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SOLID STATE STUDY OF THE INCLUSION COMPOUNDS OF *ALPHA*-, *BETA*- CYCLODEXTRIN WITH D-, L-TRYPTOPHAN ISOMERS

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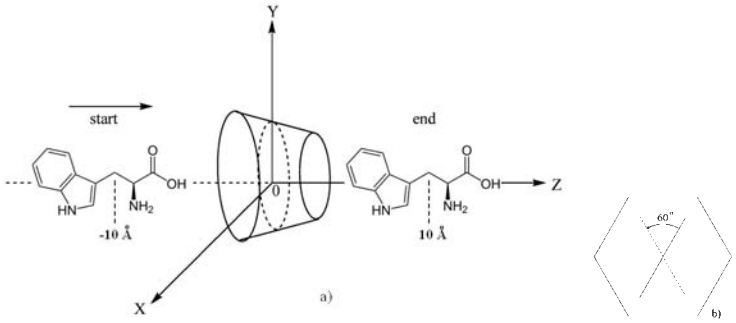
The solid inclusion complexes of α- and β-cyclodextrins (CD) with stereo isomeric tryptophan (Trp) amino acids (L-tryptophan and D-tryptophan) were studied by using Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FT-IR) and simulation methods. The data reveal that selective inclusion complex formation of α- and β-cyclodextrins with L-tryptophan and D-tryptophan amino acids takes place. The correlation of the experimental and theoretical data shows that the complexation between the tryptophan and cyclodextrin could be explained by geometrical differences giving higher stability for the systems including β-cyclodextrin.



INTRODUCTION

Cyclodextrins (CD) (cyclomaltooligosaccharides) are macrocyclic oligosugars capable to form inclusion complexes with small hydrophobic molecules, thus improving their properties. Cyclodextrins can be obtained from starch by means of enzymatic conversion. The enzyme is modifying the polysaccharide, turning it into a cyclic oligomer with a proper number of glycopyranoside units. The cyclic oligomers consisting of six, seven and eight glycopyranoside units are the most used and are called α-, β- and γ- cyclodextrin, respectively.[1] Cyclodextrins are shaped like an intrusive truncated cone which is a relatively hydrophobic in the middle and relatively hydrophilic on the outside because of hydroxyl groups.[2] The most important factor in the guest selectivity of the cyclodextrin is the size of the cyclodextrin cavity matching that of the guest molecule. Thus, cyclodextrins are designed to have a high ability to complex a wide range of molecules with different degree of hydrophobicity. The inclusion complexes thus formed, also known as guest–host complexes, can have highly different properties compared to that of the guest molecule alone, including altered solubility, stability, reactivity, volatility and bioavailability.[1] Tryptophan (Trp) is an essential amino acid for many organisms and participates as a building block in protein biosynthesis and in production of other important biochemical compounds, especially L-stereoisomer of Tryptophan; D-stereoisomer is occasionally found in naturally compounds. Tryptophan possesses a hydrophobic indole group attached to its β carbon; indole is a common component and the precursor to many pharmaceuticals; indole nitrogen is capable of acting as a hydrogen bond donor.

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Even though the characterization of cyclodextrin inclusion complexes with amino acids was accomplished in many papers,[3-6] more studies are still needed to understand the complex formation and to reveal their thermodynamic stability domains. In the present paper, D- /L- Trp amino acids were chosen as the guest molecules in order to explore the effects of their size and shape upon the formation and stability of inclusion complexes. In order to obtain further insight into physical mechanisms explaining the formation of the solid inclusion compounds α-CD/D-Trp, α-CD/L-Trp, β-CD/D-Trp and β-CD/L-Trp, combined DSC, FTIR and quantum chemistry calculations were performed and the data were analyzed to make clear the relationship between the thermodynamic stability, the structural factors and the probability of the complex formation.

MATERIALS

D-Tryptophan (purity 99%), L-Tryptophan (purity 98%), α-Cyclodextrin (purity 98%), β-Cyclodextrin (purity 97%) were purchased from Sigma Aldrich Chemical Company and used without further purification.

Mixtures of Trp/CD molar ratio 1:1 in aqueous solution were obtained by co-precipitation. Solid powders resulted by vacuum evaporation of the solvent.

METHODS

A Perkin-Elmer power compensated differential scanning microcalorimeter model 8500 has been used for the thermal behavior investigation and enthalpies measurement of D-Trp, L-Trp, α-CD, β-CD solid powders and 1:1 molar ratio solid inclusion complexes between tryptophan enantiomers and cyclodextrins. DSC data were recorded using standard aluminium pans with crimped lids. All samples masses have been between 1 and 3 mg and were scanned in flowing nitrogen atmosphere (20 mL min^{-1}). The calorimeter was calibrated using indium samples (ΔH_{fus}= 28.46 J g^{-1}). In all measurements the calorimeter was operated at a scanning rate of 10 °C/min. The heat flow curves were processed with Pyris Software for Windows. Before starting the measurements all the compounds were dried in vacuum at room temperature.

FT-IR spectral data of pure compounds and inclusion complexes were recorded at room temperature by Nicolet iS10 FT-IR Spectrometer covering the range of 4000 to 600 cm^{-1}. The spectra were acquired with an average of 32 scans at a resolution of 4 cm^{-1}.

The interaction tryptophan-cyclodextrin was also characterized by quantum chemical calculations using the software package GAMESS.[7] Restricted Open Hartree-Fock calculations were performed, in the evaluation being used triple zeta valence (TZV) wave function sets. In the optimizing process of the geometries, to short the calculation time, we used progressively STO-3G, STO-6G and N311 wave functions, only the final calculations being performed with TZV sets.

RESULTS AND DISCUSSION

DSC analysis

DSC analysis was done for pure components α-CD, β-CD, D-Trp and L-Trp, and for inclusion complexes CD/Trp, Figs. 1 and 2.

Thermal behavior of cyclodextrins has been described by several processes observed in the temperature range between 30 °C and 500 °C. For α-CD the first dehydration process occurs in two stages, Fig. 1. The first stage of dehydration starts before the temperature of 100 °C, followed by another stage attributed to the elimination of water molecules from cyclodextrin cavity taking place at a higher temperature (the onset temperature is 121 °C). This last stage may be due to both working conditions (closed crucibles) and the manner of binding of water molecules inside the α-CD cavity. After dehydration stages an endothermic phase transition (started at 179 °C) occurs in α-CD due to a phase change of the anhydrous α-CD used in the analysis. This endothermic phase transition was also reported in other studies[8,9] being related to different types of commercial materials. In α-CD the thermal process between 288.19 °C - 289.11 °C is due to the thermal decomposition and is described by an enthalpy of decomposition of 37.92 J g^{-1}.

In Fig. 2, the thermogram corresponding to β-CD indicates several weak endothermic processes followed by thermal decomposition; in the temperature range between 130 °C to 170 °C have been identified two successive endothermic phenomena attributed to dehydration by loosening the deep water molecules from β-cyclodextrin cavity, in agreement with literature data.[8] The melting-decomposition thermal process for β-CD is noticed in the temperature range of 265.42 °C - 266.77 °C and is characterized by a value of the decomposition enthalpy of 22.87 Jg^{-1}.

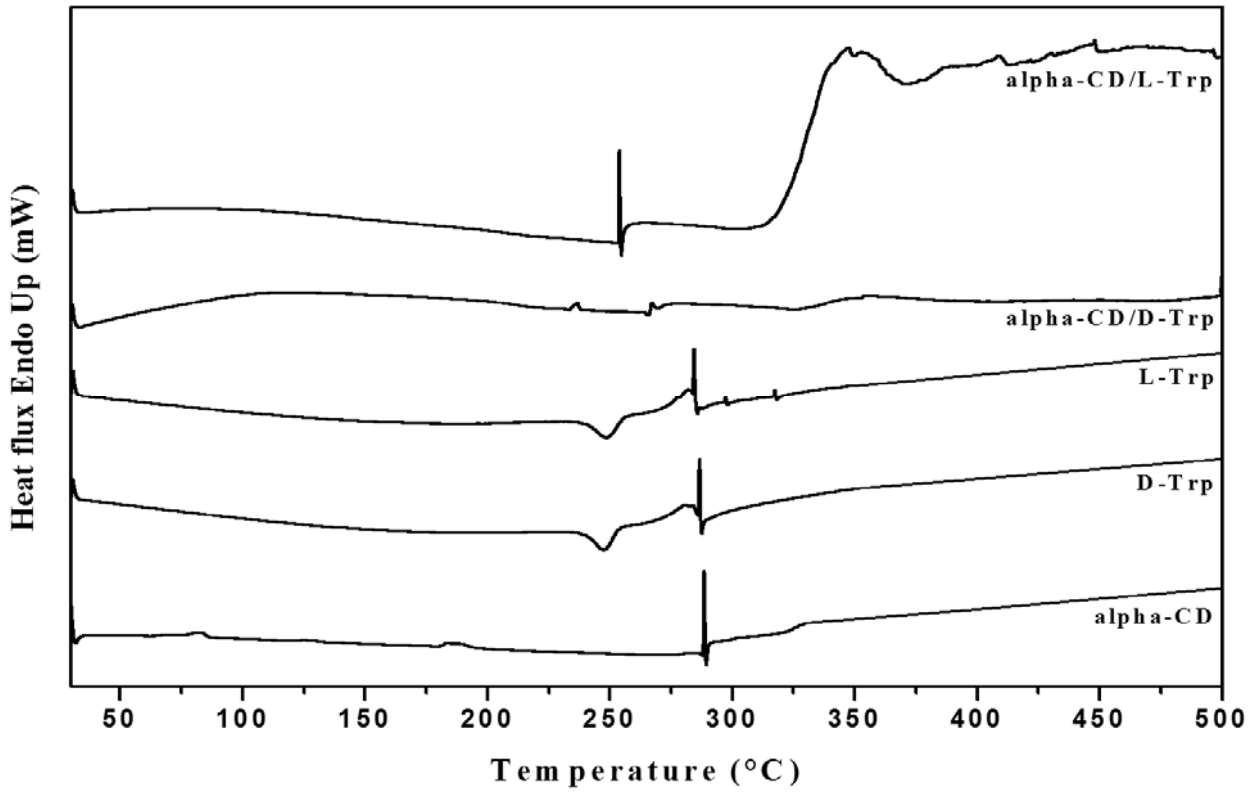

Fig. 1 – DSC curves for pure compounds: α-cyclodextrin (alpha-CD), L-tryptophan (L-Trp), D-tryptophan (D-Trp), and for 1:1 Trp/CD mixture: alpha-CD/L-Trp, alpha-CD/D-Trp.

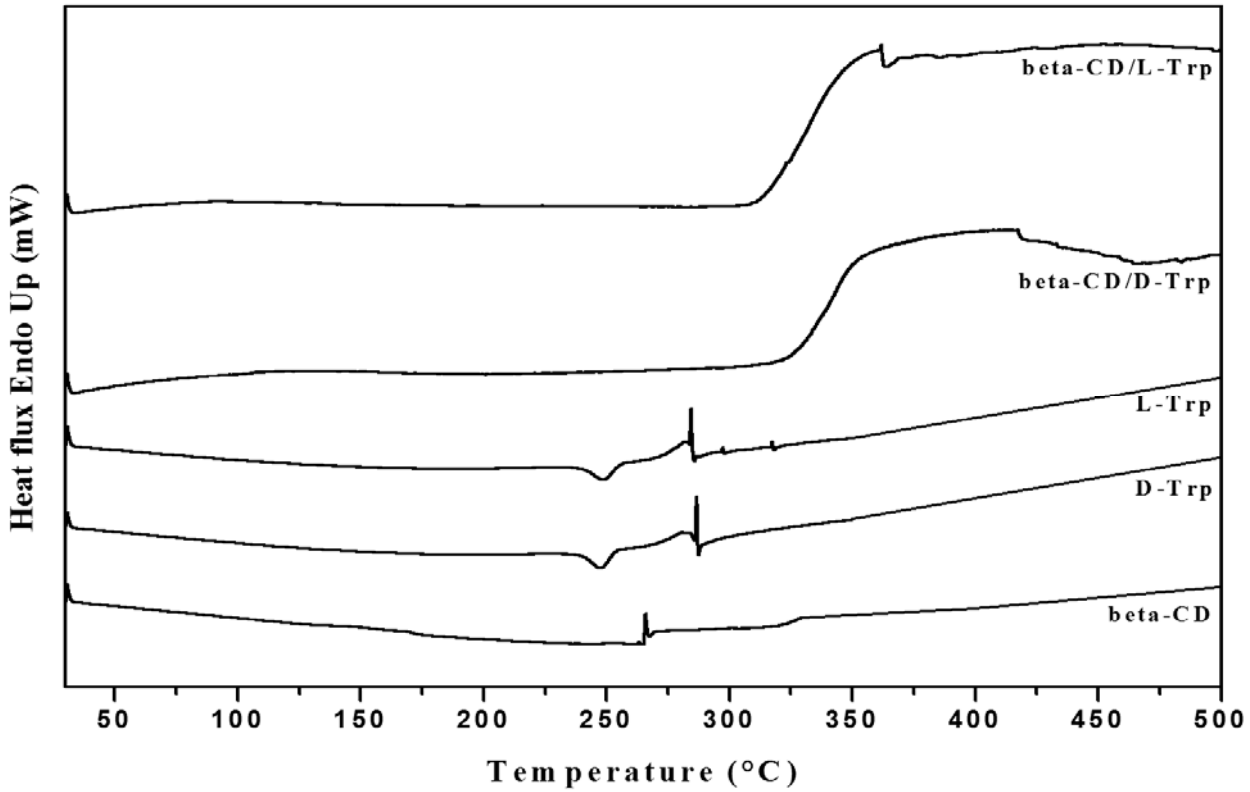

Fig. 2 – DSC curves for pure compounds: β-cyclodextrin (beta-CD), L-tryptophan (L-Trp), D-tryptophan (D-Trp), and for 1:1 Trp/CD mixture: beta-CD/L-Trp, beta-CD/D-Trp.

The thermograms corresponding to tryptophan isomers both reveal an exothermic process which is a thermal effect due to a rearrangement of chemical bonds. For levogir isomer of Trp (L-Trp) this thermal process runs in the temperature range of 241.71 °C - 255.07 °C and is characterized by enthalpy of transition ΔH_{tr} = -150.23 J g^{-1}. For dextrogir isomer of Trp the temperature range is 240.91 °C - 253.05 °C with enthalpy of transition ΔH_{tr} = -154.68 J g^{-1}. The peaks of the transition temperatures for both D- and L- Trp isomers have close values (T_{peak} = 248.64 °C for L-Trp and T_{peak} = 247.69 °C for D-Trp). As one can observe, the melting process is suddenly followed by thermal decomposition. For D-Trp thermal decomposition occurs between 286.29 °C-287.36 °C (T_{peak} = 286.69 °C) and is characterized by a decomposition enthalpy value of 35.17 J g^{-1}. For L-Trp can be observed more peaks describing a thermal decomposition process running in several steps and the main process holds in the range 284.03 °C-285.09 °C with T_{peak} = 284.47 °C having a decomposition enthalpy value of 27.36 J g^{-1}.

The results of the thermo-analytical curves of the systems formed with α-CD, Fig. 1, show some differences from that found for the single components. In thermogram corresponding to α-CD/D-Trp system, the first endothermic effect is characterized by the enthalpy value of 21.04 Jg^{-1} and the second endothermic process is confirmed by enthalpy value of 18.92 Jg^{-1}. These two thermal processes are followed by another endothermic effect started at 327.8 °C corresponding to decomposition of α-CD/D-Trp system which takes place at a temperature with approximately 40 °C higher than the decomposition temperature of α-CD. The thermal modifications showed for α-CD/D-Trp system could be explained by the existence of interactions between the host and the guest molecules without a fair inclusion complex formation.

The thermogram corresponding to α-CD/L-Trp system indicates a sharp melting endothermic peak with an onset temperature of 253.6 °C and a value of melting enthalpy of 47.89 Jg^{-1}. After this peak, the thermal decomposition of the complex α-CD/L-Trp is starting at the onset temperature of 311.9 °C, which is slightly higher (approximately 25°C) than that of the parent α-CD. Even though there is a reduced ability for a complexation process, the disappearance of L-Trp melting peak and the appearance of a thermal effect at 311.9 °C are proves for the inclusion process between L-Trp and α-CD.

The thermal curves corresponding to inclusion complex β-CD/D-Trp and β-CD/L-Trp, respectively, displayed a thermal behavior which can be attributed to a melting-decomposition phenomenon that begins at 321.6 °C for β-CD/D-Trp and at 311.2 °C for β-CD/L-Trp. Looking at the registered results one can observe that new compounds with a different thermal behavior from pure components were obtained. In both β-CD systems the stage corresponding to the thermal decomposition is shifted to higher temperature and the melting peak of the guests disappear. This behavior is also observed in other CD's systems.[10-12] DSC results suggest that β-CD/L-Trp and β-CD/D-Trp systems get the possibility to form inclusion complexes with a higher ability and thermal stability compared with α-CD/L-Trp and α-CD/D-Trp inclusion complexes.

FTIR analysis

FT-IR technique has been used to study the formation of inclusion complex in solid phase. This technique is also used to point out the contribution of the different functional groups of the tryptophan and cyclodextrin molecules in the inclusion process. The FT-IR spectra of the pure D-, L- tryptophan, α-CD, β-CD, as well as the FT-IR spectra of the cyclodextrins complexes are given in Fig. 3.

As one can see in Fig. 3, spectral difference between the two isomers of tryptophan is almost indistinguishable. Both D- and L- tryptophan have the IR absorption peak of the carboxylic acid group at 1662 cm^{-1} and the deformation vibrations in the region between 700 and 600 cm^{-1}. In the D- /L- Trp spectra, the vibrational bands of the indole group in the low frequency region 1300-650 cm^{-1} are easily observed. The bands obtained at 1230, 1160, 1115 and 1045 cm^{-1} are assigned to the in-plane deformation of C-H (βCH) in indole ring.[13,14] Another important characteristic in the FT-IR spectra of solid D- /L- Trp belonging to bending out-of-plane indole C-H was observed at 742 cm^{-1}. This peak is also due to the out-of-plane deformation of C-H (γC-H) in indole ring and the band located at 1355cm^{-1} is assigned to the stretching vibration of C=C indole ring.[15] The D- /L- Trp FT-IR spectra show an intensive peak at 3400 cm^{-1} of N-H (νN-H) stretching vibration of indole ring.

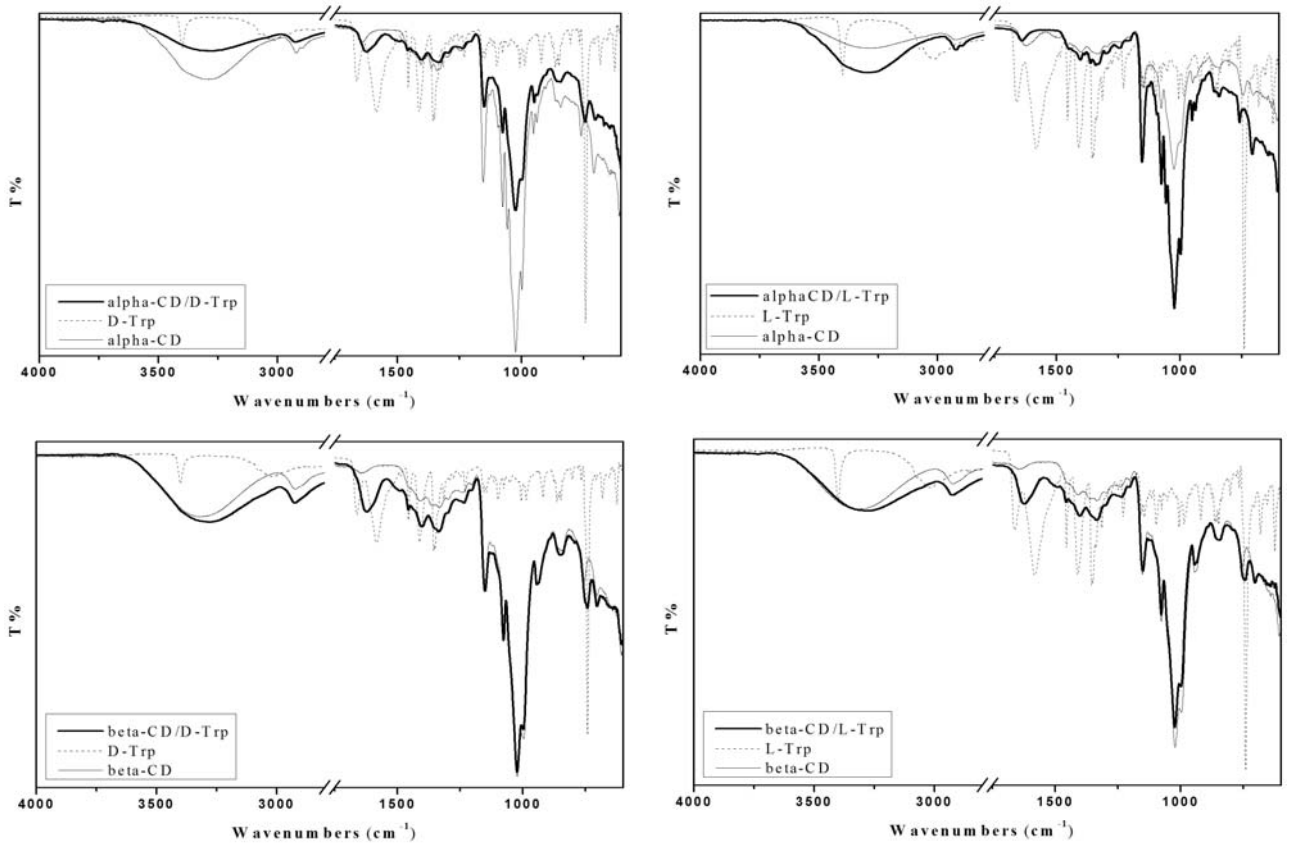

Fig. 3 – FT-IR spectra of solid powders of pure substances and CD/Trp inclusion complexes.

In the FT-IR spectra of pure α-CD and β-CD, Fig. 3, the absorption bands in the region 950-650 cm^{-1} belong to the deformation vibrations of the C-H bonds and the pulsation vibrations in glucopyranose cycle. In the interval 1100-1020 cm^{-1}, the absorption bands of the valence vibrations of the C-O bonds in the ether and hydroxyl groups of α-CD (1076 and 1022 cm^{-1}) are registered; identical wave numbers for β-CD are observed. In the region 1400-1150 cm^{-1}, the absorption bands of the deformation vibrations of the C-H bonds in the primary and secondary hydroxyl groups of α-CD are at 1154 cm^{-1} and 1341 cm^{-1}, and for β-CD at 1152 and 1332 cm^{-1}, respectively. In the recorded spectra, the valence vibrations of the C-H bonds in the CH and CH_2 groups cause in the α-CD an absorption band with maximum at 2919 cm^{-1} and in the β-CD an absorption band with maximum at 2920 cm^{-1}. The pure CDs spectra also show a characteristic large band with the absorption maximum at 3288 cm^{-1} for α-CD and at 3314 cm^{-1} for β-CD. These bands were assigned to symmetric and anti-symmetric O-H stretching modes[16,17] and may be affected when complexation will be done.

The FT-IR spectra of the cyclodextrins complexes showed variations compared with those of the corresponding pure α- /β-cyclodextrin especially in hydrogen bonded-OH region. The observed changes in the spectrum can be attributed to interstitial and intracavity interactions between the Trp isomers and CDs. In the FT-IR spectra the band around 3,300 cm^{-1} is assigned to symmetric and asymmetric –OH stretching modes for cyclodextrins and thyptophan isomers. For each of the α-CD/L-Trp, β-CD/D-Trp and β-CD/L-Trp systems the position of this band is shifted to smaller peak wavenumbers due to an increase in the number of molecules involved in the network establishment by hydrogen bonds; for the α-CD/D-Trp system are established less intense associations which indicates the weakening of the hydrogen bonding network. Aromatic C–C bands appear as sharp bands around 1660–1585 and 1456, 1413, 1358 cm^{-1} in D- /L-Trp and are also present in the CD/Trp spectra inclusion complex, although some depressed. As one can see the spectra of CD/Trp inclusion complexes resemble that of pure CD, another indication for encapsulation of Trp into the CD cavity.

Quantum chemical calculations

To test interaction tryptophan – cyclodextrin, see Fig. 4, the tryptophan was passed through the cavity of cyclodextrin from the left towards the right from -10 Å to 10 Å, with a step of 0.5 Å. As suggested in Fig. 4b in each position the tryptophan molecule was rotated by a step angle of 5^o till the rotation was 60^o. The total energy is computed for each configuration of the assembly α-CD/Trp, both molecules having optimized geometries. Then, the energy of the assembly is compared to the sum of the energies of the two isolated molecules. In the following this sum is named "reference energy" (RE) and the total energy of the assembly "assembly energy" (AE). The procedure described above was applied both to L-Trp/α-CD and D-Trp/α-CD assemblies. In calculations we considered both the case when the indolic part of the Trp molecule enter into the wider base cavity of CD's as well as the case when the indolic part of the Trp molecules enter into narrow base cavity of CD's.

In Fig. 5, the difference between the reference and assembly energies, for α-CD and β-CD, is plotted. As seen in all the analyzed cases this energy difference is negative. This means that the energy of the assembly Trp/CD is larger than the sum of the energies of the isolated molecules and, consequently, the configuration of the isolated molecules is more stable than the assembly. Finally, this agrees that the interaction between the two molecules inside the assembly is repulsive most likely due to multipolar interaction between the charged atoms belonging to the two molecules. Should be noted that the very small energy variations due to rotation of Trp molecule into CD cavity, around 2-3 kJ/mol, does not significantly change the shape of the graphics in Fig. 5. This is an apparent repulsion. In any of their positions the two molecules are rigid, with optimized geometries and fixed relative positions. If it is allowed to the atoms to have small displacements along to the resultant forces which act on them, it is possible to finally obtain a configuration of the two molecules that has a lower energy than their reference energy. This may happen because the two molecules one could form one, two, three or even more hydrogen bonds. As one can see in Fig. 6, these hydrogen bonds are established between the intra- or inter-glycosidic oxygen and the hydrogen atoms which belong to aminic or/and carboxylic groups.

We have to mention that the final conformations of the assembly CD/Trp were obtained starting from several initial relative positions of the CD/Trp assembly. These were similar to those presented in Fig. 4; the distance between the two molecules is in the range of -4 Å to +4 Å, being varied with a step of 2 Å. There were taken into consideration both the configuration of tryptophan presented in Fig. 4, as well as the reverse configuration (that is obtained reversing its 'z' coordinates).

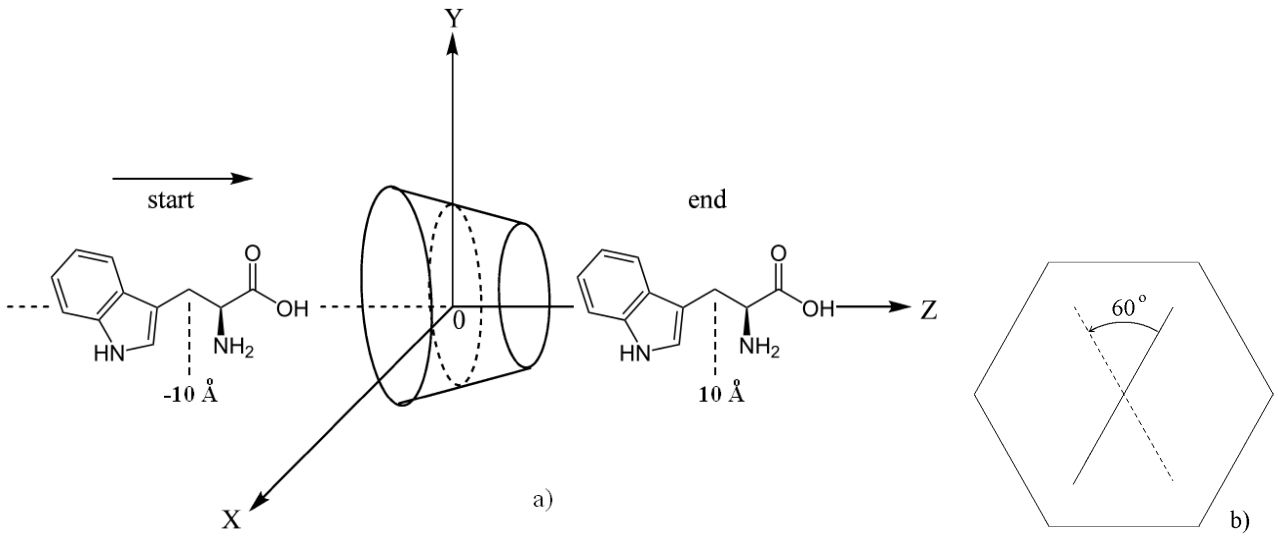

Fig. 4 – Tryptophan displacement through α-CD cavity.

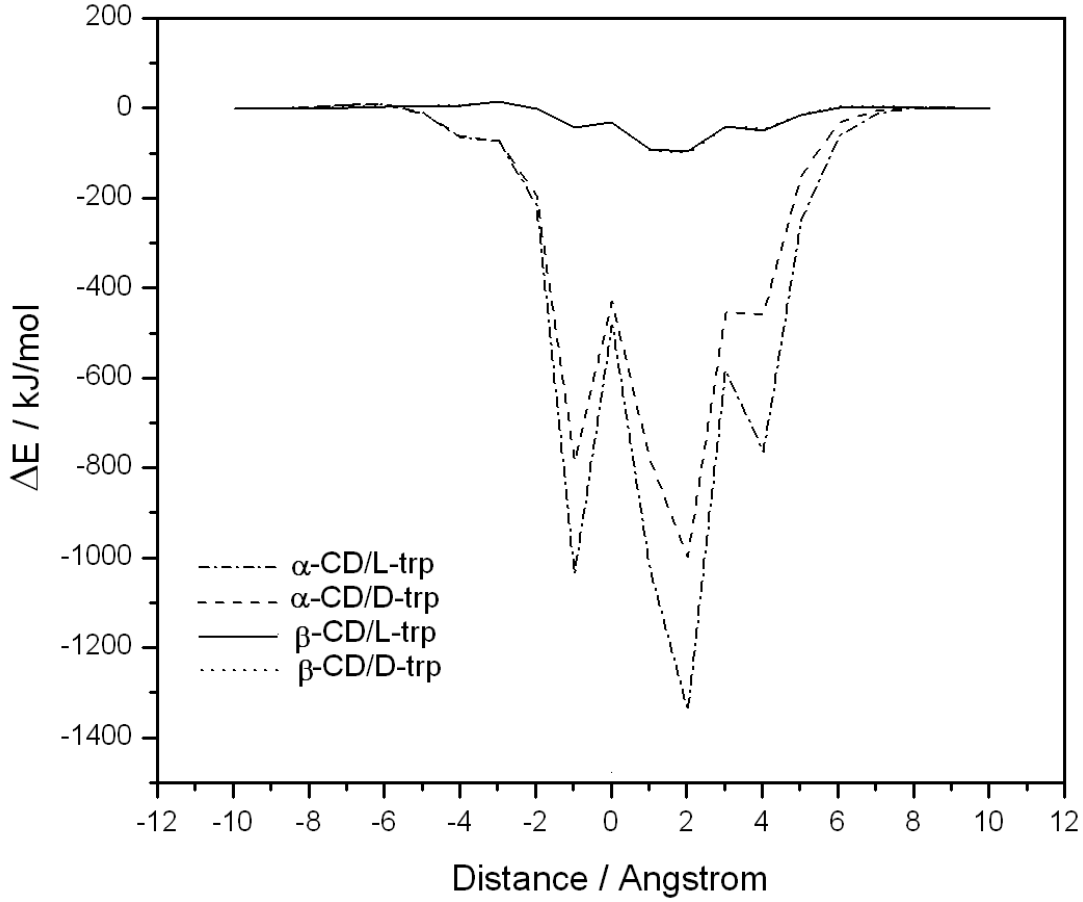

Fig. 5 –The difference ΔE = RE - AE as a function of the distance between tryptophan and cyclodextrin.

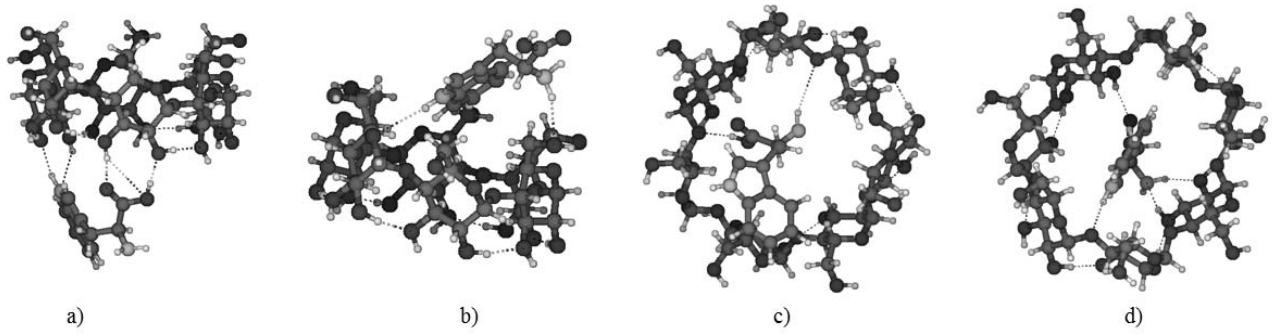

Fig. 6 – Several configurations of the assembly tryptophan-CD characterized by the highest interaction energies.
a) and b): α-CD/L-Trp; c) and d): β-CD/L-Trp.

In agreement with Nishijo and Tsuchitani[4] we have shown that hydrogen bonds cannot exist between the Trp and CD when Trp is located inside the CD cavity. These type of bonds can exist only when Trp is outside CD cavity. As one can see in Fig. 6 (a, b), in the case of α-CD the highest interaction energies correspond to some assemblies where L-Trp is located outside of CD. For any configuration that corresponds to α-CD/L-Trp assembly where L-Trp is located inside of α-CD cavity, the interaction energy is negative (repulsive interaction). In spite of this repulsion, the tryptophan molecule is not expelled outside the cavity, probably due to a local minimum that exists on the potential energy surface. The interaction energy between α-CD and L-Trp varies between -35 and +88 kJ/mol, the average interaction energy being 10 kJ mol^{-1} (14 different configurations there were taken into consideration).

In the case of the β-CD/L-Trp complex Fig. 6 (c, d), the interaction energies for the configurations are highest comparatively with

those obtained for the α-CD/L-Trp complex. In Fig. 6c and Fig. 6d, the L-Trp is located inside of the CD cavity. In our opinion this is possible because the cavity of β-CD is larger than that of α-CD, at the same time β-CD being more flexible than α-CD. The result is in agreement with recent NMR spectroscopy and UV dichroism molecular structure information for the interaction between CD and aromatic aminoacids.[18,19]

CONCLUSIONS

This paper constitutes a study on 1:1 complex formation of α-CD and β-CD with stereo isomers of tryptophan in solid state and was carried out by DSC and FT-IR techniques, and quantum chemical calculation. The ability of α- and β- CDs to form inclusion complexes with optical isomers of Trp is influenced by the thermodynamic interactions between the components and the steric factors. Considering the dimension and the symmetry of D-/L- Trp molecule, there is a low possibility of accommodation of D- /L- Trp molecule completely in the cavity of α-CD molecule. The results indicate that in the case of α-CD/D-Trp system, the D-Trp molecule is disposed outside the α-CD cavity and the intermolecular interaction is mediated by hydrogen bonds between terminal groups of D-Trp and the α-CD external surface, while the L-Trp isomer involves a possible inclusion of the indole moiety. The best complexation of the Trp isomers occurs with β-CD with no observed enantiomeric preference. It is possible that β-CD to display higher possibilities to form stable inclusion complexes by both the flexibility of the ring which reduces the interactions of rejecting the Trp molecules and the deeper insertion of indole group into macrocyclic cavity.

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