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Structural Analysis of Host–Guest Systems. Methyl-substituted Phenols in β-Cyclodextrin

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Abstract. Complexation trajectories and the variation of induced circular dichroism are calculated for the docking of phenol and 2,4,6-trimethyl-phenol with β-cyclodextrin. The results are compared to experimental chirality data to elucidate the mechanism of nonspecific molecular recognition processes in aqueous solution. Large geometrical changes along nearly isoenergetic Dynamic Monte Carlo trajectories show the conformational flexibility of such host–guest systems. This proves diffuse intermolecular interactions, van der Waals or electrostatic in nature, as the main contributions to the binding energy. The number and position of the methyl substituents of the guest reduces the complexity of the conformational space as the guest's position becomes fixed by steric constraints.

The solvation free energy is calculated from the solvent accessible surface area weighted by respective atomic solvation parameters. Considering the solvation term in the dynamic simulations restricts the conformational flexibility of the macromolecular system. The relative importance of various contributions to the solvation energy is discussed and it is shown that those terms arising from the interaction of hydrophobic groups with the aqueous environment are essential for the determination of the complex structure. Considering these terms in the dynamic simulation model, the sign and strength of the calculated rotatory strength is in perfect agreement with induced circular dichroism obtained from experimentally determined averaged spectra. The results demonstrate the accuracy of the geometrical properties of host–guest systems obtained from these simulations.

Key words: Cyclodextrins, host–guest systems, molecular recognition, molecular surfaces, solvation.

1. Introduction

Self-assembly of macromolecular systems is of great importance in chemistry and molecular biology as it provides the physical basis for molecular recognition, e.g., in the formation of enzyme complexes with substrates. This process is also related to protein folding and the determination of the three-dimensional structure of macromolecules in general. Two crucial steps are involved in the formation of supramolecular assemblies: molecular recognition of a guest by the host due to molecular surface properties and the dynamic flexibility of the resulting complex. Besides intermolecular interactions the solvent environment also essentially determines the structure and stability of such complexes. These effects are related to the

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structure of the solvent water and are thus considerably less amenable to physical definition.

A valuable model system for studying molecular self-assembly is inclusion complex formation of cyclodextrins as hosts and appropriate organic guest molecules. Cyclodextrins are cyclic oligosaccharides with six (α-CD), seven (β-CD) and eight (γ-CD) α-1,4-glycosidically linked glucopyranose units [1–3]. This results in truncated cone shaped structures, additionally rigidized by superimposed H-bonds between the 2′- and 3′-hydroxy groups of adjacent subunits [4, 5]. The most important geometrical feature of cyclodextrins is the hydrophobic inner cavity with a diameter of approximately 5 to 8 Å, depending on the number of glucose moieties. Small, hydrophobic molecules can be nonspecifically complexed in this cavity and stable, low energy structures result. Such complexes are frequently considered in experimental and theoretical investigations of inclusion phenomena and cyclodextrins have gained great importance in fundamental as well as applied research [6–12]. Recent data also revealed the formation of higher order structures, e.g., the 1 : 2 guest to host complexes for α-CD and aromatic guests such as indole and 2-naphthol, 1 : 2 complexes of C_{60} fullerene with γ-CD [11], or tubular structures of up to 20 Å in length [13]. CDs also gained importance for technological applications, e.g., as mediators for solubilization and for chiral recognition [14].

CDs are most suitable for spectroscopic studies extending into the far UV as they show no absorption in this region. Besides electronic absorption and fluorescence, the circular dichroism induced in the UV absorption band of an appropriate aromatic guest by the chirality of the glucose subunits of the macromolecular host is used to study complexation. The sign and strength of the induced circular dichroism (ICD) is essentially dependent on the distance and relative orientation between the aromatic transition dipole and the various bonds of non-symmetric carbons. ICD-spectra are thus strongly dependent on the molecular geometry of the host–guest system and can therefore be applied to elucidate structural properties.

Induced circular dichroism spectra of such systems were thus measured frequently and a general scheme was developed by Kodaka, relating the sign of the measured ellipticity to general geometrical features [15]. It allows one to decide whether the long axis of the guest molecule is oriented preferentially along or perpendicular to the axis of CD. It should also support conclusions placing the molecule within the cavity or attached to one rim of the cone. In recent work [10,11,16], we showed that a technique calculating spectral properties from optimized complex structures, which are obtained from molecular force field computations, can predict the sign and strength of the ellipticity of inclusion complexes. Nevertheless, the agreement of calculated results with experimental ones depends essentially on the flexibility of the system. Molecular mechanics and dynamics calculations of the geometry of CD complexes showed that the flexibility of these host compounds leads to a large variety of low energy conformations [6,8,17–19]. In particular β-CD is highly nonsymmetric in solution, i.e. the cone is not circular in shape and the tilt angle between adjacent glucose units is not identical [17,19]. Solvent effects

were studied in molecular dynamics simulations on phenol complexes with α-CD and a molecular representation of water and showed a large number of different low energy minima, successively occupied by the host [18]. The rigidity of the system depends on the molecular shape and the van der Waals volume of the guest, as it is a consequence of the molecular fit of the guest to the host's inner cavity and the concomittant deformation of the flexible macrocycle.

In this paper, molecular docking between β-CD and methyl-substituted phenols is discussed by a Dynamic Monte Carlo approach and solvation effects are included by a continuum model. The guest molecules phenol, 4-methylphenol (4-MP), 2,4-dimethylphenol (2,4-DMP), 3,5-dimethylphenol (3,5-DMP), 2,6-dimethylphenol (2,6-DMP), 4,6-dimethylphenol (4,6-DMP), 3,4,5-trimethylphenol (3,4,5-TMP) and 2,4,6-trimethylphenol (2,4,6-TMP) provide a wide variability of molecular shape to study steric constraints. A variety of physicochemical properties and thermodynamic data are available for CD complexes formed by these guest molecules [20]. The reliability of low energy complex structures is judged by a comparison of the calculated ICD to experimentally obtained spectra. Differences in the conformational space of complexes formed with phenol and bulky 2,4,6-TMP are compared for solvated and isolated species and the influence of the aqueous environment on complex structures is discussed.

2. Theoretical Methods

Molecular mechanics calculations are performed using Allinger's MM2-87 and MM3-92 force fields [21–23]. The reference β-CD-structure for all calculations is obtained by minimizing a crystallographic geometry [5] by steepest descent, Newton–Raphson and block diagonal minimization. Minimum energies for β-CD of 262.30 kJ mol^{-1} and 292.11 kJ mol $^{-1}$ result for these force fields, respectively. In general, the main structural features of CD and the CD complexes investigated do not depend on the force field release used.

The following routines are used to find low energy geometries of complexes and to sample the conformational space under different conditions:

– Complexation pathways [12,16,24], obtained from a stepwise transfer in 1 Å increments of the guest molecule through the CD cavity, show the coarse location of local minima. Runs are started from a host to guest distance of 10 Å, for which the potential energy is nearly the sum of the individual contributions of guest and host. The host to guest distance is defined by the distance between the center of CD, which is the mean position of the glycosidic oxygens, and the center of mass of the aromatic moiety of the guest. A positive distance value indicates a guest located at the secondary hydroxyl rim of CD; a negative one locates it at the primary hydroxyl rim side. After each translation step the system is fully minimized without constraints. The resulting geometry is the starting structure for the next translation. These pathways are calculated within the MM2-87 force field.

– In a second routine, Dynamic Monte Carlo simulations [25,26] (DMC) are performed with 1000 steps per run at a constant temperature of 300 K. They are started at an initial host to guest distance of 5 Å with random relative orientation. The conformational space in the vicinity of this starting geometry is then explored by stochastic mutation of: (i) the dihedral angles between adjacent glucose subunits; (ii) of the host to guest distance; and (iii) of the relative orientation of host and guest. All complex geometries generated are fully minimized within the MM3-92 force field.

To include solvation effects arising from the aqueous environment, a continuum solvation model [27] is implemented in the DMC runs [28]: the solvent accessible molecular surface area is calculated using the QCPE program MSEED [29] with a probe radius of 1.4 Å. This is the same as the value used by Wesson and Eisenberg to obtain their set of atomic solvation parameters (ASPs), which were deduced from transfer experiments of model compounds from vapour phase to water [30]. The ASPs are assigned to the respective surface areas and are used to calculate solvation energies. In our solvation model, the total free energy change ΔG_{tot} of two conformations is given by:

$$\Delta G_{\text{tot}} = \Delta E_{\text{conf}} + \Delta G_{\text{solv}}.$$

The conformational energy is computed by the MM3-92 force field, the free energy of solvation results from the summation of the contributions of individual atoms derived from their solvent accessible surface area S_i multiplied by the respective atomic solvation parameter σ_i:

$$\Delta G_{\text{solv}} = \sum_i \sigma_i \Delta S_i.$$

A modified Metropolis criterion is used in the DMC routine to consider contributions arising from solvation:

$$P(\Delta E, \Delta G_{\text{solv}}) = \exp(-\Delta E/RT) \exp\left[\left(w \sum_{i-1}^{n} \Delta S_i \sigma_i\right) / RT\right].$$

The probability $P(\Delta E, \Delta G_{\text{solv}})$ of accepting a stochastically generated conformation is calculated as the product of the probabilities arising from differences in potential (ΔE) and solvation energies (ΔG_{solv}) between the actual and the last accepted structure (R is the gas constant, T the temperature in K). The optimum balance w of both contributions to the Metropolis criterion is a crucial point for this model. Its correct choice was reported previously for peptide folding [28] and will be discussed in detail for the host–guest system below.

The solvation free energy is factorized into contributions derived from solvating hydrophilic or hydrophobic surfaces, i.e. those with negative or positive ASPs, respectively:

$$\begin{aligned}\Delta G_{\text{solv}} &= \Delta G_{\text{hydrophilic}} + \Delta G_{\text{hydrophobic}} \\ &= \left(\sum_j \sigma_j \Delta S_j\right)_{\sigma_j<0} + \left(\sum_k \sigma_k \Delta S_k\right)_{\sigma_k>0}.\end{aligned}$$

Depending on the choice of the weighting factor w in the modified Metropolis criterion, the following conditions can be selected.

(1) Setting $w = 0$, only variations of the potential energy determine the acceptance probability of a structure.
(2) Setting $w > 0$ and calculating ΔG_{solv} values summing only over atoms with positive ASPs, i.e. the carbon atoms in the ASP set used: this accounts for contributions from hydrophobic groups to the solvation term and corresponds to a cominimization of the hydrophobic surface area and the potential energy. This setup will be termed 'hydrophobic solvation'.
(3) Setting $w > 0$ and summing over all atoms leads to cominimization of potential and total solvation energy: the latter is calculated from the total solvent accessible surface area arising from all atoms, i.e. carbons and oxygens. Intermolecular, specific solute–solvent interactions, like hydrogen bonding, are herein taken into account.

The sign and strength of the resulting ICD of complexes are calculated by the Kirkwood–Tinoco exciton chirality method [16,31] for geometries obtained by these three different DMC variants. ICD is the most direct method to determine association constants [32] and is used to study the structural properties of such host–guest systems. Since ICD results from dipole–dipole interactions between the transition moment of the guest and induced transient dipoles in the bonds of the chiral groups of the host, i.e. of the glucose units of β-CD, this technique provides direct information on the relative position of the achiral guest in the CD cavity [10,11,15,16]. The rotational strength, calculated by the Kirkwood–Tinoco expression for the exciton chirality, is directly proportional to the molar ellipticity and, therefore, correlated with the area and sign of the ICD band. Details of the formalism of ICD calculations are reported in Ref. [16].

3. Results and Discussion

3.1. COMPLEXATION PATHWAYS

These pathways show the general tendency of complexation and locate energy minima along the main reaction coordinate. Low energy β-CD complexes with phenol are found near 133.76 kJ mol^{-1} and at small host to guest distances around the center of the macromolecule. The guest molecule is thus fully embedded in the CD cavity.

The pathways obtained for 4-MP, 2,4-, 3,5-, and 4,6-DMP show a similar shape, and their minimum regions are only slightly shifted off the CD center. The values obtained for the intermolecular host to guest distance and for the potential energy in the minimum geometry are 1.8 Å, 120.80 kJ mol^{-1} for 2,4-DMP, -0.6 Å, 142.96 kJ mol^{-1} for 4-MP, 1.9 Å, 122.06 kJ mol^{-1} for 3,5-DMP and 2.3 Å, 120.38 kJ mol^{-1} for 4,6-DMP. The methyl groups of the guest tend to relax in a plane defined by the 2′-3′-OH or the 6′-OH of β-CD.

Another type of reaction pathway is found for 2,6-DMP, 3,4,5-TMP and 2,4,6-TMP. The corresponding values are: -3.2 Å, 125.82 kJ mol^{-1} for 2,6-DMP, -3.0 Å, 139.61 kJ mol^{-1} for 3,4,5-TMP and -2.8 Å and 110.35 kJ mol^{-1} for 2,4,6-TMP. For these guests a significantly high energy barrier is found at the center of the cavity and low energy regions are located on both sides of it. These complexation diagrams show the relative location of important minimum energy regions and give a preliminary insight into the conformational flexibility of the complexes. A broad region of low potential energy near the center of the cavity is obtained for complexes of guests with small van der Waals volume, while several quite different geometries are indicated for bulky guests. Interconversion between these low energy regions is unlikely to occur. The energy landscape appears highly rugged and the minima are not accessed via the calculated low energy pathways. The two following sections will show this in more detail.

3.2. Potential energy surfaces

To obtain information about the conformational flexibility of the complexes, potential energy surfaces are calculated for various geometrical parameters. Figure 1 shows two examples, one for the phenol–β-CD system (A) and a second for 2,4,6-TMP complexes with β-CD (B).

Both surfaces are realized by moving the guest through the CD-cavity in 1 Å increments and twisting the molecule in 36 steps of 10° about a theoretical axis through the cavity. The tilt angle is defined as the angle between the long axis of the phenolic moiety [C4—OH] and that through the center of the macrocycle, normal to the mean plane through the glucosidic oxygens. This procedure is done for distances between 2.5 to -2.5Å, which yields 180 structures for each system. The geometries generated are minimized without constraints and this results in 148 nonidentical structures for phenol and 78 for 2,4,6-TMP within an energy range of 70 kJ mol^{-1} above the lowest energy found, which is 121.64 kJ mol^{-1} for phenol and 109.52 kJ mol^{-1} for 2,4,6-TMP as guests. The potential energies are then plotted versus the host–guest distance (Å) and the tilt angle of the guest, which are determined for each geometry of the complex after minimization.

The lowest energies found by this method are in general agreement with those obtained from complexation pathways. The reduced conformational flexibility of the 2,4,6-TMP system shows up again by the smaller number of structures found

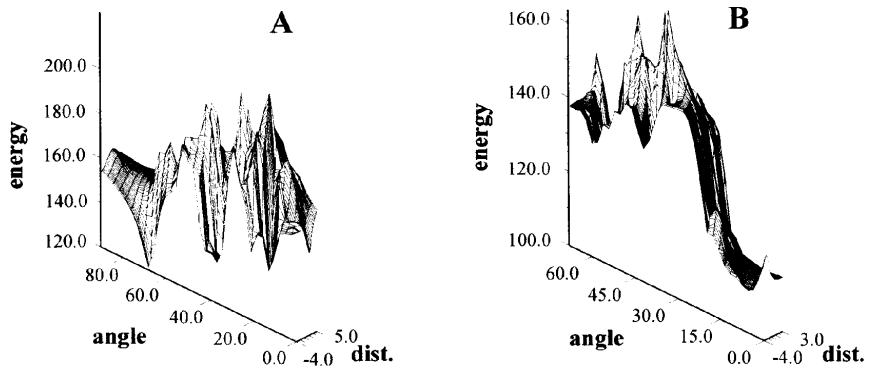

Figure 1. Plot of the energy surface for the phenol–β-CD system (A) and the 2,4,6-TMP–β-CD system (B) versus the intermolecular distance between host– and guest–molecule given in Å and the tilt angle of the guest molecule versus the axis through the CD cavity in degrees (for definition see text). The energy is given in kJ mol^{-1} and is obtained by the MM2-87 force field.

within the energy range of 70 kJ mol^{-1}, i.e. 78 compared to 148 structures obtained for phenol–CD complexes.

For phenol as guest (A), energetic minima are located near to the center of the cavity, but are also found at comparatively large distances, e.g. at -3.5 Å. Nearly isoenergetic minima appear for three different tilt angles at around 15°, 40°, and 65°.

The difference in the conformational space of phenol and 2,4,6-TMP complexes is evident from Figure 1. For the latter lowest energy complexes are found at a host to guest distance around 1.8 Å and a tilt angle of 15°. Interestingly, the two other tilt angles found for the phenol system are realized, too, and are located around 40° and 60°. The respective energies are, however, at least 30 kJ mol^{-1} higher. The minimum energy of the 2,4,6-TMP complex is 12 kJ mol^{-1} lower than that of the phenol complex. This yield of potential energy is largely based on increased intermolecular forces, as the analysis of the force field calculations show. Bulky 2,4,6-TMP fits tighter into the β-CD cavity and this increases the strength of unspecific molecular interactions of the van der Waals type between the two entities.

The complexity (ruggedness) of the surfaces is directly related to the number of methyl groups on phenol. The more substituents, the more steric effects determine the molecular interaction: the number of local minima (i.e. the possible positions of the guest in the cavity) decreases and the height of separating energy barriers increases. Complexes with phenol and with 2,4,6-TMP best reveal these contrary characteristics and are therefore chosen in particular chosen for the following studies.

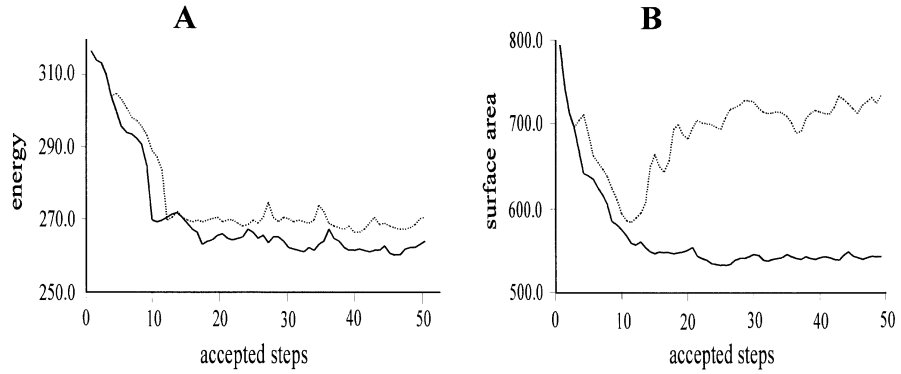

Figure 2. Comparison between DMC docking in which both the total potential energy and the hydrophobic solvation energy (full line) are minimized, and a run perfomed under identical conditions, minimizing only the potential energy (broken line). Shown are: (A) the potential energy from the MM3-92 force field in kJ mol^{-1}; (B) the total surface area in $Å^2$ for 50 successively accepted structures during the docking of phenol and β-CD.

3.3. DYNAMIC MONTE CARLO DOCKING AND IMPLEMENTATION OF THE SOLVATION MODEL

Dynamic Monte Carlo (DMC) simulations are performed to study docking pathways and the probability to reach certain local minimum structures. A further goal was to explore the topography of the conformational space in the vicinity of distinct minima on the energy surface and their possible interconversion. In these calculations a maximum translation of 0.5 Å of the guest relative to the host in all three dimensions, a 5° maximum change of the relative tilt angle of the guest, and 2.5° changes in the torsional angle between two neighboring glucose units are allowed in successive Monte Carlo steps. The algorithm was started from a guest position 5 Å outside the cavity and low energy structures are obtained within 1000 iteration steps. Figure 2 compares two DMC runs for the phenol–β-CD system, started from identical geometries: in the first computation only the conformational energy is minimized and in the second the hydrophobic solvation energy term is added in the Metropolis criterion, i.e. variant (2) described in the methods section. One intrinsic problem, which arises when conformational and solvation energy, calculated from different sources, are combined is the correct choice of the weighting factor w. In all these runs, w is taken as 0.2. A more detailed discussion on the choice of w will be given below.

Both runs show the initial docking phase. The potential energy, shown in the left graph, decreases similarily for both simulations, but after docking has occured the conformational energy is $\approx$6 kJ mol^{-1} lower when hydrophobic solvation is taken into account.

Important differences between the two methods are observed for the corresponding variation of the solvent accessible, hydrophobic surface area (see Figure 2B):

it decreases initially in both cases but rises again in the first case as the simulation proceeds. When only the potential energy is minimized, the phenol molecule is first included in the cavity but can easily leave it again. The local minimum initially reached is located within the cavity and has a molecular surface area of 585 Å^2. The complex relaxes, however, to an even more stable structure, in which the guest is just attached to the rim of the macrocycle at a distance of 3.1 Å and a larger surface of 710 Å^2. In the second case, when potential and hydrophobic solvation energies are cominimized, the guest remains well embedded in the CD cavity after the initial docking and the solvent accessible surface stays nearly constant at 540 Å^2. Hydrophobic solvation thus restricts the movement of the hydrophobic guest relative to the center of CD. Complexes in which the guest molecule lies within the cavity have similar potential energy to those in which it is just attached to one hydroxylic rim. Due to the lack of tight fit between phenol and β-CD, the attractive potential energy surface is flat and molecular motion can only be restricted by solvent effects. A minimum hydrophobic molecular surface area leads to a well defined geometry with only little flexibility.

This solvation model corresponds to a minimization of the hydrophobic contact surface with the aqueous environment and hence to a classical view of the hydrophobic effect [33]. A more detailed solvation model could be achieved by calculating and minimizing the total solvation energy, i.e. summing over all hydrophobic and hydrophilic groups. Such DMC variants, however, do not yield significantly restricted motion of the guest. In this case, the effect resulting from burying hydrophobic groups is at least partially counteracted by forces maximizing solvent interactions with the hydrophilic groups. Comparing the three variants, low energy structures within host to guest distances of -4 to 4 Å are obtained when only the conformational energy is minimized, but within -1.5 to 1.5 Å and -3 to 3 Å as hydrophobic or total solvation energy is added, respectively.

The same characteristic features of the three variants of the Dynamic Monte Carlo method are found for 2,4,6-TMP complexes with β-CD. The geometries resulting from the three variants for the complexes of the bulky guest have comparable energies around 255 kJ mol^{-1} and hydrophobic solvation confines their structural variance.

3.4. CALCULATION OF INDUCED CIRCULAR DICHROISM SPECTRA

Recent investigations [10,11,16] showed that the ICD spectra of cyclodextrin complexes can generally be predicted from structures obtained from a force field minimization and an implementation of the exciton chirality method. This method proved to be selective for different complex geometries. The general scheme [15], that ICD of CD complexes is solely determined by whether the chromophore is placed inside the cavity or is attached to the rim of the host, was supported for ideally cone-shaped CD. This method provides a valuable tool for a preliminary interpretation of the observed ellipticity data. β-CD is, however, is highly flexible

and the sign and intensity of the ICD spectra change significantly when the position and orientation of the molecule change within the cavity.

The two lowest phenol bands, denoted as S_1 and S_2, are derived from the transitions to the B_{2u} and B_{1u} states in parent benzene and molar ellipticities of $\Delta\varepsilon = -0.28\ \mathrm{M}^{-1}\mathrm{cm}^{-1}$ and $\Delta\varepsilon = +0.65\ \mathrm{M}^{-1}\mathrm{cm}^{-1}$ were determined experimentally for the β-CD complex [16]. No ICD band was found for the S_1 transition of 2,4,6-TMP complexes, but the second transition gives rise to a large molar ellipticity of $\Delta\varepsilon = +0.71\ \mathrm{M}^{-1}\mathrm{cm}^{-1}$. The lack of a first band was explained by the existence of several complex structures and effective cancellation of oppositely signed rotational strengths [16].

To prove the possibility of predicting complex structures by DMC simulations, the molar ellipticity is calculated for the geometries obtained from different runs. In every simulation 1000 steps are sampled at 300 K. The same start coordinates were used for the three different variants to allow their comparison and to study the influence of the weighting factor w. The resulting structures have comparable conformational energies ranging from 250 to 270 kJ mol^{-1} for phenol and from 245 to 265 kJ mol^{-1} for 2,4,6-TMP complexes with β-CD. The molar ellipticity was then calculated for 100 representative minimum structures for every run. The results are summarized in Table I. The correspondence of calculated and measured ICD spectra is judged according to the sign of the induced circular dichroism, i.e. the sequence $+/-$ or $0/+$ for S_1/S_2 transitions in phenol and 2,4,6-TMP, respectively. Also the range of the variation of potential and solvation energy and some geometrical data are given for each calculation in Table I.

When the weighting factor w is increased from 0.0 to 0.6 in DMC runs of variant (2), the final hydrophobic solvation energy decreases as the solvent accessible, hydrophobic surface of the complex decreases. The respective values are slightly larger for the 2,4,6-TMP system. This is mainly due to the additional methyl substituents and the deformation of the CD cone. Hydrophobic solvation is only a small contribution to the total solvation energy, which is nearly one order of magnitude larger for both systems.

Variants (1) and (3) lead to a comparable number of complex structures with correctly predicted ICD signs and their conformational energies, host to guest distances and tilt angles have similar values. For both methods, however, the calculated ICD spectra deviate significantly from the experimental results. But when hydrophobic solvation is considered (variant 2) and when w is chosen sufficiently large, the experimentally obtained signs of the ellipticity for the phenol complexes with β-CD are determined correctly. For a weighting factor $w = 0.6$ all predicted structures of the phenol complex show the correct sign and similar good agreement is obtained for the 2,4,6-TMP complex, but already with $w = 0.2$. This inverse proportionality of hydrophobic surface area and weighting factor can also be found when considering the other substituted phenols and is also evident considering other host–guest systems as complexes of C_{60} fullerene with γ-CD [11].

Table I. Summary of calculated spectroscopic and geometrical features of phenol and 2,4,6-TMP complexes with β-CD, using the three different DMC variants (denoted as 1–3), minimizing the potential energy term alone (1) or additionally the hydrophobic and the total solvation energy, (2) and (3), respectively. The number of ICD signs correctly predicted refers to the experimental values which are $+/-$ for the phenol system and $0/+$ for the 2,4,6-TMP system. Potential energies and solvation energies are given in kJ mol^{-1}, the distances in Å, the tilt angles in degrees.

Guests	DMC variant	Weight factor w	Correct ICD-signs[a]	Potential energy	Solvation energy	Distance	Tilt angle
Phenol	1	0.0	14	262.5–269.6	28.4–36.8[b]	1.2–3.9	20–80
Phenol	2	0.2	4	255.0–265.4	27.2–28.8[b]	−2.5–0.9	20–70
Phenol	2	0.4	76	258.3–265.0	27.2–28.0[b]	−2.4–0.9	20–50
Phenol	2	0.6	100	259.6–266.7	26.8–27.2[b]	−2.1–(−0.9)	20–40
Phenol	3	0.2	36	257.1–263.8	−239.1–(−228.2)[c]	0.3–2.7	20–80
2,4,6-TMP	1	0.0	18	252.5–261.3	29.3–34.3[b]	1.0–3.1	25–35
2,4,6-TMP	2	0.2	98	252.9–265.0	28.4–29.7[b]	−1.1–0.6	35–40
2,4,6-TMP	3	0.2	18	252.9–261.3	−225.7–(−221.1)[c]	1.0–2.8	25–35

[a]Out of 100 structures.
[b]Solvation energy of hydrophobic surface parts.
[c]Total solvation energy.

The range of host to guest distances and tilt angles found for these complex geometries are given in Table I. They show the comparatively small conformational space in which these structures with correct ICD signs are realized. This subset of geometries is also obtained by DMC runs using variants 1 or 3, but only with low probability.

The failure to find good coincidence between measured and calculated ICD spectra when total solvation energy is calculated reflects shortcomings of the solvation model used. Based on the large negative atomic solvation parameter for oxygen, -0.116, compared to 0.012 for carbon, the molecular surface areas of hydroxylic groups tend to become as large as possible. This can be interpreted in such a way that hydrogen bonding with water molecules is preferred over intramolecular hydrogen bonding. The secondary hydroxy groups, however, can also form intramolecular hydrogen bonds between adjacent glucose subunits. The formation of such a hydrogen bonded rim was demonstrated by molecular dynamics simulations [4] and was also confirmed by crystallographic data [5]. These hydrogen bonds rigidize the cone shaped structure of β-CD [19]. Considering total solvation, all these intramolecular hydrogen bonds appear to be broken in the calculations favouring hydrogen bonding with the aqueous environment. To model such an intramolecular hydrogen bonded system, the atomic solvation parameter of such hydroxylic groups should be taken as nearly zero and this is fulfilled in variant 2. These results indicate that the secondary hydroxylic rim remains intramolecularly hydrogen bonded in an aqueous environment and this is an important prerequisite to obtain the right complex structure. These intramolecular hydrogen bonds also cause the low solubility of β-CD in comparison to the α- and the γ-derivative [1, 3].

Representative structures for the phenol and 2,4,6- TMP complexes with β-CD, found by the DMC procedure, are depicted in Figure 3. Phenol is embedded in the cavity but its molecular surface does not tightly fit the inverse surface of the host. Bulky 2,4,6-TMP, however, is attached to one side of the cavity and the macrocycle is distorted from a circular shape. The 2-methyl and 4-methyl substituents are placed near a plane defined by the $2'$- and $3'$-OH-groups of the host. Formation of several hydrogen bonds between adjacent secondary hydroxylic groups is evident in both cases. Intermolecular hydrogen bonding between the phenolic OH group and the aqueous environment is possible and most likely to occur.

4. Conclusion

It has been demonstrated in the example of phenol and 2,4,6-TMP complexes with β-CD, that the sign of the induced circular dichroism spectra of such inclusion complexes can be predicted by the present method if the conformational space is sampled by a dynamic method and solvation in aqueous environment is taken into account.

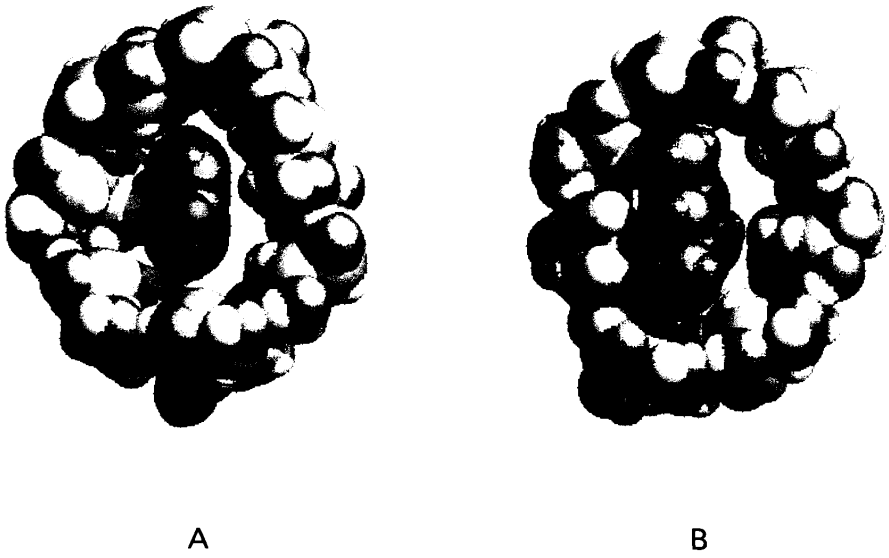

Figure 3. Optimized structures of the phenol (A) and the 2,4,6-TMP complex with β-CD (B) yielding correct ICD signs calculated by the DMC method minimizing potential and hydrophobic solvation energy (variant 2, see text). The complexes are viewed from the 2′-3′-OH rim. The potential energy (MM3-92) of the phenol–system is 265.93 kJ mol^{-1}, the free energy contribution from solvation of hydrophobic surfaces is 26.92 kJ mol^{-1}. The respective values for the 2,4,6-TMP complex are 254.44 kJ mol^{-1} and 28.67 kJ mol^{-1}.

Solvation reduces the highly complex conformational space of phenol inclusion complexes *in vacuo* to a small subspace, which is occupied most of the time during the dynamic simulation. Complexes formed between bulky, methyl-substituted phenols and CD exhibit different dynamic properties as only a limited number of well defined structures is available, and direct interconversion between them is improbable. In both cases solvation selects these structures, which yield the correct ICD spectra, out of that large set of low energy configurations. One important dynamic feature of these complexes is the stability of the intramolecularily hydrogen bonded, secondary hydroxylic rim.

Optical absorption and fluorescence spectra of inclusion complexes of simple aromatic compounds with cyclodextrins are often narrower and show more vibronic features than in pure aqueous solutions or other polar solvents [12,16]. The observed ICD spectra are likewise well defined and often show vibronic resolution [20]. Furthermore, a single fluorescence lifetime is frequently found for these complexes and the decay time varies in accordance with its variation replacing water by less polar solvents like alcohols or ethers [12,16]. Such spectral effects are hard to explain assuming a wide variety of different complex structures with different spectral properties coexisting in solution. On the contrary, broading of spectra, loss of vibronic structure, a distribution of lifetimes, etc. should result. The observed

spectroscopic properties support our conclusion, that complex geometries exhibit only small structural variety in aqueous environment.

The main constraints for the dynamics of such molecular assemblies are perfectly described by minimizing the hydrophobic surface area. These results suggest that the hydrophobic effect, described by minimized perturbation of the water structure, is of crucial importance for the structural and dynamic properties of these supramolecular systems in aqueous solution.

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