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Modelling of molecular interactions and inclusion phenomena in substituted β-cyclodextrin: from simple probes to proteins

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SUMMARY: The ability of β-cyclodextrin (βCD) to form stable complexes with α-interferon was investigated. By using simple molecular mechanics approach interaction energy profiles of simple probes passing the center of βCD ring cavity along the main molecular symmetry axis were evaluated first. A computational study of host-guest inclusion complexes between βCD and L-α-aminoacids and some selected pentapeptides was also carried out and aimed at understanding the nature of the driving forces and mechanism, leading to their formation. Relative complexation energies for the complexes and the solvation Gibbs free energies for single L-α-aminoacids were calculated. Both the aminoacid residue inside the βCD cavity and neighbouring residues were found to contribute to the stabilization of βCD complexes with the side-chain of aminoacids present on the surface of α-interferon. The most appropriate number of host βCD molecules for the encapsulation in the first shell of one α-interferon molecule resulted to be 25.

Introduction

Interferons are proteins which exhibit antiviral, antitumor and immunomodulating activities[1)]. Interferons include fibroblast-produced interferon or α-interferon (IFNα), leukocyte interferon (IFNβ) consisting of a family of proteins and immune or γ-interferon (IFNγ)[2)]. They have found several medical applications but there still is a need to improve their efficacy as drugs[3)]. Encapsulation could be one method to increase their biological activity. Preliminary docking calculations[4)] on INFα by one molecule of β-cyclodextrin (βCD) have shown that their interaction energy ranges from -58 to -226 kJ mol^{-1}. It appears that cyclodextrins may exert an appreciable stabilizing effect on protein system such as interferon. The cyclic oligosaccharide βCD consists of seven α-D-glucopyranose residues covalently bound by α-1,4-linkages, thus forming a ring-shaped structure with a central cavity of about 0.7 nm diameter. βCD may form inclusion complexes with many guest molecules and

stabilize peptides and protein drugs[5-12]. In addition, several interesting papers report on both theoretical and experimental studies of the interaction of different class of molecules with βCD[13-16] and also on structural effects, docking and drug binding, quantum mechanical studies, hydrophobic potential calculations etc.[17-19].

To evaluate the nature of the interactions between βCD and IFNα, a detailed molecular modeling study has been performed focused on:

i) investigation of the molecular interactions and inclusion phenomena of simple probes, such as H_2O, C, CH_4, C_6H_6, NH_4^+, $HCOO^-$, within the βCD cavity[20];
ii) modelling of the molecular interactions of βCD molecule with the side chains of α-L-aminoacids in model molecules and in pentapeptides[21];
iii) evaluation of the interaction energies and analysis of the energetic contributions in 1:1 complexes between βCD and IFNα at different docking sites[22];
iv) evaluation of the energies of complexes between n βCD and one molecule of IFNα varying the number n of βCD molecules placed in the first encapsulating shell[22].

Computational Methods

Molecular mechanics simulations on free βCD were carried out with the Discover program[23] using an all-atom model for βCD and the probes. Consistent-valence force field (CVFF)[24] and atomic charges, without non-bonding interaction cutoff were used. The crystal structure[25] of βCD was relaxed using an effective dielectric constant of 4 to account for the dielectric shielding. Initial optimization of hydrogen atom positions was followed by steepest descent and conjugate gradient minimizations of the whole βCD molecule till convergence at the gradient of 0.04 $kJ \cdot mol^{-1} \cdot Å^{-1}$ was reached. Molecular dynamics simulations were carried out for the isolated βCD molecule ($\varepsilon = 4$) and for βCD hydrated by 133 water molecules in a periodic box for 100 ps at a temperature of 300 K.

To analyze the nature of intermolecular interactions between the probe approaching the center of the βCD ring cavity in a perpendicular direction to the plane of the host molecule the interaction energy profiles were simulated along this path. The profile of interaction energy, E_{int}, calculated for 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 force field. In this relatively simple approach the probe orientation was preserved and the molecular geometries of the probe and βCD were kept fixed at each point of the path. Adaptation of the host molecule to the guest was not taken into account. The solvation Gibbs free energy, G_{solv}, of the probes was calculated in the framework of the Polarizable Continuum Model[26,27] with the dielectric constant of 80 representing water as the solvent and includes electrostatic, dispersion-repulsion and cavitation terms.

All structures of the model L-α-aminoacids (AA) and βCD/AA complexes were modeled using the Insight II molecular modelling package of BIOSYM/MSI[28]. Molecular mechanics

calculations of model α-aminoacids and βCD/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[23]. In Model 1, 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 βCD ring cavity. The reported energy values refer to these optimized structures.

To analyze in detail the nature of interactions of βCD 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 βCD cavity (Model 2). In both cases the complexation energies were calculated from the molecular mechanics energies of the βCD/AA complex ($E_{AA/\beta CD}$), βCD ($E_{\beta CD}$) and model AA (E_{AA}) using the relationship:

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

where E_{compl} [AA] is the complexation energy for a given α-aminoacid, either isolated or at the center of a pentapeptide, inside the βCD cavity. The relative complexation energies (ΔE_{compl}) of the guest L-α-aminoacid species with respect to the smallest L-α-aminoacid, glycine (Gly) was calculated as

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

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 $(E^{d,r}/E^{int})_{compl}$ of the complexation energy of each species, with a view to analyze in detail the contributions of non-bonding interactions to the stabilization of the inclusion complex. The $(E^{d,r}/E^{int})_{compl}$ ratio is defined as:

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

where $E^{d,r}_{compl}$ is the dispersion-repulsion contribution and E^{coul}_{compl} is the non-bonding coulombic contribution to the complexation energy. The solvation Gibbs free energies (ΔG_{solv}) for the Model 1 α-aminoacids were calculated using the Polarizable Continuum Model[26,27]. The solute was represented by a set of point atomic charges derived from the CVFF force field and was placed in a cavity of realistic shape composed of intersecting spheres with van der Waals radii centered on individual atoms. The solvent was represented by a homogeneous dielectric medium of permittivity ε = 80 (water).

The 3-D structure of IFNα was established by computational studies[29]. In the inclusion complexes of βCD, organic guest molecules may move into within the ring cavity from either the upper side, containing secondary hydroxyl groups (model A), or from the lower side containing primary hydroxyl groups (model B). The 1:1 inclusion complexes (one molecule of βCD and one molecule of IFNα) were constructed by including the most representative residues of 20 natural L-α-aminoacids from various region of the IFNα surface within the cavity of βCD. In both models (A and B), the complexation energy (E_{compl}) was calculated from the molecular mechanics energies of the βCD/IFNα complex ($E_{\beta CD/IFN\alpha}$), βCD ($E_{\beta CD}$) and INF-α ($E_{IFN\alpha}$) by using the relationship:

$$E_{compl}[AX] = E_{\beta CD/IFN\alpha} - E_{\beta CD} - E_{IFN\alpha}$$

where $E_{compl}[AX]$ is the complexation energy of an aminoacid residue (AX) of IFNα.

In order to compare differences between inclusion complexes with aminoacids bonded to the protein molecule and with isolated L-α-aminoacids, the relative complexation energy ($\Delta E_{compl}[AX]$) was also calculated by the following equation:

$$\Delta E_{compl}[AX] = E_{compl}[AX] - E_{compl}[GlyAX]$$

where $E_{compl}[GlyAX]$ is the complexation energy for the complex between βCD and gly44 in model A. The total interaction energy of βCD with different IFNα aminoacid residues ($E_{int}[AX]$) can be divided into two main components according to the relation:

$$E_{int}[AX] = E_{\beta CD/AX} + E_{\beta CD/IFN\alpha}$$

where $E_{int}[AX]$ is the total interaction energy calculated by a custom-written program, $E_{\beta CD/AX}$ is the energetic contribution to the interaction between βCD and the included aminoacid residue and $E_{\beta CD/IFN\alpha}$ includes the interactions among βCD and neighboring IFN-α residues. In addition, dispersion-repulsion ($E^{d/r}$) and non-bonding coulombic (E^{coul}) contributions to $E_{\beta CD/AX}$ and $E_{\beta CD/IFN\alpha}$ were calculated from the equation:

$$E_{int}[AX] = [E^{d/r}]_{\beta CD/AX} + [E^{coul}]_{\beta CD/AX} + [E^{d/r}]_{\beta CD/IFN\alpha} + [E^{coul}]_{\beta CD/IFN\alpha}$$

To evaluate the appropriate number n of host molecules required to encapsulate INFα, the shape of IFNα was approximated to a cylinder having base diameter d = 30 Å and height h = 50 Å. β-Cyclodextrin molecules were represented by discs with effective diameter D = 14 Å (approximate diameter) + 4 Å (inter-discs spacing) = 18 Å. The computational simulation started from n = 16, then βCD molecules were gradually added to the system until n = 30 was reached. All structures were minimized by fully relaxed geometrical optimization. For each system the complexation energy $[E^n]_{compl}$ and the average energetic contribution by one βCD to the stabilization of one molecule of IFNα ($\Delta[E_{\beta CD}]_{compl}$) were calculated. $\Delta[E_{\beta CD}]_{compl}$ is defined as the ratio:

$$\Delta[E_{\beta CD}]_{compl} = [E^n]_{compl}/n$$

where n is a number of molecules of βCD used for the encapsulation of IFNα and $[E^n]_{compl}$ is the total complexation energy defined by the relation:

$$[E^n]_{compl} = [E^n]_{\beta CD/IFN\alpha} - n\,[E^n]_{\beta CD} - E_{IFN\alpha}$$

The above equation includes the molecular mechanics energies for the system IFNα/n βCD ($[E^n]_{\beta CD/IFN\alpha}$) and for the isolated βCD ($E_{\beta CD}$) and IFNα ($E_{IFN\alpha}$). To analyze interactions within the inclusion complexes in the liquid phase, water as a solvent was simulated by a homogeneous dielectric medium of permittivity ε = 80 using the consistent valence force field (CVFF).

Results and Discussion

Small molecular probes. The energy minimized structure of βCD forms a ring with an inner diameter of approximately 10.1 Å and a height of about 5.5 Å. The inner ring diameter and the ring height determine the size of the host cavity. Thus βCD can accommodate 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 a limit on the guest molecule size. The investigated probes (H_2O, C, CH_4, C_6H_6, NH_4^+, $HCOO^-$) are small molecules that can easily fit into the βCD ring cavity, therefore no steric factor affected the simulated guest-host interaction energy profiles. During the simulations of the inclusion complex formation probes penetrated into the empty βCD cavity, i.e. no water molecules was displaced by the entering probe. Thus the energy profiles reflected the net effect of guest-host non-bonding interactions and were analyzed in terms of coulombic and dispersion-repulsion components.

The interaction energy profiles (E_{int}) of the probes entering the cavity of βCD along the path perpendicular to the βCD ring plane are shown on Fig. 1, together with coulombic (E_{coul}) and dispersion-repulsion ($E_{d,r}$) components. The interaction energy of the guest-βCD 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 βCD axis.

The interaction energy profile for the water probe, the symmetry axis of H_2O was parallel to the βCD axis, indicates that the inclusion complex of βCD with one water molecule located in the ring cavity center is stabilized by an interaction energy of E_{min} = -10.0 kJ·mol^{-1} and the molecule is trapped within the cavity by an energy of -10.5 kJ·mol^{-1}. Coulombic and dispersion-repulsion interactions contribute almost equally to the total interaction energy and both contributions show a minimum at $R_{min} \approx 0.5$ Å. The interaction energy profiles of three hydrophobic probes C, CH_4 and C_6H_6 with βCD show similar features (Fig. 1). The interaction energies of these hydrophobic probes with βCD are composed entirely from dispersion-repulsion interactions in both the short and long range portion of the reaction path. The coulombic component of the interaction energy cancels out due to the symmetry of the neutral probes and the βCD ring.

The interaction energy profiles of the charged hydrophilic probes NH_4^+ and $HCOO^-$ are both non-symmetric, contain a barrier with a maximum close to the βCD cavity opening (R ≈ 6.0

Å) and a minimum at the opposite side of the βCD ring opening (outside the cavity). Both interaction energy curves are dominated by the coulombic component. Therefore, neutral small hydrophobic molecules are attracted towards the βCD cavity center by dispersion forces whereas 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 βCD ring captivated by the electrostatic forces.

Small molecules such as the considered probes possess enough freedom to adjust their position and orientation inside the cavity and at the faces of the βCD ring, therefore, the reported interaction energies evaluated for fixed probe orientation and molecular geometries of guest and host molecules represent only a rough approximation of the complexation energies. If global minimization is performed by releasing the fixed coordinates in the position of minimum energy, then a further stabilization is obtained (e. g. 15.1 kJ·mol^{-1} for benzene). However, this is not significant for the present investigation that is focused on the evaluation of interaction profiles passing along the internal cavity axis.

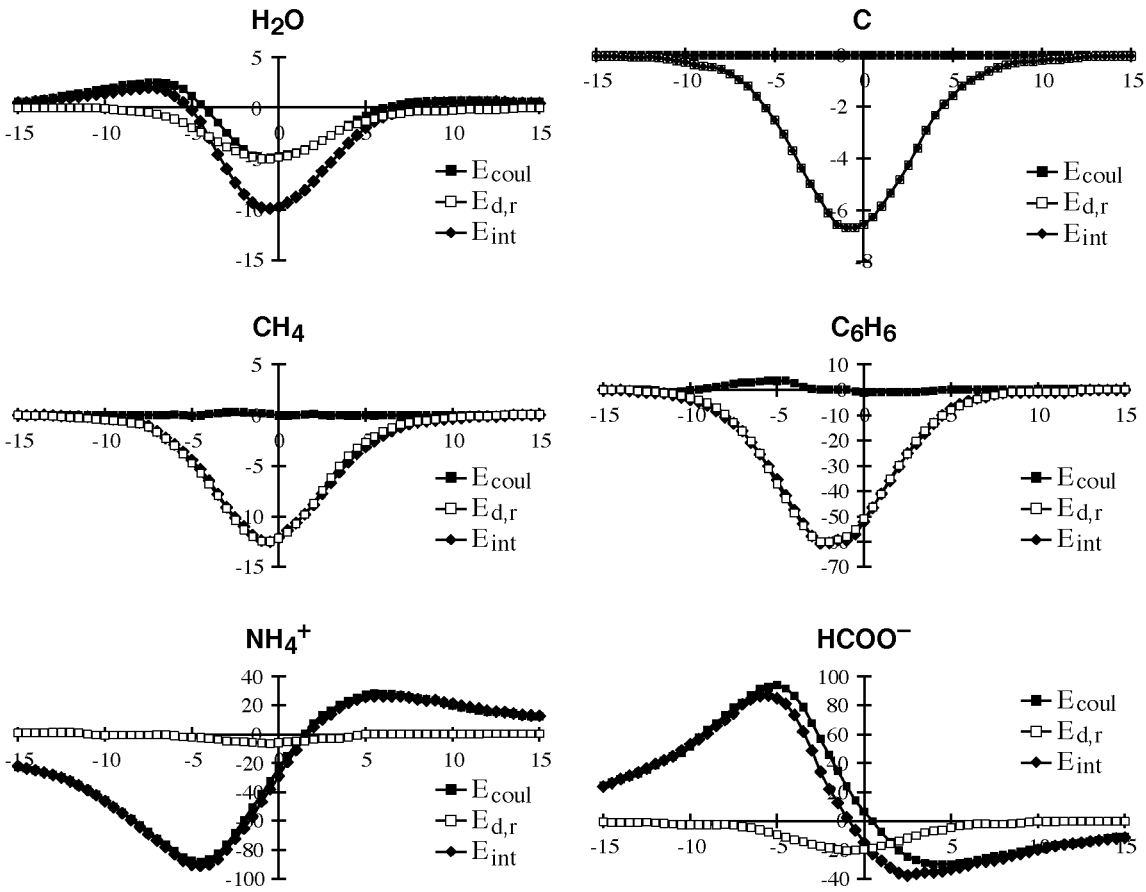

Fig. 1: Interaction energy (in kJ/mol) profiles of simple probes entering the βCD cavity along the symmetry axis perpendicular to the ring plane (coordinate on the x-axis with the origin placed in the cavity center is in Å).

Moreover, in solution most of the hydroxyl groups of βCD will be engaged in intra- or intermolecular hydrogen bonding with water molecules and therefore will not be entirely exposed towards the approaching probe. Nevertheless, this approach describes the essence of βCD inclusion complexes formation and enables a consistent comparison between host molecules of different charge, symmetry and hydrophobicity.

Formation of an inclusion complex in solution will depend on the competition between the stabilization of the probe inside of the βCD 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} (that approximately correspond to the energy of inclusion complex formation, neglecting the entropy contribution to the complexation reaction) should be compared to the solvation Gibbs free energies in water, G_{solv}, for the considered probes. This comparison revealed that polar and charged molecules (such as H_2O, NH_4^+, $HCOO^-$) prefer the hydrophilic solvent (water) environment to the hydrophobic βCD cavity and non-polar probes (such as C, CH_4, C_6H_6) will rather prefer the βCD cavity interior to the bulk solvent.

Analysis of the net effects of molecular properties, such as total charge, polarity, and charge distribution symmetry in the probes, upon the shape and magnitude of the interaction energy profiles with βCD which possesses a high cylindrical symmetry of the electric field along its symmetry axis, 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, the degree of symmetry of charge distribution determines the existence of "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, and SF_6, and low symmetry non-polar small molecules tend to form "hydrophobic" inclusion complexes, while low symmetry polar molecules with permanent dipole moments tend to form "dipole-dipole" complexes.

Electrostatic interactions between highly symmetric polar probes and host molecules cancel out and the formation of guest-host inclusion complexes is dominated by the dispersion-repulsion interactions between the probe and βCD which reach the maximum stabilization at the closest distance, i.e. in the center of the βCD ring cavity. For a low symmetry polar probe the guest-host interactions are dominated by the stronger electrostatic interactions which reaches the maximum stabilization at the minimum of the MEP profile. Thus non-symmetric probes form "dipole-dipole" rather than the "hydrophobic" inclusion complexes.

Inclusion complexes of L-α-aminoacids with β-cyclodextrin. The computed complexation energies (ΔE_{comp}) for inclusion complexes of the side chains of L-α-aminoacids in which both the carboxyl and the amino group have been replaced by methyl groups with βCD (Model 1)

together with the calculated $(E^{d,r}/E^{int})_{compl}$ ratio and solvation Gibbs free energy (ΔG_{solv}) are given in Tab. 1. The ΔE_{compl} computed for the side chains of the same L-α-aminoacids when placed at the centre of a methyl end-capped symmetric pentapeptide, containing two alanine residues on both sides, (Model 2) together with the $(E^{d,r}/E^{int})_{compl}$ ratios are also reported in Tab. 1. The reported values suggest that neutral aliphatic non-polar α-aminoacids such as Gly, Ala, Val, Leu, Ile form weak hydrophobic inclusion complexes with βCD. The corresponding solvation Gibbs free energies are quite low compared to the ΔE_{compl} values suggesting that formation of the inclusion complex is preferred to the solvation in bulk water.

Tab. 1. Computed complexation energies (in kJ mol^{-1}) for inclusion complexes of the side chains of L-α-aminoacids with βCD.

α-Amino	Model 1			Model 2	
Acid	ΔE_{comp}	$(E^{d,r}/E^{int})_{compl}$	ΔG_{solv}	ΔE_{compl}	$(E^{d,r}/E^{int})_{compl}$
Gly	0.0	1.0	0.0	0.0	1.0
Ala	-4.2	0.9	-3.8	-17.2	0.7
Val	-29.7	1.0	-8.8	-14.2	0.9
Leu	-37.7	1.0	-10.9	-22.2	0.9
Ile	-37.3	1.0	-10.9	-13.4	0.9
Pro	-43.5	0.8	-20.9	-28.5	1.0
Phe	-69.9	0.9	-15.1	-54.0	0.9
Tyr	-71.2	0.9	-22.2	-63.2	0.9
His	-55.7	0.8	-19.3	-47.7	0.9
Trp	-72.4	1.0	-22.2	-92.1	0.8
Ser	-22.6	0.5	-10.9	-29.3	0.7
Thr	-32.7	0.7	-15.1	-24.3	1.0
Cys	-20.1	0.8	-3.8	-13.8	0.9
Met	-26.0	0.9	-7.1	-22.2	1.0
Asn	-38.5	0.7	-18.8	-27.2	0.7
Gln	-36.0	0.9	-20.1	-48.6	0.7
Lys	-157.8	0.2	-164.9	-110.5	0.6
Arg	-155.7	0.3	-157.4	-117.2	0.7
Asp	-62.0	0.3	-185.0	-117.9	0.3
Glu	-53.6	0.5	-178.7	-114.3	0.3

The ΔE_{compl} values indicate that also L-α-aminoacids inserted in CH_3-Ala-Ala-AA-Ala-Ala-CH_3 structures form weak hydrophobic inclusion complexes. The guest-host interaction in

the optimized complex is composed mostly by the dispersion-repulsion term as indicated by the $(E^{d,r}/E^{int})_{compl}$ ratio that is close to 1. The $(E^{d,r}/E^{int})_{compl}$ ratio obtained for guest-host interactions only is 0.7, indicating that the coulombic part of stabilization is almost fully compensated by the destabilization of βCD when engaged in the formation of the complex.

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 and in the pentapeptide, as indicated by their very high ΔE_{compl} values. The complexation energy increases (in magnitude) with the size of the side chain. Dispersion-repulsion interactions are the dominating interactions also for aromatic side chains.

Polar α-aminoacids such as Ser, Thr, Cys and Met were found to yield only low ΔE_{compl} values, indicating that the corresponding inclusion complexes are relatively weak. Also in these cases, dispersion-repulsion interactions play a major role and the hydrophobic nature of the internal cavity of βCD is the factor governing guest-host interactions[30].

Polar amides such as Asparagine (Asn) and Glutamine (Gln) were found to form weaker inclusion complexes as compared to the corresponding Trp derivatives. The $(E^{d,r}/E^{int})_{compl}$ ratios for these systems show a slight decrease, particularly in the single α-aminoacid case, suggesting a significant contribution by electrostatic interactions.

The acidic protonated α-aminoacids Lys and Arg should give strong inclusion complexes with βCD in the gas phase, as indicated by their large negative ΔE_{compl} values. The $(E^{d,r}/E^{int})_{compl}$ 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. Calculation of solvation Gibbs free energy (ΔG_{sol}) for these cationic species yielded large negative values, suggesting the occurrence of strong interactions with bulk water. Very likely in aqueous solution protonated Lys and Arg have a larger preference for the solvent bulk rather than for the formation of inclusion complexes. The decrease of the $(E^{d,r}/E^{int})_{compl}$ ratios is less dramatic when these L-α-aminoacids are inserted in the pentapeptide chain, probably due to the interactions of the backbone and side chains of the Ala-Ala segments.

The negatively charged basic residues Asp and Glu also showed significant ΔE_{compl} values, indicating strong interactions with βCD. 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 suggesting that these α-aminoacids will prefer to stay in the bulk solvent rather than forming hydrophobic inclusion complexes. Asp and Glu exhibit strong interactions with βCD in the pentapeptide form and in fact the complexation energies for Model 2 are much higher than those obtained for 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 of the pentapeptide backbone. The $E^{d,r}/E^{int}$ ratios for Asp and Glu in Model 1 and Model 2 forms are very low and hence it may be concluded that the major interactions of these residues with βCD are electrostatic in nature.

The molecular mechanics optimized structure of βCD indicates that 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. Therefore, it is reasonable to assume that H-bond interactions of the peptide backbone with the upper opening of the cavity have to compete with the existing intramolecular hydrogen bonding among the hydroxyl groups of free βCD or with the βCD/water hydrogen bonding. The geometry optimized structures of the inclusion complexes of the pentapeptides (CH_3-Ala-Ala-**AA**-Ala-Ala-CH_3) with βCD show that four of these intramolecular H-bonds have been released to form four strong intermolecular hydrogen bonds with the carbonyl oxygen atoms 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 βCD. However, the coulombic interaction is more or less completely compensated by the loss of the intramolecular hydrogen bonding of free βCD and hence the calculated $(E^{d,r}/E^{int})_{compl}$ ratio is close to 1 for Gly-containing Model 2.

Inclusion complexes of α-interferon with β-cyclodextrin. Evaluation of the interaction energies in 1:1 complexes between βCD and IFNα was performed on inclusion complexes containing gly44, ala97, val99, leu3, leu30, pro109, pro137, phe27, tyr89, his7, trp76, ser115, met111, asn156, lys70, lys134, glu78 and glu113 as guest residue. Calculated complexation energies on both models A and B range from -136.0 to -277.1 kJ mol^{-1}. The average value of E_{compl} is -207.5 kJ mol^{-1} (model A) and -181.5 kJ mol^{-1} (model B). The complexes with leu30 (model A), tyr89 (model A), ser115 (model A), asn156 (models A and B), lys70 (model A) and glu113 (models A and B) show large E_{compl} values. The complex tyr89 (model A) possess the highest E_{compl} value (-277.1 kJ mol^{-1}), and it is remarkable that the E_{compl} difference between model A and model B of the complex of tyr89 is 81.2 kJ mol^{-1}. Rather large differences can also be detected for the two models of ala97 (66.1 kJ mol^{-1}) and met111 (66.6 kJ mol^{-1}) complexes. On the other hand, in the case of asn156 this difference is only 0.8 kJ mol^{-1}. These results seem to demonstrate that the interaction between βCD and IFNα aminoacid residues is more effective when the upper opening of the host βCD molecule approaches the IFNα surface (model A). The ala97 (model B), leu3 (model B), pro137 (model A), pro137 (model B) and met111 (model B) complexes exhibit small E_{compl} values, the met111 complex (model B) showing the lowest one (-136.0 kJ mol^{-1}). It appears that weakly polar aromatic residues, such as phe, tyr, his etc. and charged glu and lys are the most appropriate central residues, that is the ones placed within the βCD cavity.

The calculated relative complexation energies range from -68.6 to 72.5 kJ mol^{-1}. Their average value is -2.2 kJ mol^{-1} (model A) and 19.6 kJ mol^{-1} (model B). The rather large difference

between the average values of the two models confirmed that model A may better simulate the encapsulation process than model B. The values of E_{int} range from -132.0 to -323.5 kJ mol^{-1}. Also the average value of E_{int} is higher for model A (-244.5 kJ mol^{-1}) than for model B (-209.7 kJ mol^{-1}).

Complexes with the same type of central residue, for instance leu3 and leu30 or glu78 and glu113, showed large E_{int} differences, in accordance with the existence of strong interactions among βCD and IFNα aminoacid residues neighbouring the encapsulated one. To evaluate a total interaction profile we have calculated the interaction energies for each inclusion complex from its output geometry minimized by molecular mechanics calculations (Tab. 2).

Although residues located inside the βCD cavity give the greatest contribution to E_{int}, their values do not exceed 45.3 % of the total energy for all the calculated species. The central residues phe27 (models A and B), tyr89 (model A), his7 (model A), trp76 (models A and B), glu78 (models A and B) and glu113 (models A and B) give high energetic contributions to the total E_{int}. The residue glu113 (model B) has the highest value of $E_{\beta CD/AX}$ (-116.8 kJ mol^{-1}). On the contrary, residues gly44 (models A and B), ala97 (models A and B), pro137 (model A), ser115 (models A and B) and met111 (model B) have small $E_{\beta CD/AX}$ values. The residue ser115 shows the smallest $E_{\beta CD/AX}$ (-10.6 kJ mol^{-1}). For all complexes approximately 90 % of the total E_{int} arises from the dispersion/repulsion component.

Weakly polar cyclic and aromatic residues, such as phe27 (models A and B), tyr89 (models A and B), his7 (model A) and trp76 (models A and B) and the negatively charged glu78 (models A and B), and glu113 (models A and B) exhibits very strong hydrophobic interactions as indicated by their very high $E^{d/r}$ values. Trp76 (model A) possess the highest $E^{d/r}$ value (-108.1 kJ mol^{-1}). The residues glu78 (model B) and glu113 (model B) also have rather high values of the coulombic component E^{coul} (-46.8 kJ mol^{-1} and -44.2 kJ mol^{-1}), but most residues have rather small values of E^{coul} that do not contribute much to the total E_{int}.

Since the calculations on the inclusion complexes have displayed better results for the model A, the system n βCD/IFNα was designed accordingly, that is each βCD molecule was placed on the surface of IFNα by the upper opening which has only secondary hydroxyl groups. $\Delta[E_{\beta CD}]_{compl}$ increases gradually by adding βCD molecules until the number n = 25 is reached (Fig. 2). Further addition of βCD molecules causes a decrease of $\Delta[E_{\beta CD}]_{compl}$ and a destabilization of the encapsulated system. The system 25 βCD/IFNα has the highest value of $\Delta[E_{\beta CD}]_{compl}$ (-245.2 kJ mol^{-1} for ε = 4 and -235.5 kJ mol^{-1} for ε = 80) and the number n = 25 can be considered as a limit for the coverage of the first encapsulating shell of IFNα.

We have tried to explain this finding by a decomposition of the interaction forces within the systems 16 βCD/IFNα (ε = 4) and 25 βCD/IFNα (ε = 4). This simple analysis has shown that for the systems 16 βCD/IFNα and 25 βCD/IFNα, respectively 7 % (-16.4 kJ mol^{-1}) and 13 % (-31.9 kJ mol^{-1}) of $\Delta[E_{\beta CD}]_{compl}$ (-233.9 kJ mol^{-1}) arise from interactive forces among cyclodextrins (βCD/βCD interaction).

Tab. 2. Calculated interaction energies (in kJ·mol^{-1}) within 1:1 βCD/IFNα complexes[a)].

Residue	$[E^{coul}]_{\beta CD/AX}$	$[E^{d/r}]_{\beta CD/AX}$	$[E^{coul}]_{\beta CD/IFN\alpha}$	$[E^{d/r}]_{\beta CD/IFN\alpha}$	E_{int}
gly44-A	-2.3	-24.5	-22.6	-185.9	-235.3
gly44-B	0.4	-23.2	0.3	-157.9	-179.3
ala97-A	0.4	-31.9	-32.0	-172.4	-236.6
ala97-B	-4.3	-30.7	0.2	-122.7	-157.5
val99-A	-4.4	-48.5	-8.6	-154.9	-216.3
val99-B	-5.1	-48.5	-1.3	-143.7	-198.5
leu3-A	-4.7	-51.4	-22.4	-134.5	-212.9
leu3-B	-0.02	-50.9	-26.1	-149.3	-175.4
leu30-A	-4.7	-66.8	-5.9	-177.9	-250.6
leu30-B	0.8	-61.2	-19.9	-151.1	-231.5
pro109-A	-5.4	-46.6	-21.5	-131.9	-205.3
pro109-B	1.1	-41.6	10.0	-105.5	-132.0
pro137-A	-0.1	-29.9	-20.6	-134.3	-184.9
pro137-B	3.6	-53.9	2.8	-141.9	-196.6
phe27-A	-1.1	-87.9	2.9	-114.2	-200.3
phe27-B	-4.1	-77.2	-14.5	-83.4	-179.3
tyr89-A	-3.4	-74.7	-17.5	-198.8	-323.5
tyr89-B	-2.1	-68.7	4.1	-154.2	-216.7
his7-A	-3.3	-72.0	-12.6	-173.3	-261.2
his7-B	0.1	-59.4	0.4	-131.2	-190.2
trp76-A	-1.1	-108.1	-16.1	-153.8	-279.1
trp76-B	-2.8	-86.1	-36.3	-120.1	-245.3
ser115-A	3.8	-14.4	-71.4	-180.7	-265.1
ser115-B	-1.1	-20.1	-27.8	-196.5	-245.3
met111-A	-0.4	-39.3	-64.1	-141.9	-244.8
met111-B	-0.3	-34.2	5.7	-130.7	-159.4
asn156-A	-2.6	-44.8	-48.7	-156.2	-252.3
asn156-B	1.7	-44.4	-58.7	-175.9	-277.2
lys70-A	7.5	-47.5	-51.7	-177.7	-269.3
lys70-B	-4.4	-43.7	-15.7	-176.5	-240.4
lys134-A	-14.2	-53.4	-5.7	-178.7	-252.1
lys134-B	-23.2	-48.6	4.4	-160.2	-227.6
glu78-A	-22.1	-74.2	-3.3	-145.8	-245.4
glu78-B	-46.8	-65.8	-9.0	-131.2	-252.8
glu113-A	-12.0	-69.2	-40.3	-147.2	-268.7
glu113-B	-44.2	-72.6	-16.7	-160.5	-294.0

[a)] The A and B suffixes refer to structures calculated using model A and B, respectively. E_{int} is the total interaction energy. $[E^{d/r}]_{\beta CD/AX}$ and $[E^{coul}]_{\beta CD/AX}$ are the dispersion-repulsion and the coulombic component of the interaction energy between βCD and a central residue AX of IFNα. $[E^{d/r}]_{\beta CD/IFN\alpha}$ and $[E^{coul}]_{\beta CD/IFN\alpha}$ are the dispersion-repulsion and the coulombic component of the interaction energy among βCD and neighboring IFNα residues.

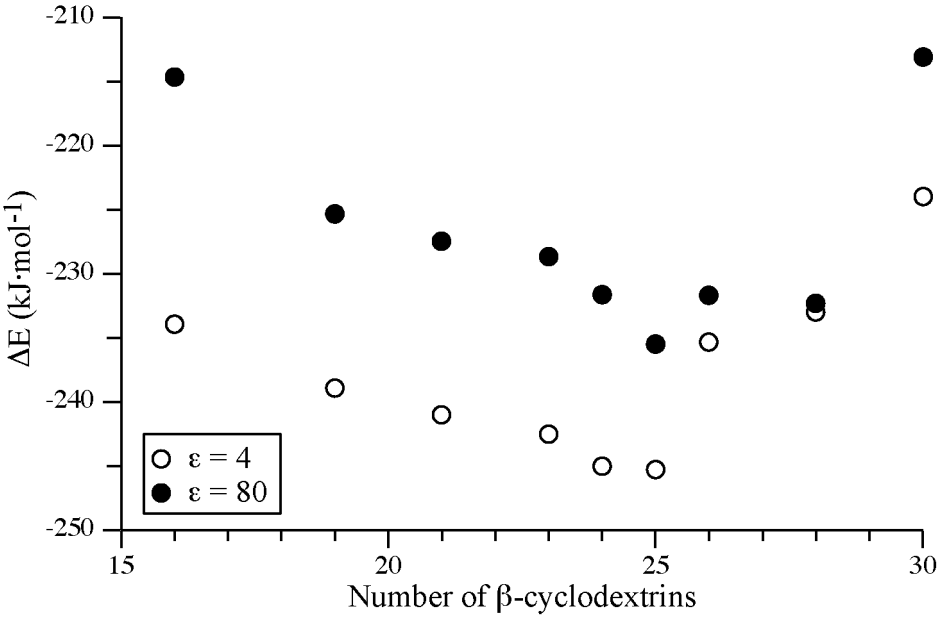

Fig. 2: Variation of the complexation energy of the system n βCD/IFNα with number n of βCD molecules, in a continuum with dielectric constant 4 and 80.

Cyclodextrins are not very close to each other in the system 16 βCD/IFNα and addition of another molecule of βCD to the system fills up empty space on the IFNα surface and increase the interaction area among cyclodextrins. It appears that the attractive force arising from βCD/βCD interactions reaches a maximum at n = 25. A further increase of n up to 30 results in an increase of the repulsion forces among cyclodextrins.

Concluding Remarks

The results obtained in a molecular mechanics investigation of the interaction energy profiles of host-guest inclusion complexes of βCD with simple probes, α-aminoacids, pentapeptides and α-interferon, allow to draw the following concluding remarks.

i) Symmetric hydrophobic probes are predicted to form stable inclusion complexes with βCD, the probe position will be typically near the cavity center. The stability of the inclusion complexes will increase with increasing size and hydrophobic character of the probe. Polar and charged probes are predicted to prefer the interaction with bulk water rather than the formation of inclusion complexes.

ii) Guest-host interactions are almost entirely dominated by dispersion interactions in stable inclusion complexes with hydrophobic probes.

iii) Strong inclusion complexes are 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 water thus preventing the formation of an inclusion complex. In

pentapeptides, interactions of the peptide backbone with βCD contribute to the stabilization of the inclusion complex. However, also intermolecular and intramolecular hydrogen bonding must be taken into proper account.

iv) The surprisingly small coulombic interactions, not taking into account charged residues, of the 1:1 βCD/IFNα host-guest system may be caused by the symmetry of the host. Indeed βCD possesses a high cylindrical symmetry of the electric field along its symmetry axis. Thus electrostatic interactions between host and guest molecules cancel out and the formation of inclusion complexes is dominated by dispersion-repulsion interactions.

v) For the system consisting of one IFNα molecule, the most suitable number of host βCD molecules in the first encapsulating shell is 25.

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