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Computer simulation of the cyclodextrin–phenylalanine complex

J. Raul Grigera *, Ernesto R. Caffarena †, Santiago de Rosa

Instituto de Física de Líquidos y Sistemas Biológicos (IFLYSIB) UNLP-CONICET and Departamento de Ciencias Biológicas, Universidad Nacional de La Plata, c.c. 565, 1900 La Plata, Argentina

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Abstract

The results of the molecular dynamics simulations of the complexes of α-cyclodextrin-L-phenylalanine and β-cyclodextrine- L- phenylalanine in vacuo and in aqueous solution are presented. The trajectories of the insertion angle, rotation of the aromatic ring of the phenylalanine inside the macrocycle and the dihedral angle χ^2 (Cα–Cβ–Cγ–C_{D2}) describing the relative movement of the aromatic ring with respect to the polar region give detailed information of the dynamics of the complexes. It is found that the complex with α-cyclodextrin in water is not stable, in agreement with experimental data, while in all other situations studied the complex is stable within the computational limits. Comparing the different cases and the experimental evidence it comes out that a simulation of the complexes without an explicit treatment of the solvent gives unreliable results.

Keywords: Molecular dynamics; Cyclodextrin; Phenylananine–Cyclodextrin complexes; Inclusion complexes

1. Introduction

Cyclodextrins are a matter of interest since these compounds can spontaneously form inclusion complexes with a variety of guest molecules in aqueous media. They are obtained through enzymatic degradation of starch by a particular type of amylases known as glucotransferases. Inclusion complexes of cyclodextrins are highly relevant to several branches of industry because the interaction with these cyclic molecules leads to important modifications of the properties of the guest compound, allowing the fixation of volatile materials, the protection against oxidation and photolysis, modification of the reactivity and of the biological properties [1–4]. These kind of complexes are of interest to the pharmaceutical, medical, cosmetic, and food industry and they have also application in agriculture [5,6].

The particular structure of cyclodextrins and their capacity to form inclusion complexes make them unique as solubilizing and stabilising agents [7]. However, the inclusion complexes are not only important for solubilisation and transport but also because in some cases cyclodextrins catalyse the reaction of the guest molecule [8].

These cyclic oligosaccharides are formed by a number of glucose units linked by α-(1→4) glycosidic

* Corresponding author. Fax: +54-21 25 73 17; e-mail:grigera@iflysib1.unlp.edu.ar

† Also at the Facultad de Ingeniería, Universidad Nacional de La Plata, La Plata, Argentina.

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bonds. There are three known basic structures that differ only in the number of units. The α-cyclodextrin (α-CD), of six units, β-cyclodextrin (β-CD) of seven units and the γ-cyclodextrin (γ-CD), with eight units. All of them have as a common feature the existence of a hydrophobic central cavity (basket-type shaped), of about 0.6 to 1 nm in diameter, surrounded by a hydrophilic ring formed by hydroxyl groups. Complexation processes in solution depend on the size, shape, and hydrophobicity of the guest molecule and are accompanied by an increase in strain in the CD cavity. The main forces that have to do with the complexation processes are van der Waal interactions between guest and host, responsible for the stability of complexes in water and in vacuo, and hydrophobic interactions for the case of aqueous solutions. Other important interactions are hydrogen bonding between the guest and the hydroxyls groups of the CD, release of strain energy in the molecule ring and dipole–dipole and/or coulombic interactions [9].

For the different complexes of cyclodextrin the ones in which aromatic amino-acids are involved are of interest as a model for enzyme–substrate specific binding. The molecular dynamics of the particular complex cyclodextrin–phenylalanine have been studied by carbon-13 nuclear magnetic resonance (^{13}C–NMR) spectroscopy [10]. On the other hand, molecular dynamics computer simulations have been proved to be of highly predictive value [11,12] in general and for cyclodextrins in particular [13,14], also some other inclusion compounds have been studied by different techniques including molecular dynamics simulation [5,9] and molecular mechanics [15].

In the present work we describe the molecular dynamics simulations of the complexes α-cyclodextrin-L-phenylalanine and β-cyclodextrin-L-phenylalanine.

2. Experimental

Computational method.—Simulations were carried out using the GROMOS package (Biomos n.v.Groningen) [16]. The equations of motion were solved with the leap frog algorithm, the system was weakly coupled to a thermal and a hydrostatic baths to work in the isothermal-isobaric ensemble [17] at $T = 300$ K and $p = 1.013 \times 10^5$ Pa. The time step of integration was held between 2 and 1fs. All simulation runs were made in IBM RS/6000 32 H or 350 workstations and the analysis of results in a personal computer. The force field GROMOS was used for cyclodextrin [11] in conjunction with the SPC/E water model [18]. In the GROMOS force field the interactions between non-bonded atoms are modelled with a 6–12 Lennard–Jones potential and through the coulombic electrostatic interaction between the atomic partial charges.

The molecular model of the building blocks (Glc) was kept in the $^4C_1$ conformation. The transition to other conformations was avoided by applying improper torsion potentials. As observed by Catoire et al. [9] the C_1/C_7 transitions take place in widely separate time scales, so for a simulation like this it will suffice to take the most probable conformation. Groups CH and CH^2 were considered as united atoms, and only a centre of interactions was defined for such groups. The tetrahedral character of the carbon atom was fixed using improper torsion potentials. We used the SHAKE procedure [19] to maintain rigid bond lengths. Bond angles were treated as having harmonic potentials. L-phenylalanine was taken as having the topology defined as in GROMOS.

A series of runs were done with α- and β-cyclodextrin in vacuo and in SPC/E water (218 water molecules for each molecule of α-cyclodextrin and 271 water molecules for each molecule of β-cyclodextrin). After reaching the equilibrium of the cyclodextrin system (with or without the water) the complex was built by introducing the aromatic ring of one molecule of L-phenylalanine into the cavity α or β-cyclodextrin and the simulations run in the regular way. The conformation of phenylalanine was the final state of the system that was previously equilibrated in the respective medium (vacuo or water). Each system was equilibrated for 100 ps and then the trajectories were recorded for 500 ps and 150 ps for the systems in vacuo and in water, respectively.

3. Results

In the α-cyclodextrin complex the phenylalanine has a different behaviour in vacuo and in solution. In solution the amino acid migrates towards the exterior of the cavity during the run and after 100 ps the phenylalanine is completely out of the cavity. In absence of water, however, the complex is stable. For the β form in all cases the complex remains stable during the whole run.

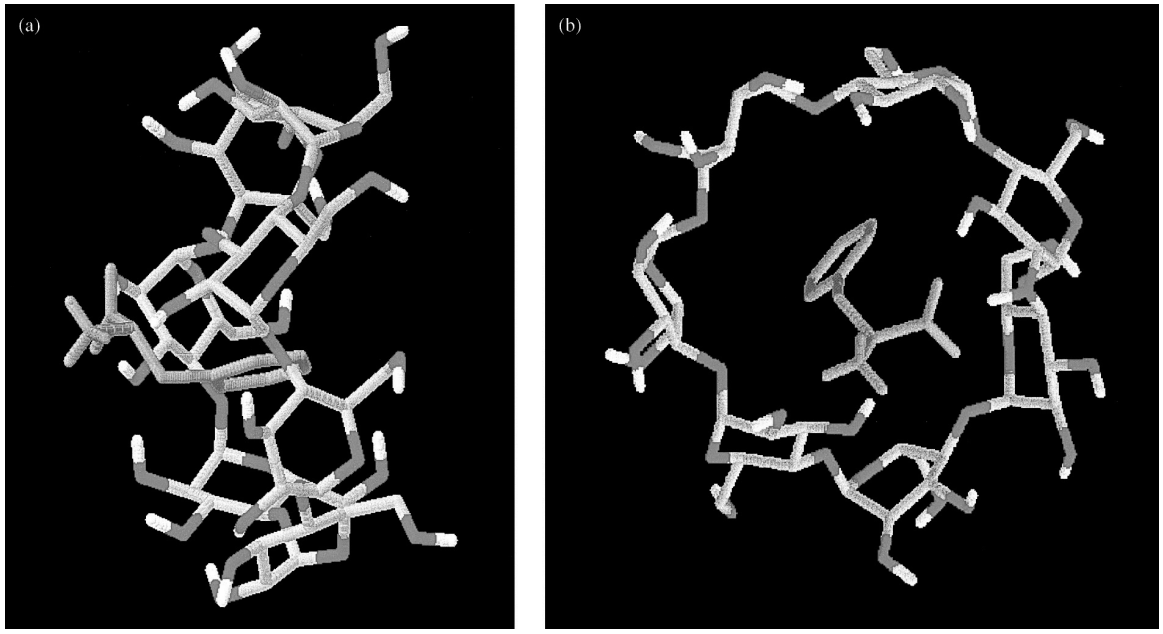

Fig. 1. Views of the β-cyclodextrin-L-phenylalanine complex: (a) lateral view; (b) axial view.

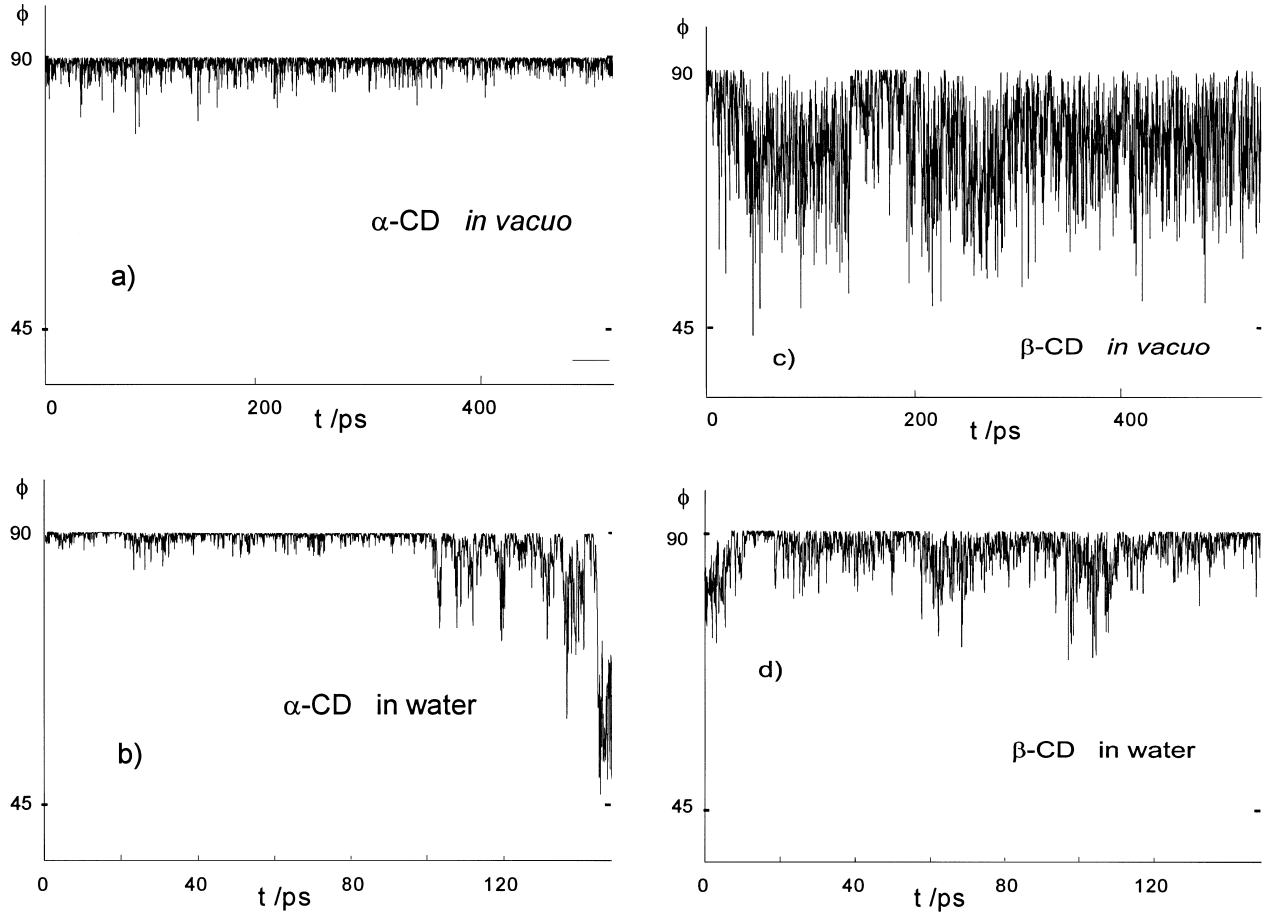

Fig. 2. Trajectories of the insertion angle ϕ of the aromatic ring of phenylalanine into the complexe cyclodextrin–phenylalanine for different cases: (a) α-cyclodextrin in vacuo; (b) α-cyclodextrin in water; (c) β-cyclodextrin in vacuo; (d) β-cyclodextrin in water. Only the positive values are shown, although it is assumed a symmetric movement around 90°.

Fig. 1 shows a scheme of the L-phenylalanine–β-cyclodextrin complex in aqueous solution after 150 ps simulation time. It shows that the aromatic ring is inserted into the cavity while the hydrophilic groups are exposed to the environment. In solution the phenylalanine can interact with the water but also can make hydrogen bonds with the outer ring of the cyclodextrin.

To follow the structure and dynamics of the complex we define two angles. One of them is the *insertion angle* φ defined as the angle formed between the vector normal to the aromatic ring of phenylalanine (**n**) and the axis of the cavity, and the other one is the *orientation angle* θ defined as the angle between the same vector **n** and to an arbitrary direction in a plane perpendicular to the axis of the macrocycle. The first angle (φ) describes the orientation of the aromatic ring with respect to the central axis of the cyclodextrin and the second (θ) is used to monitor the rotation of the guest molecule inside the cavity.

Fig. 2 shows the trajectories of the insertion angle for α-and β-cyclodextrin in vacuo and in solution (note that only the absolute value of the angle is shown, although it is assumed a symmetrical movement around 90°). The behaviour of the α and β form are different. We see that the insertion angle in the α-cyclodextrin complex in vacuo is almost unchanged during the complete run having at most 15°-departure at each side from the 90° angle, which corresponds to the aromatic ring parallel to the axis. When the solvent is present in the system the insertion angle remains close to 90° but there is a progressive departure from it over the 100 ps simulation time, when the complex breaks down. The potential energy of the complex slowly decreases when the phenylalnine leaves the complex. The average slope of the potential energy is −0.40302 KJ/(mol ps).

The phenylalanine is inserted into the cavity of β-cyclodextrin more freely than in the α form. The insertion angle of the complex in vacuo may change as far as 60° (typically 40°) out of the parallel alignment with the axis. The presence of the solvent defines better the insertion angle but still it changes with time up to 40° around the equilibrium position.

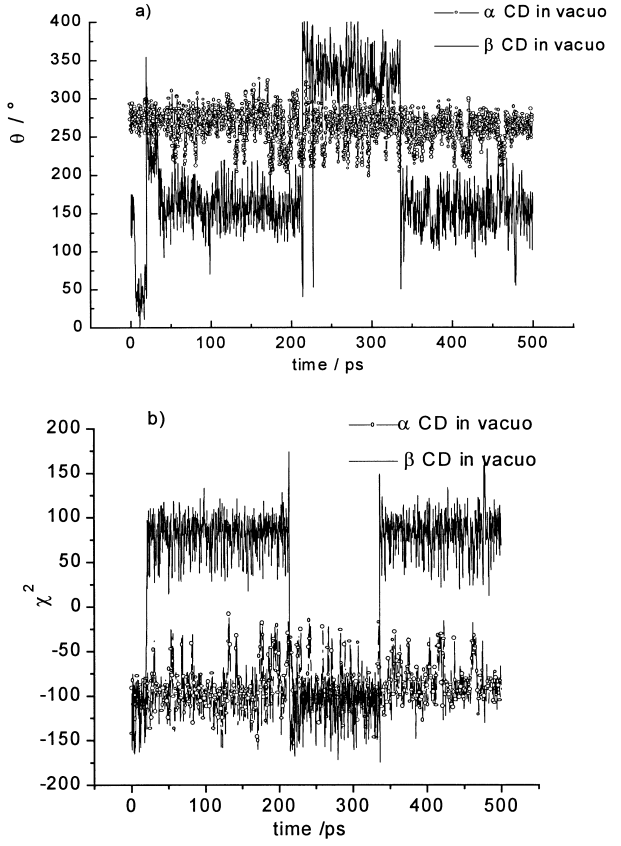

Fig. 3. Trajectories of the orientation angle θ of the aromatic ring and the dihedral χ^2 (Cα–Cβ–Cγ–C_{D2}) of the L-phenylanine during simulation of the complex with cyclodextrin in vacuo: (a) orientation angle θ; (b) dihedral χ^2.

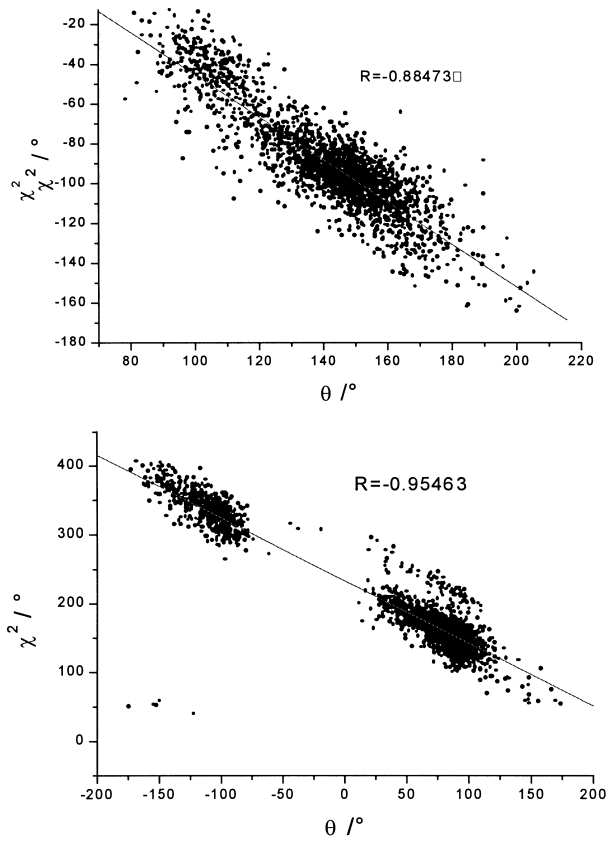

Fig. 4. L-Phenylananine dihedral χ^2 plotted against the orientation angle θ for the α and β cyclodextrin complexes. The lines correspond to the least square lineal fit and R is the correlation factor for each case.

Fig. 3(a) shows the trajectory of the orientation angle of the phenylalanine inside the macrocycle for the α and β cyclodextrin–phenylalanine complexes. Fig. 3(b) shows the corresponding trajectory of the dihedral angle χ^2 ($C\alpha$–$C\beta$–$C\gamma$–C_{D2}) of phenylalanine that characterises the relative movement of the aromatic ring with respect to the polar end of the phenylalanine. A numerical analysis of the correlations of the trajectories of both angles in vacuo for each compound shows their correlation. Fig. 4 shows the plots of the dihedral angle against the rotation time for the phenylalanine the α and β cyclodextrin complex in vacuo. This correlation suggests that the ring is moving inside the macrocycle but the polar end of the phenylalanine remains fixed to the outer border. Fig. 5 shows the insertion and dihedral angle of the complexes in water. In this case the correlation no longer exists, as is seen in Fig. 6. For the α-cyclodextrin the very large variation of both angles over long periods are a consequence of the breaking of the complex.

For the β-cyclodextrin we observe that the amplitude of the movement of the angle χ^2 is much larger than for the α form. In the water solution, and owing the fact that the β-cyclodextrin remains inside the cavity, the independent movements of the orientation angle and χ^2 suggests a rotation with respect to the cyclodextrin also of the hydrophilic region of the phenylalanine. It seems that the presence of water makes the H-bonds of the phenylalanine with the outer border of the macrocycle more mobile.

The hydration of the cyclodextrin in solution was already studied [13] and the presence of the phenylalanine inside the "cage" does not modify the general behaviour. Fig. 7 shows the average over the subunits of the radial distribution functions of water oxygen atoms around the O-3 and O-5 of the β-cyclodextrin. The α form shows the same feature. We see that the hydration structure of oxygen atoms is what we can expect. The ring oxygen (O-5) has much less defined hydration, while the O-3 shows a sharp first hydration peak and a well-defined second one. Other oxygen atoms shows a similar behaviour. This is similar to the one of glucose and other pyranoses [10,20].

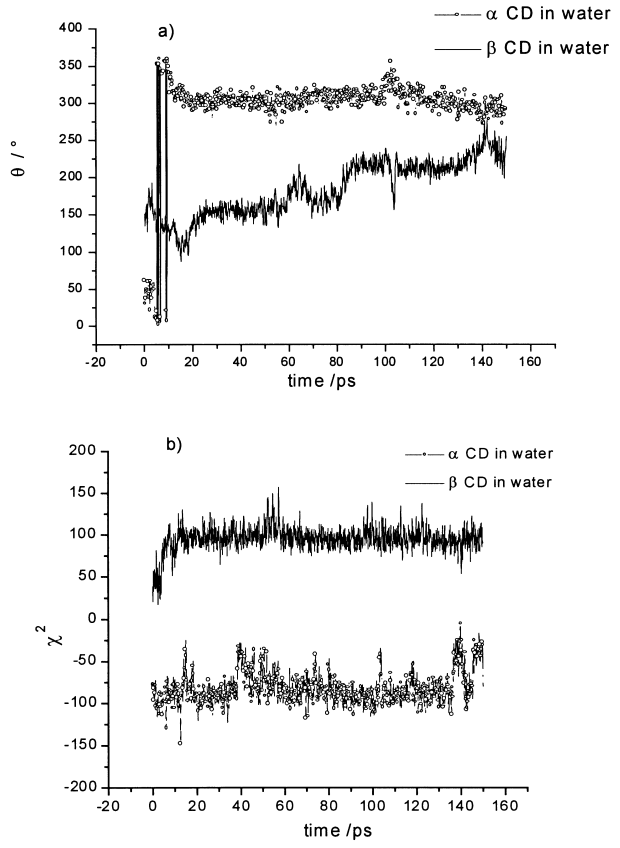

Fig. 5. The same plot as in Fig. 3 the α and β cyclodextrin–phenylananine complexes in water.

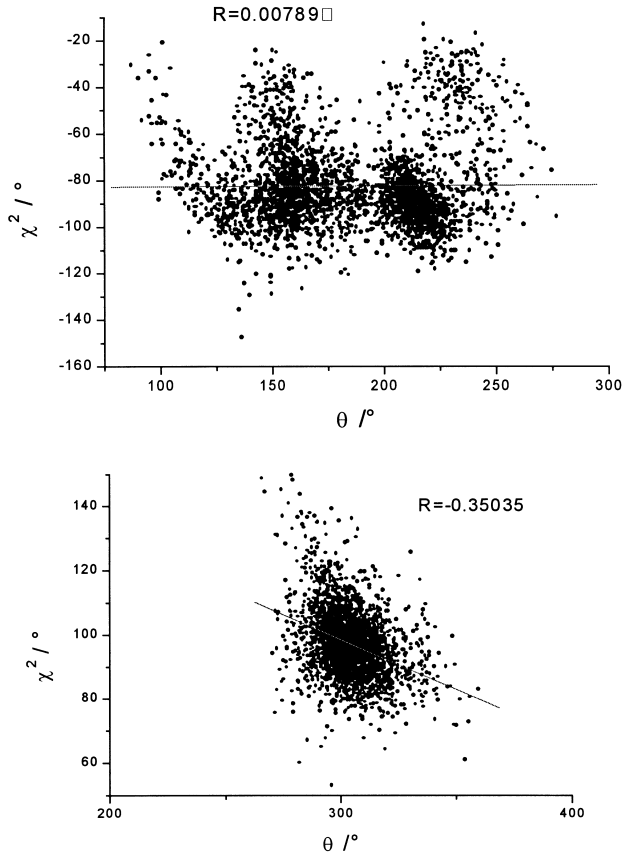

Fig. 6. Dihedral χ^2 plotted against the orientation angle θ for the α and β cyclodextrin complexes (as in Fig. 4) in water. The correlation R factors are much lower than for the complexes in vacuo.

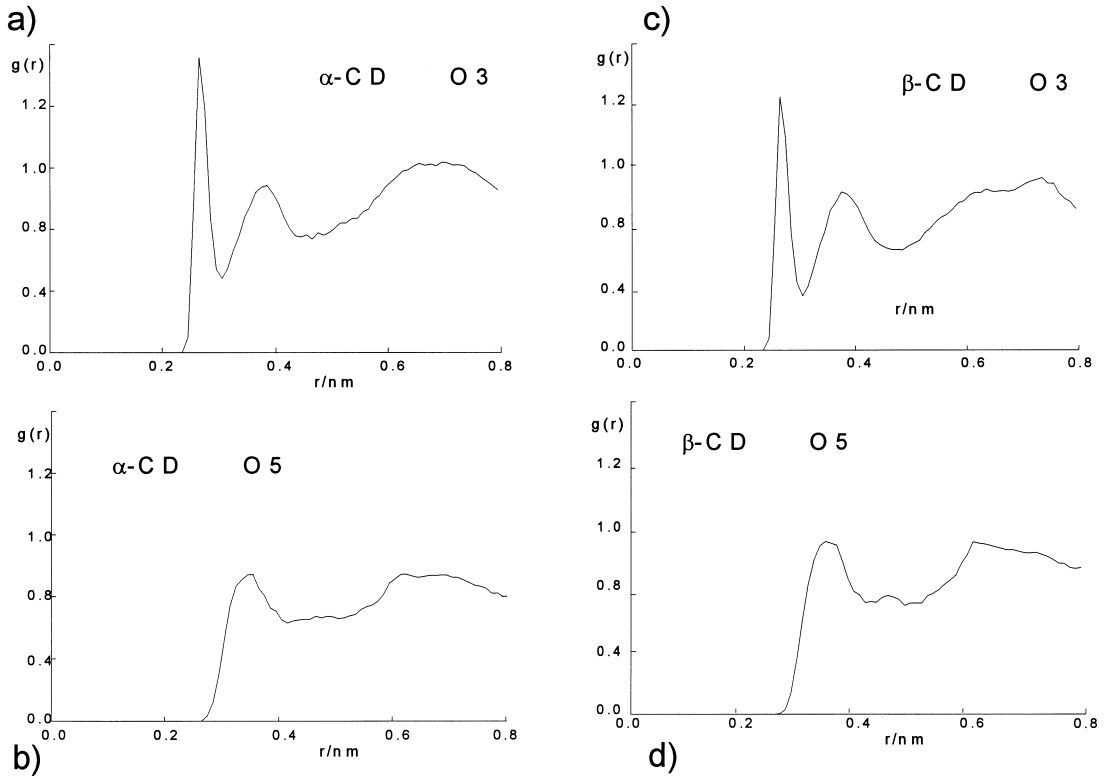

Fig. 7. Radial distribution of water oxygen atoms around the (a) O3 for the α-cyclodextrin; (b) O5 for the α-cyclodextrin in water; (c) O3 for the β-cyclodextrin; and (d) O5 for the β-cyclodextrin in water.

4. Discussion

The results show that the complexes for the α-cyclodextrin are less stable than for the β form This finding is according to what was observed by ^{13}C NMR [10]. The rather artificial cases of the simulations in vacuo are illustrative.

The forces that maintain the amino-acid inside the cyclodextrin "basket" are the hydrogen bonds formed between the polar end of the phenylalanine and the edge of the external surface of the cyclodextrin and the hydrophobic interaction of the phenylalanine ring with the interior of the cavity. In absence of solvent the hydrophobic interaction does not exist, and the hydrogen bonds appear strong enough to maintain the complex. In presence of water the cavity in the α form is not big enough to avoid steric repulsion and since water competes with the hydrogen bonds of the phenylalanine the amino-acid can be dragged out of the cavity. Fig. 5 shows that in water the rotation of the phenylalanine in the cavity and the internal rotation of the aminoacid are not correlated during the time in which the complex is formed. The cavity of the β-cyclodextrin is larger than the one of the α form, allowing a stable complex even in the presence of water. The behaviour of the hydrogen bonds is similar to the α complex. We see that in vacuo the apparent movement of the ring inside the macrocycle is larger (Fig. 5(a)), which is due to the larger size of the cavity, but the movement is highly correlated with the inner movement of the phenylalanine, with the polar ends remaining fixed. In water the movement inside the macrocyle is damped out, probably due to hydrophobic interactions, and as can be concluded from the lower correlation with the phenylalanine dihedral angle the polar end jumps between different positions on the ring edge.

When comparing these results with other simulations of cyclodextrin complexes we have to take into account, besides the possible differences of the guest molecule, the many details in the simulations. When low dielectric permittivity is used without explicit solvent [15] the electrical interaction of the complex formation (stabilised by hydrogen bonds) is overestimated. The constant pressure simulation is better than constant volume algorithms [10] with water present to produce the right system.

5. Conclusion

The results we obtained by simulation of the L-phenylalanine-(α and β)–cyclodextrin complexes in water show that the only stable complex is the one formed with the α and β-cyclodextrin. The agreement between simulation and experimental data opens the possibility of using detailed molecular information obtained by simulation for the analysis of the characteristics of the complex. The elusive theoretical treatment of the hydrophobic interaction, for instance, can be taken into account by the simulation provided that it employs an appropriated model for water. The procedure we described here can be applied to other inclusion complexes and, in view of the agreement with the experimental data for this case, the expectations of success are great. The simulation of the complexes without the inclusion of the solvent explicitly can give misleading results.

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