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Modelling of β-Cyclodextrin with L-α-Aminoacids Residues

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Abstract. A computational study of host-guest inclusion complexes between β-cyclodextrin (β-CD) and the 20 natural L-α-aminoacids and some selected pentapeptides was carried out and aimed at understanding the nature of the driving forces and mechanism leading to their formation. Relative complexation energies for the complexes with β-CD were calculated in both cases and the solvation Gibbs free energies were also evaluated for the single L-α-aminoacids. The computed results indicate strong possibilities of formation of inclusion complexes between β-CD and single L-α-aminoacids as well as pentapeptides which have hydrophobic side chains. In addition, noteworthy interactions of the side chain of the pentapeptides with the β-CD were also elucidated. A detailed molecular dynamics calculation of one of the representative pentapeptide/β-CD inclusion complex (β-CD/CH_3-Ala-Ala-**TYR**-Ala-Ala-CH_3) in aqueous solution has also been carried out. Molecular dynamics calculations support aspects connected with the formation and description of hydrogen bonds and with the role of dispersion forces in the inclusion complex in water.

Key words: β-cyclodextrin, α-aminoacids, pentapeptides, inclusion complexes, host–guest interaction, molecular simulation.

1. Introduction

In recent years extensive studies, both of theoretical and experimental significance, have been carried out on the inclusion complexes of guest molecules with the host β-cyclodextrin (β-CD) and its derivatives [1–10]. Due to its unique structural properties, β-CD can form inclusion complexes with numerous guest molecules comprising conventional drugs, L-α-aminoacids and peptide-proteic drugs. This ability can be exploited in the abatement of the toxicity and side effects of various drugs, in the increase of their bioavailability and also in the mediation of biological receptor-substrate interactions.

Various methods for the solubilization and/or the stabilization of peptides and proteins by cyclodextrin derivatives have been reported previously [11–18]. Re-

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cently experimental studies of the inclusion complexation of L-α-aminoacids with modified β-CD have highlighted interesting aspects of such interactions [19]. In our earlier paper [20] the inclusion of small probes inside the β-CD cavity was analyzed. Besides the quoted papers, contributions reporting on both theoretical and experimental analysis of the interactions of different classes of molecules with β-CD are available [21–25]. The structural effects on docking and binding of guest molecules and quantum mechanical studies relevant to hydrophobic potential calculations have also been analyzed [26–28]. It is generally assumed that the inclusion of the guest molecule in the cavity of β-CD is influenced by many factors such as steric effects, hydrophobicity and entropic factors involved in the displacement of the water from the cavity into the bulk. L-α-Aminoacids represent common building blocks of various biomolecules ranging from small peptides to large proteins displaying pharmacological activity. Therefore, information on the structure and stability of inclusion complexes of L-α-aminoacids with cyclodextrins is of high significance in designing carriers for peptide and proteic drugs and in understanding the role of host-guest interactions in stabilizing those bioactive principles.

In this paper, attention has been focussed on the nature of the interactions of a series of L-α-aminoacid guest molecules within the host β-CD cavity. We have considered the inclusion of the side chains of 20 natural L-α-aminoacids within the β-CD cavity, and two sets of calculations were performed by considering:

(i) The side chains of the various L-α-aminoacids in which both the carboxyl and the amino group have been replaced by methyl groups to evaluate the neat interaction of the side chain in the inclusion complex (Model 1).
(ii) The side chains of L-α-aminoacids at the centre of a methyl end-capped symmetric pentapeptide, containing two alanine residues on both sides, to evaluate the effect of the peptide backbone (Model 2).

The corresponding structures of the complexes of two representative cases involving tyrosine, are given in Figure 1a (Model 1) and Figure 1b (Model 2). L-α-Aminoacid side chains considered for the study include both neutral as well as charged (positive and negative) species. In addition, a molecular dynamics calculation was also carried out for a representative case of tyrosine having the extended backbone of two alanine residues each on both sides, complexed with β-CD immersed in a box of solvent consisting of 184 water molecules.

2. Computational Details

All structures of the model L-α-aminoacids (AA), β-CD and β-CD/AA complexes were modelled using the Insight II molecular modelling package of BIOSYM/MSI [29]. Molecular mechanics calculations of β-CD, model α-aminoacids and β-CD/AA complexes were carried out by using the consistent valence force field (CVFF) and all-atom model, without non-bonding interaction cut-off, employing the Discover package of BIOSYM/MSI [30]. The starting structure of β-CD

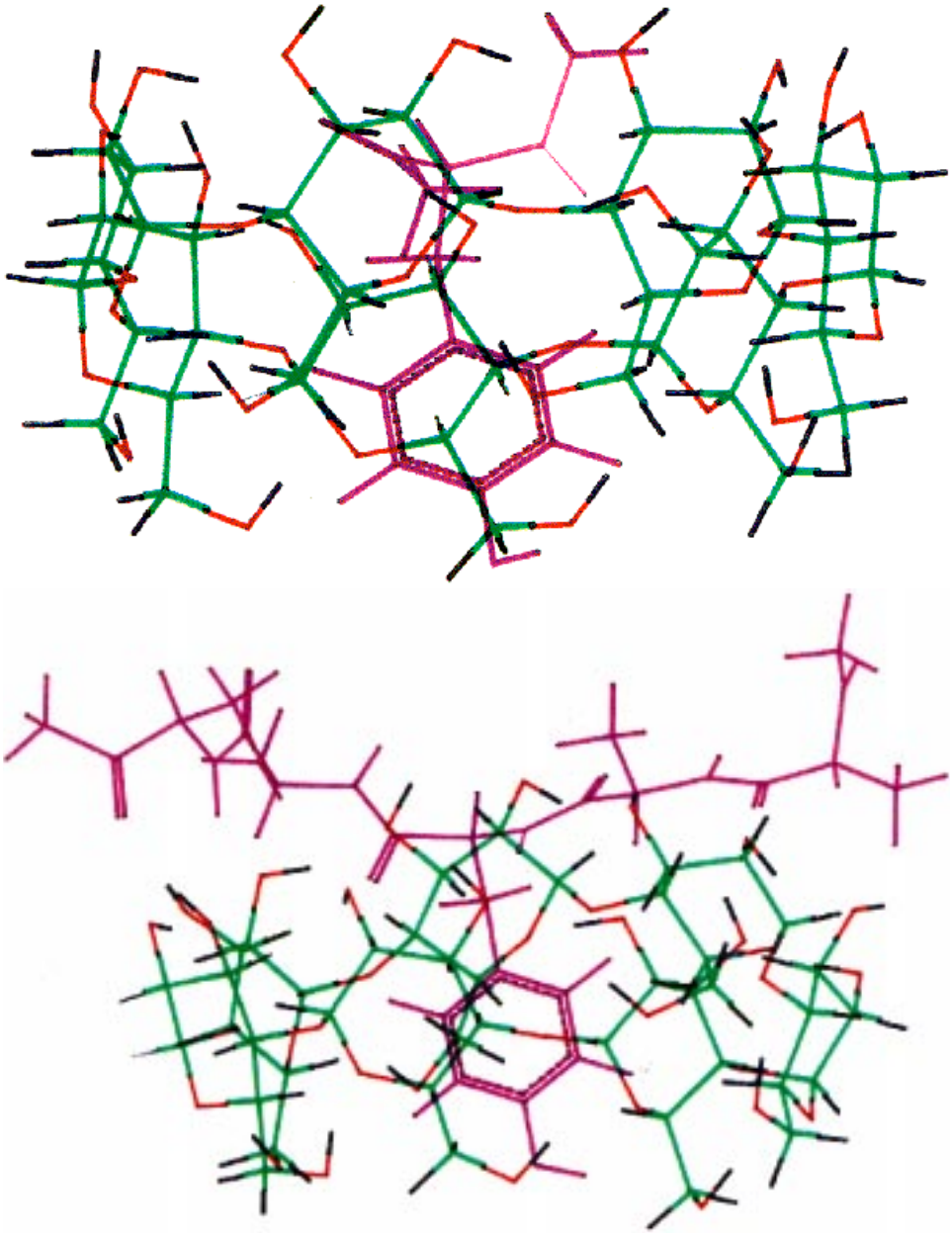

Figure 1. Optimized structures of the inclusion complexes of Model 1 (a) and Model 2 (b) with β-CD.

used for the calculations is its crystallographic geometry [31] which was gradually relaxed during the minimization process. An initial steepest descent followed by a conjugate gradient minimization was performed for all the structures using a dielectric constant, ϵ = 4. In Model 1 (Figure 1a), the α-aminoacid side chains were placed inside the cavity along the symmetry axis at the position where the maximum stabilization was observed from a previous calculation of the interaction energy profile [20]. This profile was calculated with the repulsion term scaled down by a factor of 2 only during the initial penetration process, in order to avoid repulsion of the larger and more rigid penetrating side chains. The individual α-aminoacid side chain geometries and orientations were relaxed by geometry optimization without scaling inside of the β-CD ring cavity. The reported energy values refer to these optimized structures.

To analyze in detail the nature of interactions of β-CD with the backbone of polypeptides, a similar set of calculations was performed for the complexes of the same L-α-aminoacid side chains located at the centre of a symmetrical pentapeptide constituted on both sides by two alanine (Ala) residues. The Ala residues at the terminal N- and C- ends were arbitrarily capped with two methyl groups. The general structure of the pentapeptides can be represented as follows, CH_3-Ala-Ala-**AA**-Ala-Ala-CH_3, where **AA** stands for the central L-α-aminoacid, whose side chain is inside the cavity (Model 2, Figure 1b). In both cases the complexation energies were calculated from the molecular mechanics energies of the β-CD/AA complex ($E_{\mathrm{AA}/\beta-\mathrm{CD}}$), β-CD ($E_{\beta-\mathrm{CD}}$) and model AA (E_{AA}) using the relationship:

$$E_{\mathrm{compl}}[AA] = E_{\mathrm{AA}/\beta-\mathrm{CD}} - E_{\beta-\mathrm{CD}} - E_{\mathrm{AA}}, \tag{1}$$

where E_{compl} [AA] is the complexation energy for a given α-aminoacid, either isolated or at the center of a pentapeptide, inside the β-CD cavity (for both isolated single α-aminoacid as well as L-α-aminoacid with the extended backbones on both sides). In order to facilitate the cancellation of the possible errors due to the approximate nature of molecular mechanics minimization, we have also calculated the relative complexation energies ($\Delta E_{\mathrm{compl}}$) of the guest L-$\alpha$-aminoacid species with respect to the smallest L-α-aminoacid, glycine (Gly) which is defined as,

$$\Delta E_{\mathrm{compl}} = E_{\mathrm{compl}}[AA] - E_{\mathrm{compl}}[Gly], \tag{2}$$

where E_{compl}[Gly] is the complexation energy for the Gly model. In addition, we have also calculated the ratio between the dispersion-repulsion and interaction energies $(E^{d,r}/E^{\mathrm{int}})_{\mathrm{compl}}$ of the complexation energy of each species, with a view toward analyzing in detail the contributions of non-bonding interactions to the stabilization of the inclusion complex. The $(E^{d,r}/E^{\mathrm{int}})_{\mathrm{compl}}$ ratio is defined as:

$$(E^{d,r}/E^{\mathrm{int}})_{\mathrm{compl}} = E^{d,r}_{\mathrm{compl}}/(E^{d,r}_{\mathrm{compl}} + E^{\mathrm{coul}}_{\mathrm{compl}}), \tag{3}$$

where $E^{d,r}_{\mathrm{compl}}$is the dispersion-repulsion contribution and $E^{\mathrm{coul}}_{\mathrm{compl}}$is the non-bonding coulombic contribution to the complexation energy according to Equation (1).

Table I. Charge, dipole moment, $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratio, relative complexation energies (ΔE_{compl}) and solvation Gibbs free energies (ΔG_{solv}) for the complexes of single α-aminoacid side chains with β-CD (Model 1)

α-amino acid	q	μ[a] (debye)	$(E^{d,r}/E^{\text{int}})_{\text{compl}}$	ΔE_{compl} (kJ mol^{-1})	ΔG_{solv} (kJ mol^{-1})
Gly	0	0.00	1.0	0.0	0.0
Ala	0	0.00	0.9	−4.2	−3.8
Val	0	0.02	1.0	−29.7	−8.8
Leu	0	0.05	1.0	−37.7	−10.9
Ile	0	0.05	1.0	−37.3	−10.9
Pro	0	0.88	0.8	−43.5	−20.9
Phe	0	0.07	0.9	−69.9	−15.1
Tyr	0	1.55	0.9	−71.2	−22.2
His	0	1.50	0.8	−55.7	−19.3
Trp	0	0.29	1.0	−72.4	−22.2
Ser	0	1.60	0.5	−22.6	−10.9
Thr	0	1.58	0.7	−32.7	−15.1
Cys	0	0.76	0.8	−20.1	−3.8
Met	0	0.15	0.9	−26.0	−7.1
Asn	0	3.11	0.7	−38.5	−18.8
Gln	0	3.12	0.9	−36.0	−20.1
Lys	1	–	0.2	−157.8	−164.9
Arg	1	–	0.3	−155.7	−157.4
Asp	−1	–	0.3	−62.0	−185.0
Glu	−1	–	0.5	−53.6	−178.7

[a] Dipole moments have been calculated for the systems without taking into account the amino and carboxylic groups.

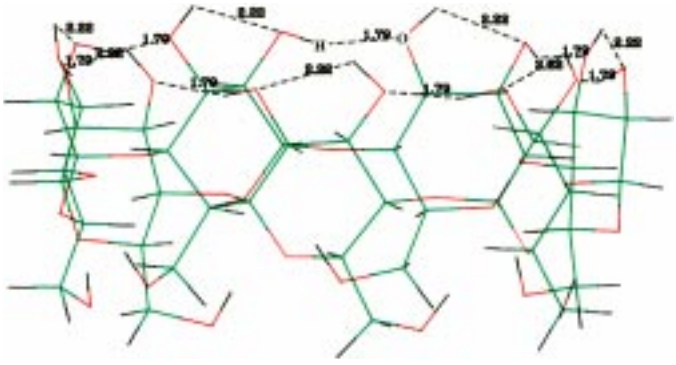

Figure 2. Optimized structures of β-CD showing intramolecular hydrogen bonding at the upper opening of the cavity.

Table II. $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratio and relative complexation energies (ΔE_{compl}) for the complexes of L-α-aminoacids having extended backbones (Model 2) with β-CD

L-α-aminoacid	$(E^{d,r}/E^{\text{int}})_{\text{compl}}$	ΔD_{compl} (kJ mol^{-1})
CH_3-Ala-Ala-**Gly**-Ala-Ala-CH_3	1.0	0.0
CH_3-Ala-Ala-**Ala**-Ala-Ala-CH_3	0.7	−17.2
CH_3-Ala-Ala-**Val**-Ala-Ala-CH_3	0.9	−14.2
CH_3-Ala-Ala-**Leu**-Ala-Ala-CH_3	0.9	−22.2
CH_3-Ala-Ala-**Ile**-Ala-Ala-CH_3	0.9	−13.4
CH_3-Ala-Ala-**Pro**-Ala-Ala-CH_3	1.0	−28.5
CH_3-Ala-Ala-**Phe**-Ala-Ala-CH_3	0.9	−54.0
CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3	0.9	−63.2
CH_3-Ala-Ala-**His**-Ala-Ala-CH_3	0.9	−47.7
CH_3-Ala-Ala-**Trp**-Ala-Ala-CH_3	0.8	−92.1
CH_3-Ala-Ala-**Ser**-Ala-Ala-CH_3	0.7	−29.3
CH_3-Ala-Ala-**Thr**-Ala-Ala-CH_3	1.0	−24.3
CH_3-Ala-Ala-**Cys**-Ala-Ala-CH_3	0.9	−13.8
CH_3-Ala-Ala-**Met**-Ala-Ala-CH_3	1.0	−22.2
CH_3-Ala-Ala-**Asn**-Ala-Ala-CH_3	0.7	−27.2
CH_3-Ala-Ala-**Gln**-Ala-Ala-CH_3	0.7	−48.6
CH_3-Ala-Ala-**Lys**-Ala-Ala-CH_3	0.6	−110.5
CH_3-Ala-Ala-**Arg**-Ala-Ala-CH_3	0.7	−117.2
CH_3-Ala-Ala-**Asp**-Ala-Ala-CH_3	0.3	−117.9
CH_3-Ala-Ala-**Glu**-Ala-Ala-CH_3	0.3	−114.3

The solvation Gibbs free energies (ΔG_{solv}) for the Model 1 α-aminoacids were calculated using the Polarizable Continuum Model [32, 33]. Here the solute is represented by a set of point atomic charges derived from the CVFF force field and is placed in a cavity of realistic shape composed of intersecting spheres with van der Waals radii centered on individual atoms. The solvent is represented by a homogeneous dielectric medium of permitivity $\epsilon = 80$ (water). Molecular dynamics calculation of one of the representative system of the guest–host complex has been carried out for the β-CD/CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 complex in water (184 water molecules in a cubic box of size 20 Å) for 500 ps at room temperature.

3. Results and Discussion

3.1. INCLUSION COMPLEXES OF L-α-AMINOACIDS WITH β-CYCLODEXTRIN

The L-α-aminoacids considered for the present study are, Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, His, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, Asp and Glu. The computed complexation energies for these systems in the isolated form (Model 1) together with the calculated $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratio, solvation Gibbs free energy, charge of the residue and dipole moment of the neutral residues are given in Table I. The ΔE_{compl} computed for the same L-α-aminoacids inserted in a pentapeptide (Model 2) together with the $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratios are reported in Table II. The ΔE_{compl} values reported in Table I suggest that neutral aliphatic non-polar α-aminoacids such as Gly, Ala, Val, Leu, Ile will prefer to form weaker hydrophobic inclusion complexes with the β-CD. The corresponding solvation Gibbs free energy for these α-aminoacids are quite low compared to the ΔE_{compl} values, suggesting the preferential formation of the inclusion complex to the solvation in bulk water. From the ΔE_{compl} values in Table II, it is clear that the L-α-aminoacids in the extended CH_3-Ala-Ala-AA-Ala-Ala-CH_3 structures also form weak hydrophobic inclusion complexes. In these complexes, as can be seen from the $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratio, the guest–host interactions in the optimized complex (relative to free β-CD and AA, see Figure 3) are composed mostly by the dispersion-repulsion term as this ratio is close to 1. The $(E^{d,r}/E^{\text{int}})$ ratio obtained for guest–host interactions only is, however, 0.7 indicating that the coulombic part of the stabilization is almost fully compensated by the destabilization of β-CD when engaged in the formation of the complex. The affinity towards β-CD is found to grow with the increase of the side chain length.

Cyclic and aromatic weakly polar L-α-aminoacids like Pro, Phe, Tyr, His and Trp form very strong hydrophobic inclusion complexes both as single α-aminoacid side chains (Table I) and in the pentapeptide CH_3-Ala-Ala-AA-Ala-Ala-CH_3 structure (Table II) as indicated by their very high ΔE_{compl} values. The complexation energy is found to increase (in magnitude) with the size of the side chain as Trp has the highest negative values of ΔE_{compl}, -72.4 and -92.1 kJ mol^{-1} for Model 1 and Model 2, respectively (see Tables I and II). Experimental studies by Prokai et al. [10] also suggest that there are strong possibilities for the formation of inclusion complexes between tryptophan and β-CD. Also for the aromatic α-aminoacid side chains the dispersion-repulsion interactions are found to be the dominating interactions, as the $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratios are quite significant.

Polar α-aminoacids such as Ser, Thr, Cys and Met are found to yield only low ΔE_{compl} values (-20.1 to -32.7 kJ mol^{-1} for Model 1 and -13.8 to -29.3 kJ mol^{-1} for Model 2 pentapeptides), indicating that the corresponding inclusion complexes are relatively weak. Also in these cases, the dispersion-repulsion interaction plays a major role and the hydrophobic nature of the internal cavity of β-CD is the factor governing guest–host interactions [13]. Polar amines such as Asparagine (Asn) and Glutamine (Gln), both as single α-aminoacid side chains and in the

pentapeptide, are found to form weaker inclusion complexes as compared to the corresponding Trp derivatives. The $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratios for these systems show a slight decrease, particularly in the single α-aminoacid case, suggesting a significant contribution of electrostatic interactions.

The acidic protonated α-aminoacid ($q = 1$) Lys and Arg in the single α-aminoacid form give strong inclusion complexes with β-CD in the gas phase, as indicated by their large negative ΔE_{compl} values (Table I). Analogous highly negative ΔE_{compl} values were also obtained for these L-α-aminoacids in Model 2 pentapeptides (Table II). The $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratios for Lys and Arg in the single α-aminoacid form are very small (0.2 and 0.3 respectively) indicating the importance of the electrostatic interaction in both cases. The calculation of solvation Gibbs free energy (ΔG_{sol}) for these cationic species (Table I) yielded very large negative values, suggesting the occurrence of strong interactions with the bulk water solvent. This observation points to the fact that in aqueous solution protonated Lys and Arg may show a larger preference for the solvent bulk rather than for the formation of inclusion complexes. The decrease of the $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratios is less dramatic when these L-α-aminoacids are inserted in the pentapeptide chain, probably due to the role played by the interactions of the backbone and side chains of the Ala-Ala-segments on both sides. The negatively charged ($q = -1$) basic residues Asp and Glu also show significant ΔE_{compl} values for single (Model 1) as well as pentapeptide structures (Model 2), indicating strong interactions with β-CD. Similar to the positively charged α-aminoacids, the ΔG_{solv} calculated for the side chains of Asp and Glu in Model 1 are rather large (in magnitude) suggesting that these α-aminoacids will also prefer to stay in the bulk solvent phase rather than forming hydrophobic inclusion complexes. Asp and Glu exhibit strong interactions with β-CD in the pentapeptide form and, in fact, the complexation energies for the relevant pentapeptides are much higher (in magnitude) than those obtained for Model 1. This is quite surprising in that the complexation energies are lower for side chains with $q = -1$ in Model 2 than for those with $q = +1$ in Model 1. Comparison of the backbone orientation of the optimized structures of Model 2 for Asp and Glu gives evidence for the existence of an extra stabilizing interaction for the backbone of the pentapeptide containing Asp, possibly due to the strain experienced by it from the interactions with the side chain inside the cavity. The $E^{d,r}/E^{\text{int}}$ ratios for Asp and Glu in Model 1 and Model 2 forms are very low and hence it may be concluded that the major interaction of these residues with β-CD is electrostatic in nature due to the charges present on the residues.

The molecular mechanics optimized structure of β-CD, given in Figure 2, indicates that the hydroxyl groups at the upper opening of the cavity are aligned in an intramolecular hydrogen bonding pattern (H-bond distance in the range, 1.7–2.4 Å) with the neighbouring hydroxyls arranged in a cyclic assembly (Figure 2). Therefore, it is reasonable to argue that any possibility of H-bond interaction of the peptide backbone with the upper opening of the cavity, may have to compete with the existing intramolecular hydrogen bonding among the hydroxyl groups of

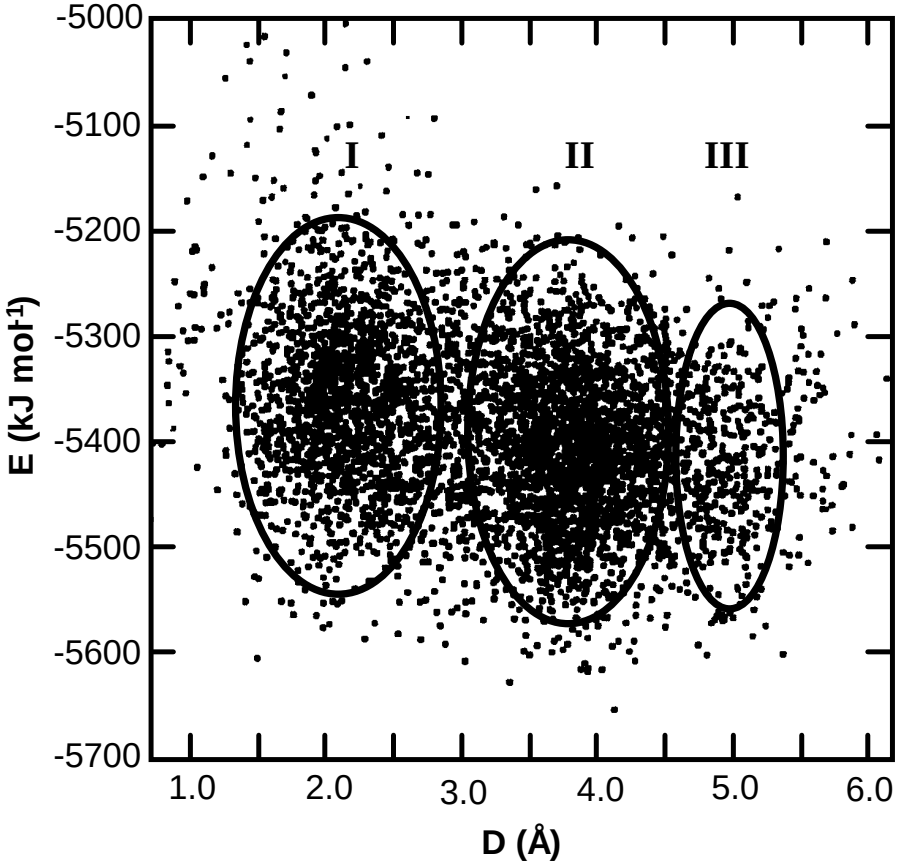

Figure 3. Variation of the energy of the Tyr-pentapeptide : β-CD complex with the distance of the C_α-carbon of the central residue from the plane of β-CD glycosidic oxygens.

the free β-CD, or with the β-CD/water hydrogen bonding in solution (see the next section of molecular dynamics).

The geometry optimized structures of the inclusion complexes of the pentapeptides (CH_3-Ala-Ala-**AA**-Ala-Ala-CH_3) with β-CD show that four of these intramolecular H-bonds have been released to form four strong intermolecular hydrogen bonds (with an average H-bond length in the range 1.8–2.3 Å) with the carbonyl oxygens of the pentapeptide backbone. This is also supported by the observation that the pentapeptide containing Gly, the simplest α-aminoacid, exhibits a complexation energy of -83.7 kJ mol^{-1} due to the stabilization originating from dispersion-repulsion and coulombic interaction of the backbone with the β-CD. However, the coulombic interaction is more or less completely compensated by the loss of the intramolecular hydrogen bonding of free β-CD, and, hence, the calculated $(E^{d,r}/E^{\text{int}})_{\text{compl}}$ ratio is close to 1 for the Gly-containing Model 2.

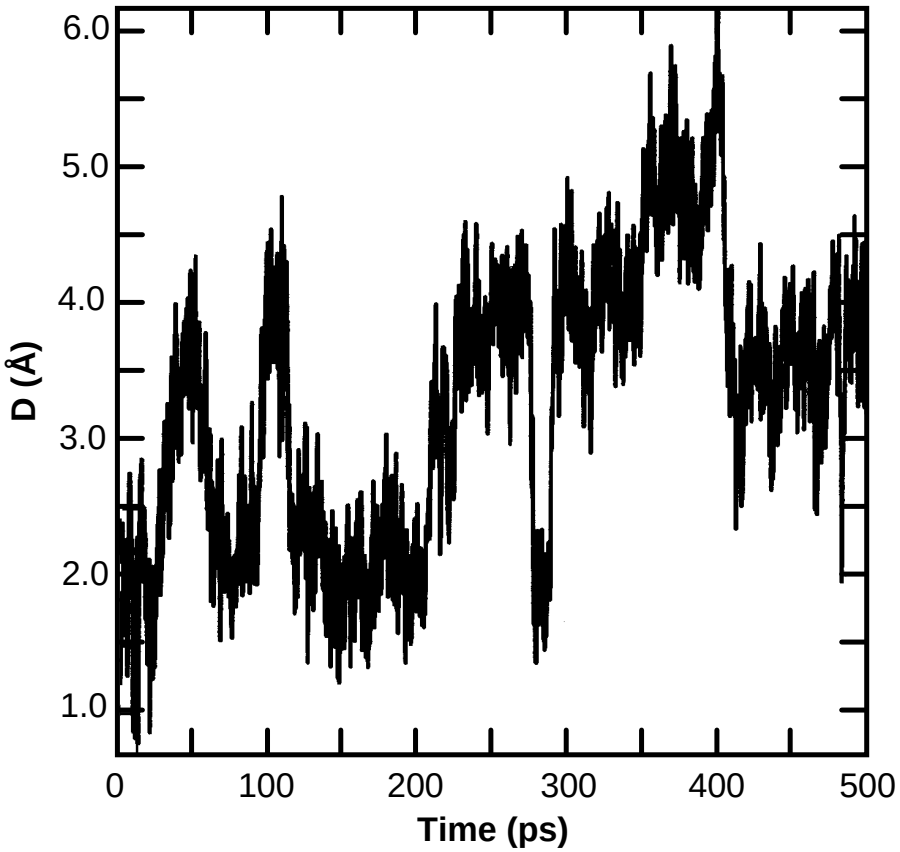

Figure 4. Plot vs. time of the distance (D) of the C_α-carbon of the Tyr-pentapeptide central residue from the plane of β-CD glycosidic oxygens.

3.2. MOLECULAR DYNAMICS STUDY OF AN INCLUSION COMPLEX WITH β-CYCLODEXTRIN IN WATER

In the present study, we have performed a molecular dynamics calculation for 500 ps at 300 K for one representative case of the guest molecules, CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 complexed with β-CD in water in a cubic box of size 20 Å containing 184 water molecules. Our goal was to understand the nature and stability of the β-CD/CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 complex when surrounded by water molecules, i.e., to address the following questions:

1. Is the inclusion complex persistent in time?
2. What is the population of the inclusion complex conformations having favourable orientation and energy?
3. What is the nature and dynamics of the interaction between the Tyr side chain and β-CD?

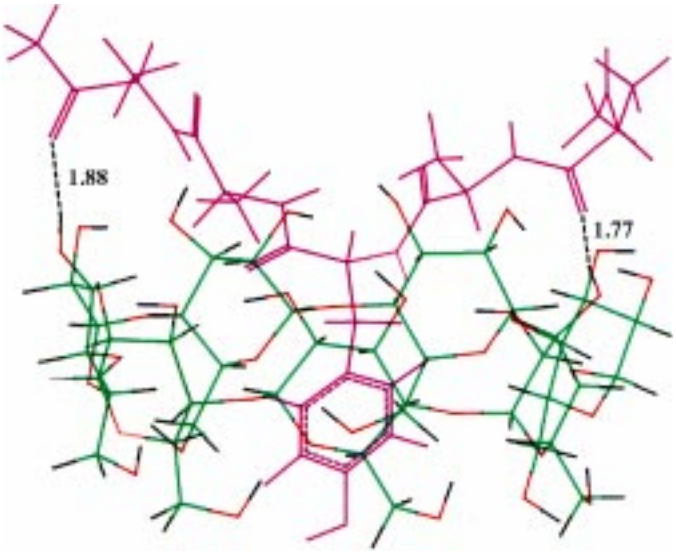

Figure 5. Snapshot of the molecular dynamics of the CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 : βCD complex in water at a distance D = 2.0 Å showing hydrogen bonding of the backbone with β-CD hydroxyl groups.

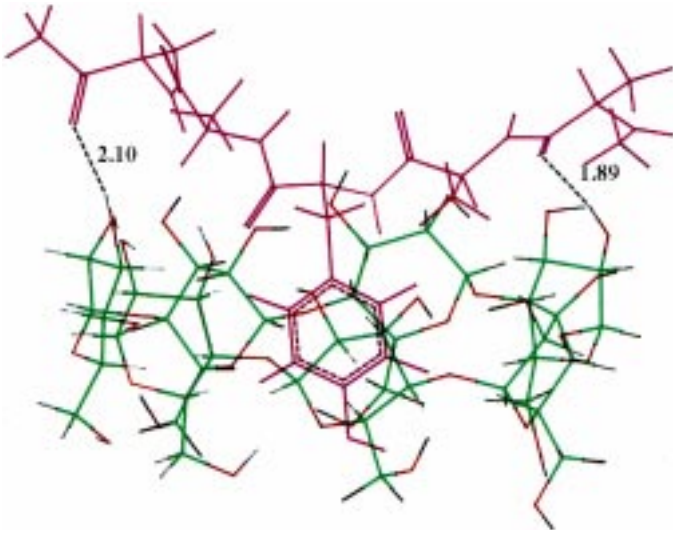

Figure 6. Snapshot of the molecular dynamics of the CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 : β-CD complex in water at a distance D = 3.9 Å showing hydrogen bonding of the backbone with β-CD hydroxyl groups.

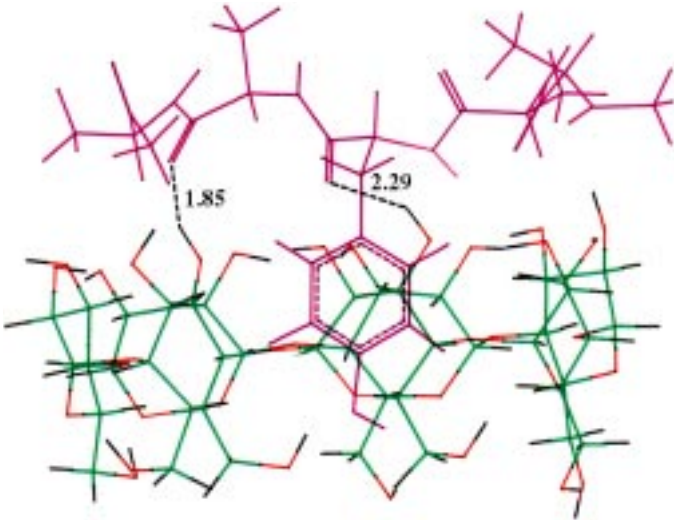

Figure 7. Snapshot of the molecular dynamics of the CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 : β-CD complex in water at a distance D = 5.0 Å showing hydrogen bonding of the backbone with β-CD hydroxyl groups.

4. What is the effect of water molecules on these interactions? i.e., are water molecules playing an active role in the interactions between the pentapeptide and β-CD in the complex?

The selected trajectories of these studies are reported in Figures 3–9. Figure 3 represents the graph of the distance (D) of the C_α-carbon atom of the Tyr residue from the plane containing three symmetric glycosidic oxygens of the cyclodextrin as a function of the potential energy of the complex, and Figure 4 represents the corresponding time vs. distance (D) plot. These two graphs show that the concentration of the maximum number of conformations is mostly around two points corresponding to $D =\sim 2$ and $\sim$3.9 Å, respectively. The corresponding energy values for these two accumulations of conformations are approximately centered around -5383 and -5446 kJ mol^{-1} (see ellipsoid areas I and II in Figure 3). There also exists another less intense grouping of conformations corresponding to a D value of 5 Å and an energy value of about -5447 kJ mol^{-1} (ellipsoid area III in Figure 3). These observations suggest that, in water, the inclusion complexes formed between β-CD and CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 are statistically favored for distances of about 2, 3.9 and 5 Å (Figures 3 and 4) with a larger probability in the first two cases, with higher preference for the second one. A snapshot of the molecular dynamics simulation corresponding to the distance D = 2 Å (Figure 5) shows that there are two hydrogen bonding interactions between the terminal carbonyl groups of the pentapeptide backbone of CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 and the hydroxyl groups of the β-CD, with H-bond distances of 1.9 and 1.8 Å. This frame also shows that there are no water molecules inside the β-CD cavity. The snapshot conformation corresponding to D = 3.9 Å (Figure 6) indi-

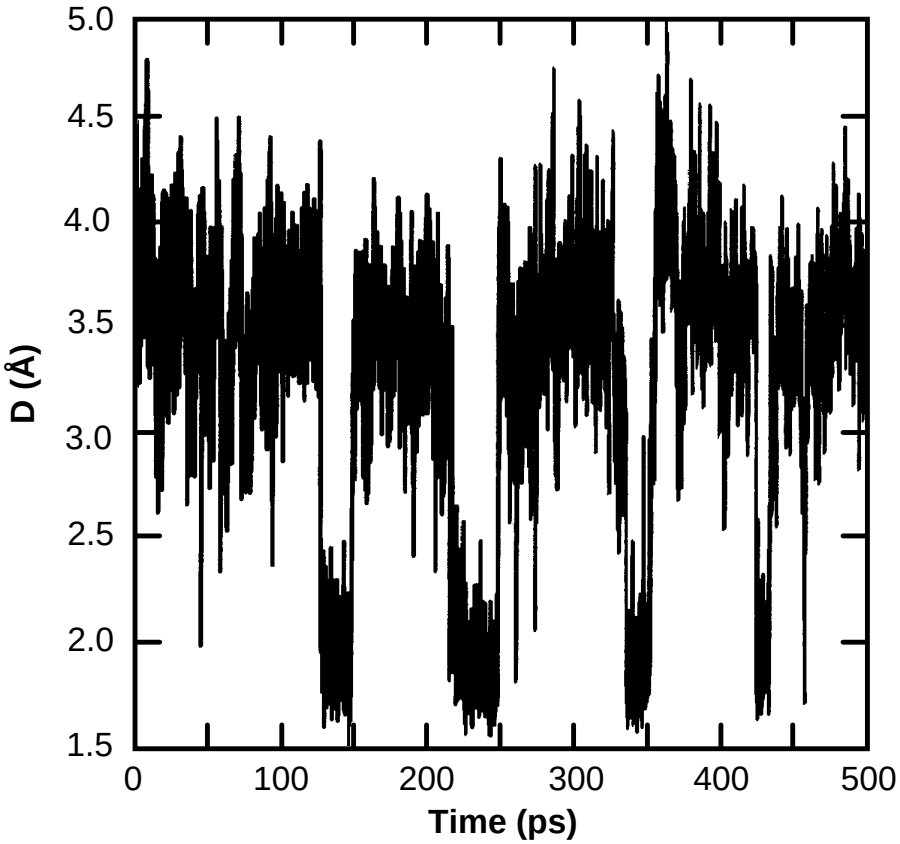

Figure 8. Variation with time of the intramolecular hydrogen bond distance between a pair of the hydroxyl groups at the upper opening of the β-CD.

cates a similar hydrogen bonding interaction between the pentapeptide backbone and the hydroxyl groups of the β-CD (H-bond distances 2.1 and 1.9 Å). Also in this case, no water molecules are present inside the cavity; however, two water molecules were found close to the bottom of the cavity in positions suitable for hydrogen bonding with the OH group of tyrosine. The representative conformation corresponding to D = 5.0 Å (Figure 7) once more indicates the presence of H-bonding between the backbone carbonyls of the pentapeptide and the rim hydroxyl groups of the β-CD. The presence of one water molecule inside the β-CD cavity (more or less close to the centre of the cavity) and of one water molecule at the mouth of the cavity between the pentapeptide backbone and the β-CD is observed. Two water molecules are also present at the bottom of the β-CD cavity in hydrogen bonding interaction with the hydroxyl groups of β-CD. These observations indicate that there is a strong possibility for the formation of inclusion complexes between the CH_3-Ala-Ala-**Tyr**-Ala-Ala-CH_3 pentapeptide (Model 2) and β-CD in water solvent, due to the large number of statistically favored conformations at distances

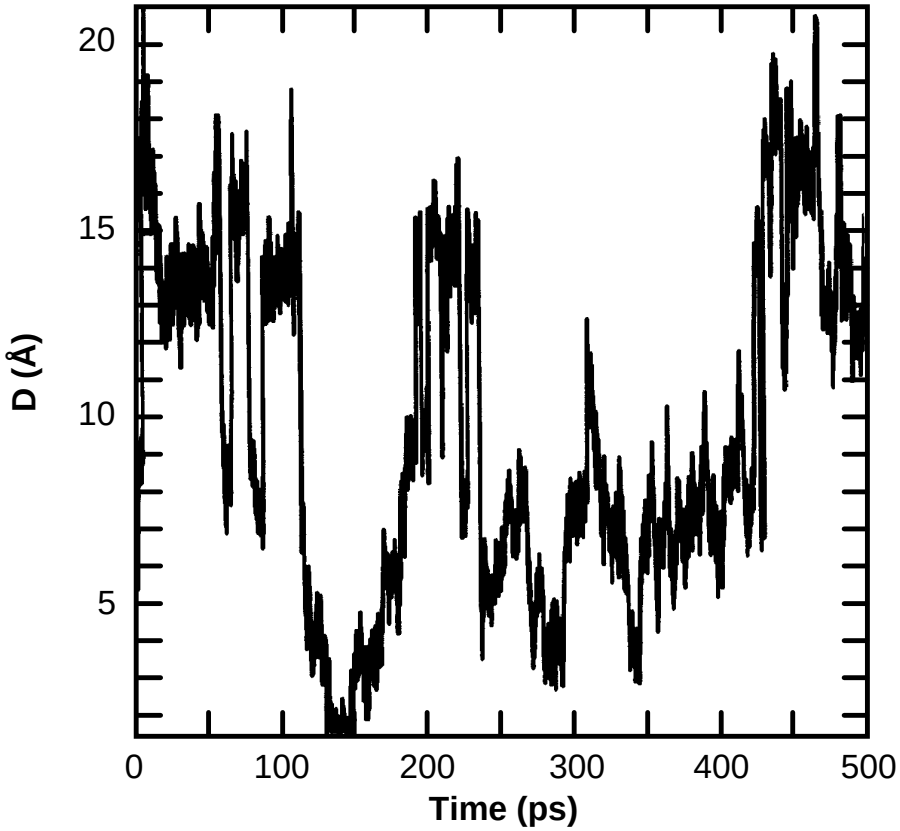

Figure 9. Variation with time of the hydrogen bond distance between one hydroxyl group at the upper opening of the β-CD and a water molecule.

of about 2 and 3.9 Å (Figures 3 and 4). Another noteworthy feature of the dynamics study is that, for both the above distances, no water molecules were found inside the β-CD cavity because the guest molecule displaced the water molecules present inside the cavity, whereas, at larger distances (e.g., $D =\sim 5.0$ Å), it is possible for water molecules to stay inside the hydrophobic cavity, but their density inside the cavity is significantly lower than that outside the space surrounding the complex.

An analysis of the nature of the intramolecular hydrogen bondings of the hydroxyl groups at the rim of the β-CD cavity during the course of molecular dynamics was also carried out. For this purpose, the distance of one intramolecular hydrogen bond (between the two neighbouring hydroxyl groups of β-CD at the mouth of the cavity) has been plotted as a function of time (Figure 8). The H-bonding distances between the considered hydroxyl groups are labeled in Figure 2. The intramolecular hydrogen bonding considered here is the one which is far from the pentapeptide backbone. Figure 8 indicates the occurrence of a break down of this bond during the dynamics simulation, very likely due to the formation of intermolecular hydrogen bonds with neighboring water molecules and to the exchange

of the corresponding donor and acceptor. These results are in agreement with the MD study of β-CD in vacuo [19].

We have also carried out a detailed analysis of the intermolecular hydrogen bonding of this β-CD hydroxyl group with an adjacent water molecule by plotting the corresponding distance versus time. Figure 9 illustrates the formation of intermolecular hydrogen bonding between the β-CD hydroxyl group and one water molecule by monitoring the movement of this water molecule during the course of the dynamics.

4. Conclusions

The major conclusions that may be drawn from the present molecular mechanics and molecular dynamics study of the inclusion complexes formed between L-α-aminoacids and β-CD are as follows: Strong inclusion complexes may be formed with L-α-aminoacids having hydrophobic non-polar side chains. For polar L-α-aminoacids, especially for the charged ones, solvation-free energy calculations of the single α-aminoacids suggest that these guest molecules will be more stabilized by the water solvent, thus preventing the formation of an inclusion complex. In pentapeptides, interaction of the backbone of the protein with the mouth of the β-CD contributes to the stabilization of the inclusion complex. However, in such cases, the competition between the intramolecular hydrogen bonding of the hydroxyls of β-CD and the interaction with the peptide backbone (also the interaction of the β-CD hydroxyls with the water molecules) has also to be taken into account. Molecular dynamics for the pentapeptide β-CD complex reveal the existence of a large number of populations of real guest–host complexes with reasonable energy indicating the formation of an inclusion complex in water as solvent. It also indicates the persistence of the inclusion complex throughout the whole of the trajectory of dynamics due to the strong stabilization effected by the interaction of the L-α-aminoacid side chain inside the cavity and also by the interaction between the peptide backbone with the mouth of β-CD.

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