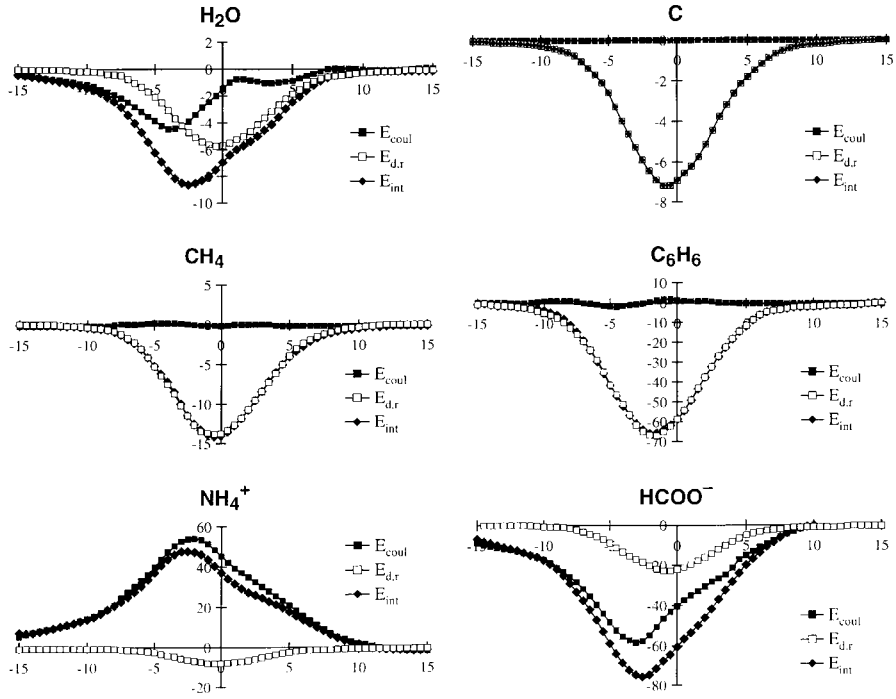


Figure 5. Interaction energy profiles of simple probes entering the cavity of substituted β-$CD_{7,OH}$ along the pseudo-symmetry axis perpendicular to the ring plane (energy on y-axis is in [kJ/mol], coordinate on x-axis with the origin placed in the cavity center is in [Å]).

compared to the non-substituted β-CD and lower V_{min}, V_{max} magnitudes (in absolute values). Thus in the substituted β-CDs the charged molecules will be more attracted to the opposite β-CD ring face compared to the non-substituted β-CD, however, the bulk hydration will be preferred even more. Both the —OH and —NH substituents favor the interaction with the $HCOO^-$ probe due to the complementarity of the $—OH^+ \cdots {}^-O—C—$ or $—NH^+ \cdots {}^-O—C—$ interaction and disfavor the interaction with the NH_4^+ probe mainly due to repulsive $—OH^+ \cdots {}^+H—N—$ or $—NH^+ \cdots {}^+H—N—$ interaction (Tables V, VI). For example, no bound state was found on the interaction energy profile of the β-$CD_{7,OH}$ and the NH_4^+ probe.

The effect of the number of substituents increasing from 7 to 14 in the β-$CD_{14,OH}$ and β-$CD_{14,NH}$ structures decreases the affinity towards the H_2O probe and has a negligible stabilizing effect upon the interaction with the hydrophobic probes (Table V). Increasing the number of the substituents does not change the overall shape of the MEP profile but significantly enhances the interaction with the charged probes and the MEP magnitudes in the cavity center. It also changes the electric field intensity vector orientation in the cavity center to the direction parallel with the β-CDs pseudo-symmetry axis (Table IV). Doubling the number of

Table V. Interaction energies of probes with substituted β-$CD_{7,OH}$ and β-$CD_{14,OH}$ structure containing neutral side chains.

Probe	R_{min}[a]		E_{coul}		$E_{d,r}$		E_{min}[b]	
	β-$CD_{7,OH}$	β-$CD_{14,OH}$	β-$CD_{7,OH}$	β-$CD_{14,OH}$	β-$CD_{7,OH}$	β-$CD_{14,OH}$	β-$CD_{7,OH}$	β-$CD_{14,OH}$
H_2O	−2.0	−4.5	−3.8	−3.3	−5.0	−3.3	−8.8	−6.7
C[c]	0.0	0.0	0.0	0.0	−7.1	−7.5	−7.1	−7.5
CH_4	0.0	0.0	0.0	0.0	−14.2	−14.2	−14.2	−14.2
C_6H_6	−0.5	−0.5	0.4	−2.1	−66.5	−64.4	−66.1	−66.5
NH_4^+	_[d]	_[d]	_[d]	_[d]	_[d]	_[d]	_[d]	_[d]
$HCOO^-$	−2.5	−3.5	−56.5	−139.3	−19.2	−18.0	−75.7	−157.3

[a] R_{min} is the distance of the center of probe to the center of the substituted β-CD cavity, in [Å].

[b] E_{min} is the total interaction energy, $E_{int} = E_{coul} + E_{d,r}$, at the position R_{min}, E_{coul} is the coulombic component, and $E_{d,r}$ is the dispersion–repulsion component, all energies in [kJ mol^{-1}].

[c] Neutral carbon atom.

[d] No minimum found on the interaction energy curve.

Table VI. Interaction energies of probes with substituted β-$CD_{7,NH}$ and β-$CD_{14,NH}$ structure containing neutral side chains.

Probe	R_{min}[a]		E_{coul}		$E_{d,r}$		E_{min}[b]	
	β-$CD_{7,NH}$	β-$CD_{14,NH}$	β-$CD_{7,NH}$	β-$CD_{14,NH}$	β-$CD_{7,NH}$	β-$CD_{14,NH}$	β-$CD_{7,NH}$	β-$CD_{14,NH}$
H_2O	−2.5	−4.0	−2.5	−1.3	−4.6	−3.8	−7.1	−5.0
C[c]	0.0	0.0	0.0	0.0	−7.1	−7.5	−7.1	−7.5
CH_4	0.0	0.0	0.0	0.0	−13.4	−14.2	−13.4	−14.2
C_6H_6	−0.5	−0.5	0.4	−1.7	−61.9	−64.9	−59.4	−66.5
NH_4^+	3.5	5.5	−16.3	−38.1	−3.3	−1.7	−19.7	−39.7
$HCOO^-$	−3.5	−4.0	−25.9	−69.0	−15.1	−15.9	−41.0	−84.9

[a] R_{min} is the distance of the center of probe to the center of the substituted β-CD cavity, in [Å].

[b] E_{min} is the total interaction energy, $E_{int} = E_{coul} + E_{d,r}$, at the position R_{min}, E_{coul} is the coulombic component, and $E_{d,r}$ is the dispersion–repulsion component, all energies in [kJ mol^{-1}].

[c] Neutral carbon atom.

[d] No minimum found on the interaction energy curve.

substituents increases the coulombic component of the interaction energy (Tables V and VI) more than twice, although the solvent effects still prevail (Table II).

3.4. SUBSTITUTED β-CYCLODEXTRINS WITH CHARGED SIDE CHAINS

Besides the β-CDs substituted by neutral side chains we have considered also substitution by a charged substituent, namely: —CH_2—COO^- and —CH_2—$COO^- \cdots NH_4^+$ (denoted in the following text as the —CO and —CN substituents) which contain an ionized carboxylic group and a carboxylic group neutralized by an ammonium cation (located between two neighboring carboxylic groups and shared also by O_2 hydroxyl and glycosidic oxygens). Four variations of β-CDs with charged substituents have been studied, namely two β-CDs containing either the —CO or —CN substituent with 7 substituents (3 substituents attached to the O_6 oxygens of residues $r_1 - r_3$, and 4 substituents attached to O_3 oxygens of residues $r_4 - r_7$, β-$CD_{7,CO}$ and β-$CD_{7,CN}$), Figure 2F and G; and two β-CDs containing either the —CO or —CN substituent with 4 substituents (2 substituents attached to O_3 oxygens of residues r_1, r_3 and 2 substituents attached to O_6 oxygens of residue r_5, r_7, β-$CD_{4,CO}$ and β-$CD_{4,CN}$), Figures 2H and I. Each of the substituted β-CDs contains substituents of only one kind.

The effect of the —CO, —CN substituent structure upon the affinity of β-CDs towards the probes, Tables VII, VIII, is again only modest in all the considered β-CDs for the hydrophobic probes (C, CH_4, C_6H_6). The substitution causes only a minor increase in the interaction energy with the hydrophobic probes compared to the non-substituted β-CD that is roughly proportional to the number of attached side chains. It, however, places some steric constraints upon the inclusion of larger probes (such as C_6H_6) since some deformation of the β-CD rings was observed due to the presence of four highly polar substituents, e.g. in the β-$CD_{4,CN}$, compared to the ideal fully symmetric β-CD ring structure.

The MEP profiles along the pseudo-symmetry axis of β-CDs with charged substituents (Figure 4F–I) show a strong dependence of the MEP profile shape and magnitudes on the substituent structure and the number of substituents (Table IV). The fully ionized acidic β-CDs such as β-$CD_{7,CO}$ and β-$CD_{4,CO}$ with the net charge of 7e and 4e, respectively, represent only models of extreme cases that describe the highest possible effect of the charged side chains (these β-CDs will be fully solvated and their charge neutralized by counterions in a polar solvent with non-zero ionic strength). Not surprisingly, both these β-CDs posses a deep MEP minimum in the cavity center and strongly attract the oppositely charged NH_4^+ probe and repel the $HCOO^-$ probe with the negative charge (Table VII). The polar H_2O probe is only weakly attracted by these β-CDs at a point more than 12.0 Å apart from the cyclodextrins at the O2, O3 ring face side. In the more realistic structures of the β-CDs with charged substituents forming ion pairs, β-$CD_{7,CN}$ and β-$CD_{4,CN}$, that describe the other extreme case with fully neutralized charge of the ionized carboxylic groups, the MEP profile in the pseudo-symmetry axis depends

Table VII. Interaction energies of probes with substituted β-$CD_{4,CO}$ and β-$CD_{7,CO}$ structure containing neutral side chains.

Probe	R_{min}[a]		E_{coul}		$E_{d,r}$		E_{min}[b]	
	β-$CD_{4,CO}$	β-$CD_{7,CO}$	β-$CD_{4,CO}$	β-$CD_{7,CO}$	β-$CD_{4,CO}$	β-$CD_{7,CO}$	β-$CD_{4,CO}$	β-$CD_{7,CO}$
H_2O	12.0	12.0	−3.3	−6.7	0.0	0.0	−3.3	−6.7
C[c]	0.0	0.0	0.0	0.0	−7.5	−7.1	−7.5	−7.1
CH_4	0.0	0.0	0.0	0.0	−14.2	−13.8	−14.2	−13.8
C_6H_6	−0.5	−0.5	1.3	−2.1	−61.5	−65.7	−60.2	−63.6
NH_4^+	0.5	1.0	−555.2	−971.1	−7.1	−6.3	−562.3	−977.4
$HCOO^-$	_[d]	_[d]	_[d]	_[d]	_[d]	_[d]	_[d]	_[d]

[a] R_{min} is the distance of the center of probe to the center of the substituted β-CD cavity, in [Å].

[b] E_{min} is the total interaction energy, $E_{int} = E_{coul} + E_{d,r}$, at the position R_{min}, E_{coul} is the coulombic component, and $E_{d,r}$ is the dispersion–repulsion component, all energies in [kJ mol^{-1}].

[c] Neutral carbon atom.

[d] No minimum found on the interaction energy curve.

Table VIII. Interaction energies of probes with substituted β-$CD_{4,CN}$ and β-$CD_{7,CN}$ structure containing neutral side chains.

Probe	R_{min}[a]		E_{coul}		$E_{d,r}$		E_{min}[b]	
	β-$CD_{4,CN}$	β-$CD_{7,CN}$	β-$CD_{4,CN}$	β-$CD_{7,CN}$	β-$CD_{4,CN}$	β-$CD_{7,CN}$	β-$CD_{4,CN}$	β-$CD_{7,CN}$
H_2O	2.0	−3.5	−0.9	−0.9	−0.9	−1.0	−1.8	−1.9
C[c]	0.0	0.0	0.0	0.0	−1.8	−1.8	−1.8	−1.8
CH_4	0.0	0.0	0.0	0.0	−3.4	−3.2	−3.4	−3.2
C_6H_6	−0.5	−0.5	0.3	−0.5	−12.5	−15.2	−12.5	−15.7
NH_4^+	−7.5	−13.0	−8.3	−4.8	−0.2	0.0	−8.5	−4.8
$HCOO^-$	5.5	−2.0	−8.4	−15.7	−0.7	−4.9	−9.1	−20.8

[a] R_{min} is the distance of the center of probe to the center of the substituted β-CD cavity, in [Å].

[b] E_{min} is the total interaction energy, $E_{int} = E_{coul} + E_{d,r}$, at the position R_{min}, E_{coul} is the coulombic component, and $E_{d,r}$ is the dispersion–repulsion component, all energies in [kJ mol^{-1}].

[c] Neutral carbon atom.

[d] No minimum found on the interaction energy curve.

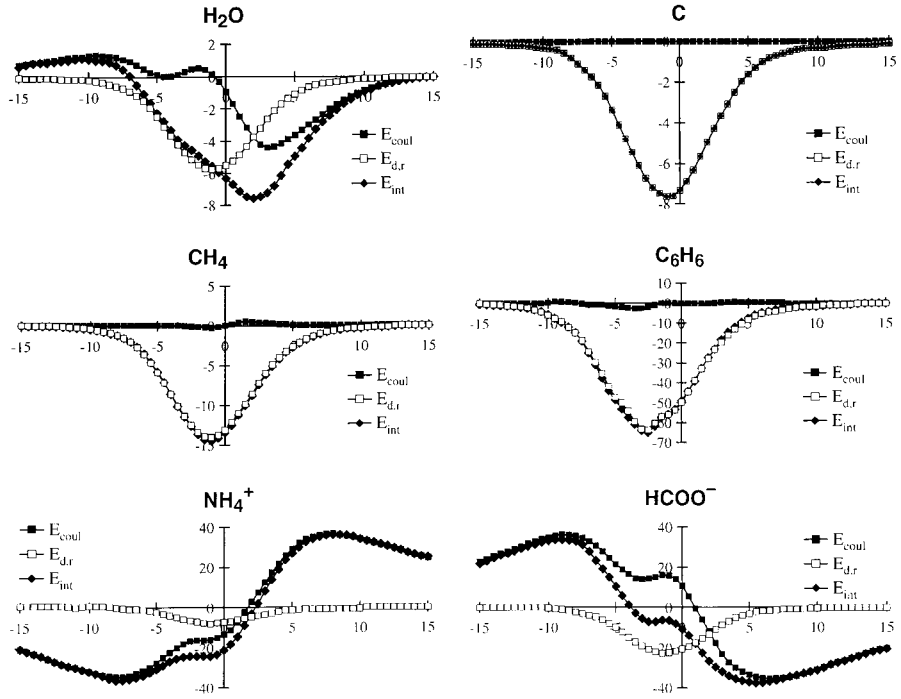


Figure 6. Interaction energy profiles of simple probes entering the cavity of substituted β-$CD_{4,CN}$ along the pseudo-symmetry axis perpendicular to the ring plane (energies are in [kJ/mol]).

significantly on the number and directionality of the substituents, which affect the symmetry of the β-CD (compare Figure 4A with Figures 4G and 4I). Four substitution in the β-$CD_{4,CN}$ (4I) causes some deformation and a decrease of the minimum and an increase of the maximum as compared to the original profiles (4A). Further substitution (β-$CD_{7,CN}$) with different orientation of the substituents leads to a larger deformation and shifts the maximum to $R_{max} = -2.0$ Å. As illustrated by the interaction energy profiles for the probes approaching the β-$CD_{4,CN}$ (Figure 6) the $HCOO^-$ probe is predicted to be attracted somewhat stronger to these two β-CDs and the NH_4^+ probe somewhat weaker than in the case of non-substituted β-CD (Table VIII), most probably due to attractive and repulsive interactions between the two probes and the positive ammonium counterions located at the β-CD ring cavity openings near the side chain ends. Bulk solvation of the charged probes still remains their preferred stabilization. The polar H_2O probe will interact with these models with about the same interaction energy as with the non-substituted β-CD.

4. Discussion

The interaction energy profiles of small molecular probes with non-substituted or substituted β-CDs along the symmetry axis of the β-CD ring (Figure 3, Table II) show that even a simple non-bonding potential energy function can describe the formation of guest-host inclusion complexes of "hydrophobic" probes residing in the cavity interior of the β-CD and predict the preferred formation of "hydrophobic" inclusion complexes rather than the "ion-dipole" or "dipole-dipole" complexes with probes trapped in the cavity or adhering to the β-CD ring faces. It should be kept in mind that this study is focused on the evaluation of interaction profiles passing along the internal cavity axis. If global minimization is performed by releasing the fixed coordinates in the position of minimum energy, then further stabilization is obtained (e.g. 15.1 kJ mol^{-1} for benzene). This, however, is not significant for the overall strategy adopted in the present investigation. Stabilizing interactions with hydrophobic probes (C, CH_4, C_6H_6) increase and stabilizing electrostatic interactions with polar and charged probes decrease when the flexibility of the β-CD ring is taken into account by the use of averaged structures $(\beta\text{-CD})_{av}$ and $(\beta\text{-CD})_{as}$.

Analysis of the net effects of molecular properties (such as total charge, polarity i.e. electronegativity of atoms composing the probe molecule, geometric symmetry, symmetry of charge distribution in the probes) upon the shape and magnitude of the interaction energy profiles with non-substituted β-CD which posses a high cylindrical symmetry of the electric field along its symmetry axis (Table IV), revealed that:

(i) the strongest effect on the interaction energy profile is imposed by the molecular charge (charged molecules tend to form "ion-dipole" type of complexes in vacuum);

(ii) for neutral probes it is the degree of symmetry of charge distribution, especially in the molecules with polar bonds (composed of atoms with higher electronegativity), that determines the existence of the "hydrophobic" inclusion complexes rather than "dipole-dipole" complexes. In other words high symmetry molecules with bonds of different polarity, such as CH_4, CF_4, SF_6 (results not shown for the last two molecules) and non-polar small molecules as well as lower symmetry non-polar small molecules tend to form "hydrophobic" inclusion complexes with the β-CD, while low symmetry polar molecules (with permanent dipole moments) tend rather to form "dipole-dipole" complexes.

Electrostatic interactions between a highly symmetric polar probe and the host molecule with high symmetry, such as β-CD, cancel out and the formation of a guest-host inclusion complex is dominated by the dispersion-repulsion interactions between the probe and the β-CD which reach the maximum stabilization at the closest distance, i.e. in the center of the β-CD ring cavity. For a low symmetry polar probe the guest-host interactions are dominated by the stronger electrostatic interaction which reaches the maximum stabilization at the points of minima of the MEP profile (located typically outside of the cavity at the pseudo-symmetry axis

near the β-CD ring faces). These non-symmetric probes thus form "dipole-dipole" complexes rather than the "hydrophobic" inclusion complexes.

With some degree of simplification we can equate the hydrophobic character of a neutral probe to the degree of symmetry of its charge distribution (taking into account the symmetry of the host system). Hence, it seems plausible to conclude that it is not the increasing dispersive interaction between the guest molecule and the β-CD cavity interior that controls the formation of hydrophobic inclusion complexes, as sometimes stated in the literature [29], but rather the cancellation of electrostatic interactions with the host β-CD due to the increasing symmetry of the guest molecule's charge distribution.

5. Conclusions

From the computer simulation of interaction energy profiles of simple probes passing through the center of the β-CD ring cavity along the perpendicular symmetry axis and the analysis of the guest–host interactions present in the inclusion complexes of the probes with non-substituted and substituted β-CDs we can conclude that:

(i) Small symmetric hydrocarbon (hydrophobic) probes (such as C, CH_4, C_6H_6) are predicted to form stable inclusion complexes with non-substituted and substituted β-CDs, the probe position will be typically near the cavity center. The stability of the inclusion complexes will increase with increasing size and aliphatic character of the probe. Small polar and charged probes (such as H_2O, NH_4^+, $HCOO^-$) are predicted to prefer the interaction with the solvent (water) in the bulk phase rather than the formation of inclusion complexes with non-substituted and substituted β-CDs;

(ii) Guest–host interactions in the stable inclusion complexes with hydrophobic probes are almost entirely dominated by dispersion interactions. This "hydrophobic" stabilization of the non-polar probes (C, CH_4, C_6H_6) is determined by their high symmetry and by the symmetry of the β-CDs. The MEP reaches magnitudes close to zero in the center of the non-substituted β-CD ring cavity in most of the studied substituted β-CDs and shows maximum positive or negative values outside of the cavity, near the ring faces;

(iii) Substitution of β-CD by neutral substituents leads to enhanced binding of hydrophobic probes and significant changes in the MEP profile along the β-CD symmetry axis. The structure of the neutral side chains is predicted to have a more important effect on the inclusion complexes stability than the number of the substituents.

(iv) Substitution of β-CD by substituents with charged side chains does not significantly affect the binding of hydrophobic probes (a slight increase similar to the non-charged substituents is observed), but has more significant consequences for the binding of polar or charged probes due to large variations of the MEP inside and outside of the ring cavity. In the more realistic models that also include

counterions the polar and charged probes are attracted to the β-CD ring faces with considerable stabilization, which is, however, still weaker than solvation in the bulk phase (water).

In the near future is seems to be possible to modify the structure of a side chain in a substituted β-CD to enhance its affinity to a particular molecule (drug) that contains non-polar and polar structural subunits. The non-polar part will be stabilized by the hydrophobic interaction in the cavity interior of a β-CD, while the polar component will be stabilized by polar interactions, enhanced due to the β-CD side chains, in a point near the O2, O3 or O6 ring faces.

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