

A Molecular Dynamics Study of the Inclusion Complexes of C₆₀ with Some Cyclodextrins

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The strongly hydrophobic C₆₀ fullerene is a carbon allotrope of huge interest in materials science and in pharmaceutical chemistry that can be solubilized in water either by extensive chemical functionalization or by inclusion in appropriate carriers such as the cyclodextrins with formation of host–guest complexes. Here we report a molecular dynamics study of the complexes formed in solution by C₆₀ with γ - and δ -cyclodextrins. The most stable host–guest complex stoichiometry is determined to be 2:1 through simulations *in vacuo* and in explicit water by the stepwise addition of the cyclodextrins to C₆₀. No *a priori* assumption about the inclusion stoichiometry and geometry is made. The equilibrium fluctuations of the complexes that can affect the system stability are also investigated within the molecular dynamics runs.

Introduction

Soon after the discovery of the C₆₀ fullerene and of its production in significant or even large quantities, its possible use in biology and medicine was actively explored.¹ C₆₀ and in particular its derivatives show in fact enzymatic inhibition of the HIV-1 protease *in vitro*, can be used in photodynamic therapy, have a promising behavior as a neuroprotector thanks to its antioxidant properties as radical scavengers, or have antiapoptotic or antibacterial activity, as reviewed for instance in refs 1 and 2. A major problem for these applications resides in the complete lack of solubility in water of strongly hydrophobic C₆₀ and its very poor solubility in polar solvents.¹ One way to improve the water solubility of fullerene is through its derivatization, even though clustering of the C₆₀ derivatives in the polar solvents is often present,^{1,2} while multiple functionalization can enhance its solubility. Another approach to carry the hydrophobic fullerene in water is to employ micellar systems or more simply to use carriers forming host–guest complexes² such as cyclodextrins, or CD for brevity. In fact, CDs are macrocyclic oligosaccharides having a truncated cone shape with a hydrophobic cavity, while the presence of primary and secondary hydroxyls at the two rims allows the solubility in a polar environment. Accordingly, they can act as stealth carriers of nonpolar molecules in water. CDs having from 6 to 9 sugar rings are usually denoted as α -, β -, γ -, and δ -CD in that order.

The first experimental report of an inclusion complex of C₆₀ with a cyclodextrin, γ -CD, dates to 1992,³ and γ -CD was shown to form a 2:1 complex with C₆₀ in a water solution,⁴ unlike β -CD which appeared to be too small for this purpose. A molecular model was also proposed by simply docking and energy minimizing the fullerene first at the secondary rim of γ -CD in a 1:1 stoichiometry and then within the cavity of two γ -CD interacting through their secondary rims in a 2:1 stoichiometry.³ Later, this stoichiometry was experimentally proved by ¹H and ¹³C NMR and by elemental analysis.⁴ Interestingly, it was recently found that a water suspension of the 2:1 complex in the presence of sodium bisulphite under a nitrogen atmosphere

produced ammonia in a 33% yield, while neither the individual γ -CD nor C₆₀ had such a catalytic effect.⁵

The adducts formed by C₆₀ and γ -CD or δ -CD were also studied in the solid state by positron lifetime measurements,⁶ which suggested that while γ -CD did form a genuine inclusion complex, δ -CD did not, at least in a significant amount. Instead, the results implied that C₆₀ was probably located in the intermolecular cavities among the δ -CD macrocycles, possibly with a sort of microphase separation. A water-soluble inclusion complex formed by the smaller sized β -CD with C₆₀ was also reported,⁷ in contrast with earlier results:^{3,4} formation of the inclusion complex was achieved through use of a toluene/dimethylformamide solution and subsequent addition of water after removal of the organic solvent, suggesting a significant difficulty of forming a stable inclusion complex by β -CD due to the small cavity size.

Molecular simulation studies offer great insight into the molecular recognition phenomena and in the formation of inclusion complexes formed in particular by cyclodextrins. In fact, we have shown that accurate and reliable predictions can be made about the possible formation of an inclusion complex through molecular dynamics (MD) techniques with no *a priori* knowledge about its existence or geometry⁸ by following a general simulation protocol.⁹ This protocol requires a preliminary energy minimization of the composite system with an outer guest facing the CD sides, and in particular its rims, in various orientations corresponding to the main geometries of approach from solution. The resulting adducts are then subjected to independent MD runs at a constant temperature (typically at room T), and the instantaneous conformations periodically saved are eventually energy-minimized in search of the most favorable geometry. In this way, we modeled the inclusion complex formed with β -CD by a glycoconjugate derivative of (–)-menthol and found an excellent agreement with experiment, in particular with the nuclear Overhauser enhancement (NOE) data obtained through NMR experiments in solution. In particular, these data were successfully interpreted through the pair distribution function giving the probability densities of finding diagnostic pairs of atoms as a function of their separation, calculated from the equilibrium trajectories.⁸ The simulations were fully performed in water, but we also showed that the same

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complex could be obtained with an implicit solvent, adopting an effective dielectric medium, and with simulations performed *in vacuo*. Solvation effects turned out to be of minor importance, only affecting to some extent the energy differences between the possible metastable arrangements and the most stable one, in particular for the higher energy geometries exposing to water the hydrocarbon moiety with an included sugar ring of the glycoconjugate derivative.

In this paper, we report the MD results about the inclusion complexes formed by C₆₀ with γ - and δ -CD. C₆₀ is a strongly hydrophobic molecule devoid of any polar groups which therefore cannot show hydrogen bonds (H-bonds) or dipolar interactions with water. Because of this feature, we can reasonably use simulations *in vacuo* to establish the preferred stoichiometry and the most stable geometry. The simulations in explicit water are obviously much lengthier due to the presence of a large number of solvent molecules. In any case, due to possible hydration effects at the hydrophilic CD rims, a full simulation in explicit water was also carried out with both γ - and δ -CD. In the following, after the methodological section we report our MD results about the formation and the stability of the complexes formed by C₆₀ with γ -CD and with δ -CD *in vacuo* and in water. In order to establish the preferred stoichiometry of the complexes, we consider the possible formation and the relative stability of a 1:1 and of a 2:1 host–guest complex, considering also the kinetics of inclusion and the equilibrium fluctuations of the guest molecule in the host cavity.

Simulation Methodology

The simulations were performed with InsightII/Discover 2000,¹⁰ using the consistent valence force field (CVFF).¹¹ This force field, satisfactorily used also in previous work,⁸ describes nonbonded interactions through van der Waals and Coulombic terms only, with no extra term for H-bonds. CVFF, originally designed to model proteins, was later augmented to include additional functional groups including the acetal moiety, thus accounting also for the anomeric effect in carbohydrates.¹² Therefore, it can be satisfactorily used for these molecules,¹³ even though this effect is not conformationally dominant in CDs because of the geometric constraint imposed by the macrocycle. Extensive tests carried out in comparison with NMR data do support the general accuracy of CVFF for oligosaccharides.^{8,14} As already described in ref 15, the geometries of γ -CD and δ -CD, generated with the available templates, were fully minimized *in vacuo* up to an energy gradient lower than 4×10^{-3} kJ mol⁻¹ Å⁻¹ and then subjected to an MD run at room temperature. Final energy minimizations of a large number of instantaneous conformations generated within these runs yielded the optimized geometries *in vacuo* of the isolated CDs. The C₆₀ geometry was taken from the templates available in Materials Studio¹⁰ and fully optimized as before by an unconstrained energy minimization *in vacuo*. The MD simulations of the whole complex with C₆₀ placed close to either rim of each CD but outside their cavities were performed *in vacuo* for 10 ns at a constant temperature ($T = 300$ K) controlled through the Berendsen thermostat, which allows for a time step of 1 fs. Integration of the dynamical equations was carried out with the Verlet algorithm, and the instantaneous coordinates were periodically saved for further analysis. Finally, the most stable geometry of each system was found by energy minimization of a large number of these instantaneous conformations (50 conformations sampled in the last half of the MD run at large time intervals after equilibration was achieved). In the MD runs the main changes took only place in the initial part of the

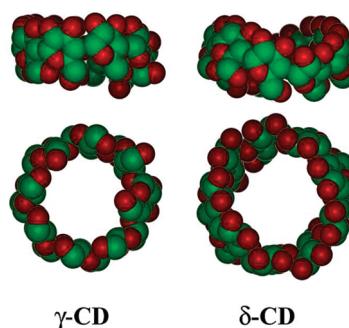


Figure 1. The most stable geometry of γ -CD (left) and of δ -CD (right) after full optimization *in vacuo*. In the side view, the primary rim is in the lower position, while the top view is taken above the primary rim. Carbons are shown in green and oxygens in red, while hydrogens were omitted for clarity.

simulation, and the system equilibration was monitored by the time change of the potential energy and of its components, and of relevant intermolecular distances, in particular between the centers of mass of C₆₀ and of the macrocycle(s). A further check of the configurational changes within the whole system and of its equilibration was also carried out through the similarity maps that show similar conformations belonging to the same family of conformers through the root-mean-square distances among selected atoms (for instance of the guest and host molecules), calculated for all pairs of instantaneous conformations of the MD trajectory. For the simulations in explicit water with both cyclodextrins, the initial C₆₀–CD assemblies, with an outer fullerene as before, were hydrated by adding about 1000 water molecules at the local density of 1 g cm⁻³ in a cubic cell with edges of 31 Å and periodic boundary conditions (constant-volume conditions). After geometry optimizations, the resulting 1:1 adducts were subjected to independent MD runs as before. The water molecules were then removed to introduce the second CD close to the initial one so that the two secondary rims faced one another, and then they were added again as before in a prismatic cell having edges equal to 33 Å × 33 Å × 35 Å for the MD runs of the 2:1 adducts leading to the final hydrated complexes.

Results and Discussion

The C₆₀ fullerene is not small enough to reasonably fit in the cavity of α -CD for steric reasons, and even partial inclusion in β -CD with a 1:2 stoichiometry is problematic, even though it was observed following a special preparation strategy.⁷ Therefore, we explored the formation of an inclusion complex formed with γ - and δ -CD. A space-filling drawing of the optimized geometries of the CDs *in vacuo*¹⁵ is reported in Figure 1. The figure clearly shows that some distortions are present in the eight-membered macrocycle of γ -CD, while δ -CD displays larger deviations from the symmetric truncated-cone shape. Even larger distortions are found in water, since most intramolecular H-bonds present *in vacuo* at the two rims are replaced by intermolecular H-bonds with water. No *a priori* inclusion geometry and kinetics of inclusion was assumed, and the simulations of the whole system were started by first placing the fullerene above either rim of a single CD for the initial geometry optimization. Afterward, we carried out long MD runs *in vacuo* of the resulting 1:1 adducts lasting for 10 ns, followed by energy minimizations of many instantaneous geometries periodically saved along the MD trajectory (50 geometries in each case) in search of the most stable arrangement, as described in the next subsection. The formation of a 2:1 host–guest

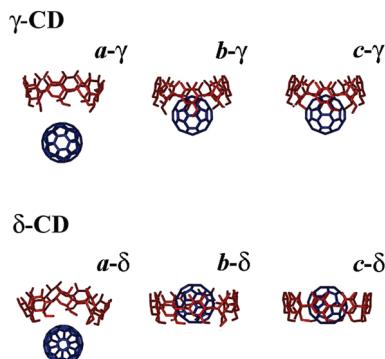


Figure 2. The 1:1 complex formed by C₆₀ with γ -CD and with δ -CD at the primary rim *in vacuo*. The figure shows the starting arrangements (a) and the complexes formed after the initial optimization (b) and after the MD runs and full optimization of the instantaneous equilibrium geometries (c). The CDs are shown in red and C₆₀ in blue for visual clarity, while the hydrogen atoms were omitted.

complex was modeled following in detail the same procedure after adding a second CD to the 1:1 adduct or complex, as described later. The complex formation of γ - and δ -CD in explicit water with the 1:1 and the 2:1 stoichiometry shall also be discussed in the same subsections.

A. Complex Formation with a 1:1 Host–Guest Stoichiometry. When C₆₀ is placed close to either rim of γ -CD, an initial adduct is formed *in vacuo* upon energy minimization, with a very shallow guest insertion in the hydrophobic cavity. An example of this geometry where C₆₀ interacts with the primary rim of γ -CD is shown in parts a- γ and b- γ of Figure 2, corresponding to the starting and to the initially optimized geometry, respectively. The analogous arrangement with C₆₀ at the secondary rim of γ -CD is not shown, but it is less stable by only 1 kJ/mol. The subsequent MD runs of these geometries, followed by energy minimizations of many instantaneous conformations thus generated did produce only minor changes, as can be seen by comparison of parts b- γ and c- γ of Figure 2. The latter is the most favorable geometry with an interaction energy $E_{\text{int}} = -241$ kJ/mol. However, the opposite arrangement with C₆₀ interacting with the secondary rim of γ -CD is less stable by only 1.3 kJ/mol after the MD runs. We defer to the next section the description of the complex formation in water. As a word of caution, we note that the interaction energies mentioned above and in the following might be somewhat affected by the approximate Lennard-Jones parameters describing the dispersion interactions, so that the differences or the ratios among the reported values are more significant than the absolute ones.

On the other hand, when C₆₀ is placed *in vacuo* near the larger sized δ -CD from either the primary or the secondary rim (see for instance Figure 2a- δ), it forms in both cases a true inclusion complex. Such complex, shown in Figure 2b- δ , did not display significant rearrangements in the subsequent MD run and geometry optimizations (see Figure 2c- δ). In this case, the interaction energy is much larger than with γ -CD in absolute value, amounting to -314 kJ/mol, in spite of the large rearrangement imposed to the macrocycle upon guest insertion, which leads to a highly symmetric host geometry. It is of interest to follow the equilibrium fluctuations of the system starting from the initial energy minimum of Figure 2b- δ in terms of the distance between the centers of mass (c.o.m.) of the macrocycle and of the fullerene monitored within the MD run *in vacuo*. The plots for the γ - and the δ -CD complexes are reported in Figure 3a,c for a comparison. The initial parts of the plots are

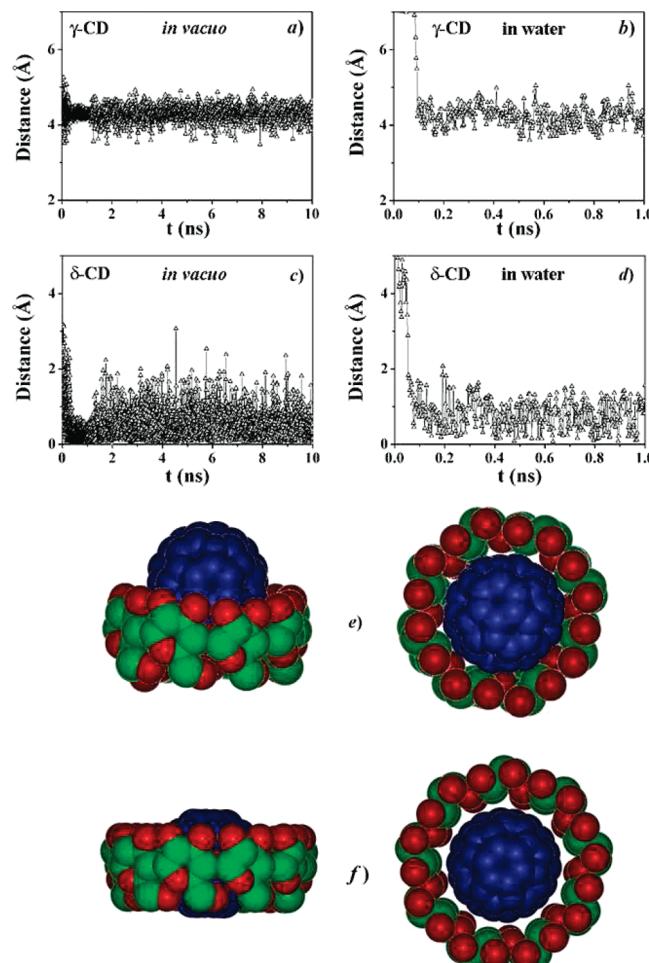


Figure 3. Distances between the c.o.m. of γ - and δ -CD and of C₆₀ in the 1:1 complex plotted as a function of time within the MD runs carried out *in vacuo* and in explicit water. The minimum-energy conformations of the complex formed by C₆₀ with γ -CD (interaction at the secondary rim) and with δ -CD *in vacuo* are shown through space-filling models (parts e and f, respectively) in a side view and viewed from above the secondary rim: for clarity, the fullerene is shown in blue, the carbons and oxygens of the CDs are in green and red, respectively, and the hydrogens were omitted. Very similar geometries were also obtained in explicit water (see text).

affected by the system optimization at room temperature, and in particular by the macrocycle relaxation to optimize its interaction with the guest, but the subsequent equilibrium fluctuations lasting for most of the runs should be noticed. In particular, the distance between the c.o.m. of δ -CD and of C₆₀ displays *in vacuo* large fluctuations in the range of ± 2 Å, with excursions up to about ± 3 Å, almost corresponding to expulsion of the guest from the cavity. These fluctuations are even larger than in the γ -CD complex, where C₆₀ is not included within the macrocycle cavity (see also the space-filling model in Figure 3e), and are allowed by the large size of δ -CD. Interestingly, the same inclusion complex of C₆₀ with δ -CD in a 1:1 stoichiometry quickly forms also in explicit water (see also later), after an initial energy minimization producing only an outer adduct. In this case, we again find larger fluctuations than in the case of γ -CD (compare parts b and d of Figure 3), even though the solvent dampens somewhat the width of the excursion of C₆₀ out of the cavity in either complex compared to the *in vacuo* runs. Still, for the δ -CD complex in water the distance between the c.o.m. shows a range of distances mostly in the range ± 1.5 Å, but also up to ± 2 Å, as shown in Figure 3d. This remarkable freedom of motion is clearly related with

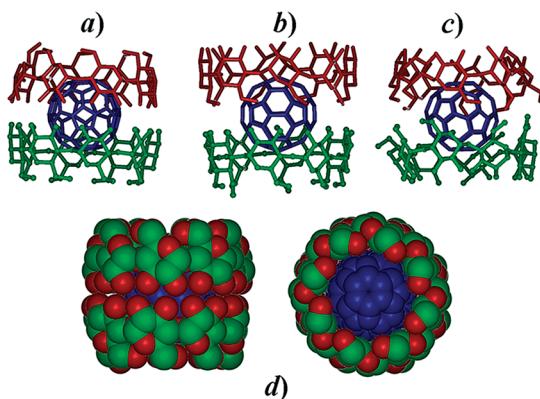


Figure 4. Possible inclusion complexes formed by γ -CD with C_{60} in the 2:1 stoichiometry *in vacuo*. The first CD having formed the 1:1 complex (see Figure 2) is shown in red, and the second one in green, using a ball and stick representation for clarity. The two CDs can interact through the secondary rims (part a, corresponding to the most stable geometry), through one primary and one secondary rim (part b), and through the two primary rims (part c). A space-filling model of the most stable arrangement is displayed in part d, where the tight fitting of the whole complex is clearly seen. Hydrogen atoms were omitted for clarity.

the empty space visible in the space-filling model of Figure 3f due to the small size of C_{60} compared to the large cavity of δ -CD.

In conclusion, the present simulations show that the interactions between C_{60} and γ - or δ -CD in a 1:1 ratio are quite large and favorable, yielding the formation of stable adducts with γ -CD and of a true inclusion complex with δ -CD *in vacuo* and in explicit water, thanks to the large cavity size of the latter macrocycle. However, the shapes of the resulting adduct with γ -CD or inclusion complex with δ -CD strongly suggest that other stoichiometries should be explored. In fact, the geometries shown in Figures 2 and 3 show that the fullerene is still mostly exposed to the outer environment; hence, it may interact with a second γ -CD. Furthermore, while C_{60} is encapsulated within the cavity of δ -CD, it protrudes through either rim with large fluctuations. Accordingly, the possible formation of host–guest complex with a 2:1 stoichiometry must be investigated, as described in the next subsection.

B. Complex Formation with a 2:1 Host–Guest Stoichiometry. In view of the above results, we placed a second CD close to the adduct or inclusion complex described in the previous section and shown in Figure 2. As starting geometries for the further simulations *in vacuo*, we considered the possible interactions of the second CD at either rim, in positions similar to those shown for instance in Figure 2a. In this way, all the unlike arrangements were attempted by facing the two CDs through their primary rims, their secondary rims, or one primary and one secondary rim with the C_{60} fullerene in between. After a full energy minimization of the whole system, each resulting adduct was subjected to a long MD run, and the most stable complex was eventually found after optimization of many instantaneous conformations, as described before. For each CD, we thus obtained three unlike arrangements of the complexes that were ranked through their energies.

In the most stable arrangement of the 2:1 complex formed by γ -CD and C_{60} *in vacuo*, the two hosts interact through their secondary rims, as shown in Figure 4a, forming a large number of intermolecular H-bonds among the secondary hydroxyls (16 H-bonds in the fully optimized structure). In this complex, the interaction energy amounts to $E_{int} = -652$ kJ/mol, a value that in its absolute value is much larger than twice the value of -241

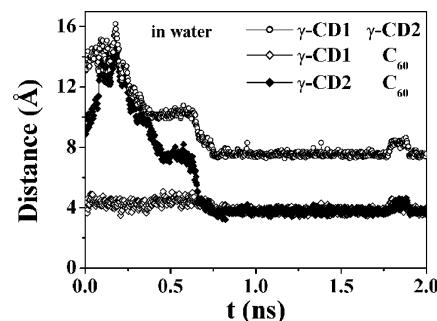


Figure 5. Distances between the c.o.m. of the two γ -CD and of each CD with the C_{60} c.o.m. in the 2:1 complex plotted as a function of time within the MD runs carried out in explicit water. The numbering of the two cyclodextrins as γ -CD1 and γ -CD2 refer to the first and to the second CD interacting with C_{60} .

kJ/mol for the 1:1 adduct. The extra stabilization is mostly due to the many intermolecular H-bonds between the two CDs, which do not undergo further significant distortions. Other less stable arrangements are also found, which include an interaction of the primary rim of one γ -CD with the secondary rim of the other one (Figure 4b) and the interaction between the two primary rims (Figure 4c). These two geometries are less stable than the preferred one by 36 and 100 kJ/mol, in that order, due to some distortions of the two macrocycles, in particular for the less stable complex, but also to a shallower penetration of C_{60} within the hydrophobic cavities. The statistical distribution of the distances between the com of C_{60} and of the two macrocycles can be monitored through the instantaneous values obtained in the MD trajectories after equilibrium was achieved. These two distances turned out to be statistically equal, with values of 3.814 ± 0.004 Å and a standard deviation $\sigma = 0.139$ Å, for the most stable geometry wherein the two CDs face their secondary rims (Figure 4a). These distance are also statistically equal in the other arrangements, amounting to 4.127 ± 0.006 Å with a standard deviation $\sigma = 0.182$ Å for the geometry with intermediate stability (Figure 4b) and to 4.025 ± 0.006 Å with a standard deviation $\sigma = 0.190$ Å for the least stable geometry (Figure 4c). We thus see that the interaction of the γ -CDs through their secondary rims leads to a deeper inclusion with a shorter distance between the c.o.m. of the guest and of the two hosts but also to a tighter structure with a narrower distribution of these distances. The tight fitting of this complex can also be gauged through its space-filling representation, shown in Figure 4d. In conclusion, the geometry shown in Figure 4a,d displays the tightest and most favored arrangement. Such issue will be further commented upon later in connection with the results for δ -CD and the experimental observations.

The simulations in explicit water did confirm the above picture. On the basis of the results of the simulations *in vacuo*, at first C_{60} was placed close to the secondary rim of γ -CD as in the starting geometries of Figure 2, and the 1:1 complex in water was formed by an MD run. Afterward, a second γ -CD was added as described in the methodological section so that the two secondary rims were facing one another. The 2:1 host guest complex in water was formed relatively slowly within a further MD run, but subsequently it was fully stable for the major part of the MD run, as shown in Figure 5. The geometry of the complex and its almost perfect axial symmetry are the same as that found *in vacuo* (see Figure 4d). Thus, the distances between the com of C_{60} and of the two macrocycles are statistically equal, with an average value of 3.810 ± 0.009 Å. This value is equal to what found *in vacuo* within the statistical error, while the standard deviation $\sigma = 0.187$ Å is somewhat larger, suggesting

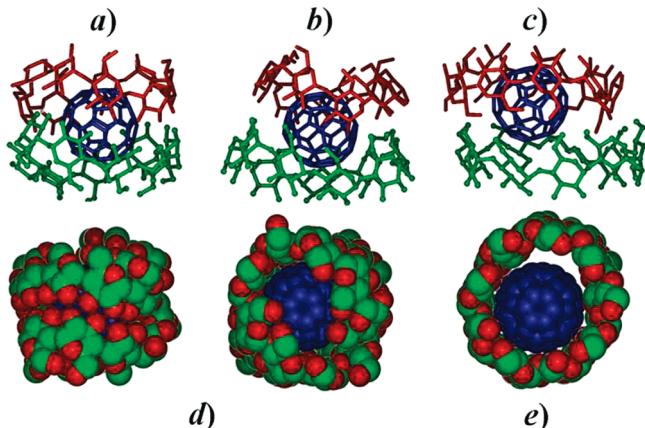


Figure 6. Possible inclusion complexes formed by δ -CD with C₆₀ in the 2:1 stoichiometry *in vacuo* (see Figure 4 for the color codes). The two CDs can interact through the secondary rims (part a, corresponding to the most stable geometry), through one primary and one secondary rim (part b), and through the primary rims (part c). A space-filling model of the most stable arrangement is displayed in part d, showing the significant distortions of the macrocycles. An “open” higher energy conformation is also shown in part e to emphasize the larger size of the cavity compare to the fullerene (see text). Hydrogen atoms were omitted throughout for clarity.

a larger CD mobility as a whole. This finding is consistent with a small number of H-bonds among the secondary hydroxyls of the two CDs, amounting to one-half those formed *in vacuo* because of the H-bonds the secondary hydroxyls form with the water molecules.

A similar procedure was adopted for the *in vacuo* complex with δ -CD (Figure 6a–c). We found that the most stable arrangement involved also in this case an interaction between the secondary rims of the two cyclodextrins, which already produced a 2:1 host–guest complex by the initial energy minimization. The complex was further stabilized within the MD run, and the most stable geometry eventually obtained by optimization of many instantaneous conformations is shown in Figure 6a. The corresponding interaction energy amounts to -637 kJ/mol; hence, it is about twice as large in its absolute value as the interaction energy of -314 kJ/mol for the 1:1 complex. Therefore, unlike what found for the γ -CD complex we do not find any extra stabilization due to the intermolecular H-bonds among the secondary hydroxyls. In fact, in the first place they are less than in the case of γ -CD, being only 12, and furthermore the corresponding energy gain is mostly canceled by the strain energy of the CDs required to optimally wrap the included C₆₀. Interestingly, in explicit water the 2:1 complex shows only minor differences compared to this arrangement (Figure 6a), as discussed later. Other higher energy arrangements *in vacuo* involve as before the interaction between the primary and the secondary rims of the two δ -CD (Figure 6b) and the interaction between the primary rims (Figure 6c). Compared to the most favorable geometry, these arrangements are less stable by 40 and 106 kJ/mol, in that order, a destabilization that is quite similar to that obtained for the complex with γ -CD.

Let us describe more in detail some features of the most stable 2:1 host–guest complex formed by δ -CD with C₆₀ upon facing their secondary rims. The cavity of this cyclodextrin is larger than the C₆₀ diameter, so that significant deformations of the macrocycle are required to achieve a fitting as tight as possible, as shown by the space-filling model reported in Figure 6d. These deformations are allowed by the significant flexibility of these

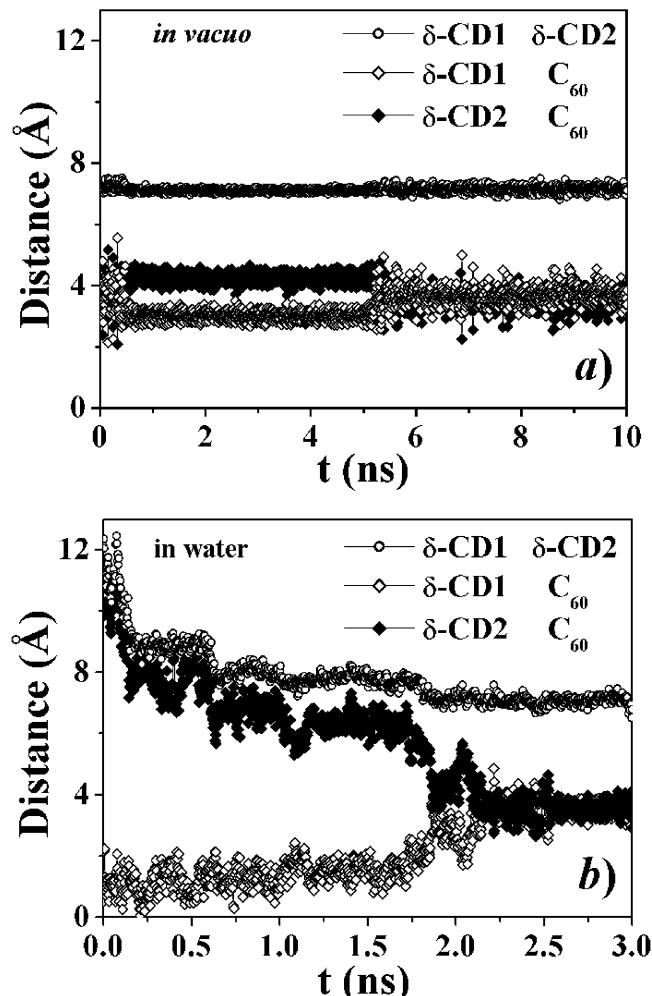


Figure 7. Distance between the centers of mass of the two δ -CD and of each CD with the C₆₀ c.o.m. in the 2:1 complex plotted as a function of time within the MD runs carried out *in vacuo* (a) and in explicit water (b). The numbering of the two cyclodextrins as δ -CD1 and δ -CD2 refer to the first and to the second CD interacting with C₆₀.

macrocycles discussed in ref 15 through MD runs carried out *in vacuo* and in explicit water. This flexibility is still largely present in the complex, allowing significant conformational adjustments to the macrocycles, and a relevant freedom of motion of the included C₆₀ within the cavity. The latter issue can be appreciated by following the equilibrium fluctuations of the distances between the c.o.m. of the two macrocycles and of C₆₀, reported in Figure 7a for the MD runs *in vacuo*. While the distance between the c.o.m. of the two macrocycles fluctuates around a constant value (uppermost points), the distance between the c.o.m. of C₆₀ and of either CD shows a bimodal distribution of values. In fact, in the first part of the MD run the fullerene is strongly displaced toward one host molecule, with average distances of 3.012 ± 0.007 and 4.248 ± 0.007 Å, respectively, but with the same standard deviation $\sigma = 0.14$ Å, indicating relatively narrow distributions. Later on, the position becomes more symmetrical, though not exactly centered within the cavity, since the average distances are 3.667 ± 0.013 and 3.506 ± 0.013 Å, with much broader distributions since they have the same standard deviation $\sigma = 0.27$ Å.

The formation of the 2:1 complex in explicit water was also modeled adding as before a second δ -CD to the 1:1 complex described in the previous section (see Figure 8). The MD run leads to the same equilibrium complex above-described, with only a minor decrease in the number of the intermolecular

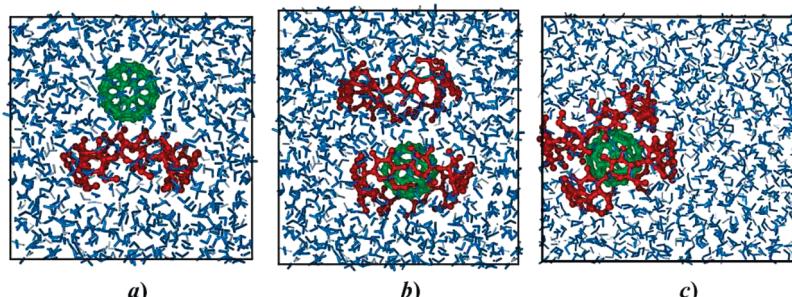


Figure 8. Hydration cell for C_{60} with δ -CD in the starting position (a) and in the 1:1 complex with an included fullerene (b) while a second CD is approaching it (view down the shortest axis of the periodic simulation cell). The final complex with a 2:1 host–guest stoichiometry is also shown in (c) (view down the longest axis of the simulation cell).

H-bonds (amounting to 10) formed by the secondary hydroxyls compared to the geometry achieved *in vacuo*. In this case, even though the average potential energy did not show any significant change after the initial 200 ps, the distance between the centers of mass (c.o.m.) of the two CDs shows a stepwise, smooth change to a final value almost equal to that achieved *in vacuo* with similar fluctuations (see Figure 7b). Furthermore, the distance between the c.o.m. of C_{60} and of the incoming CD shows a similar stepwise decrease with large fluctuations, whereas the distance with the c.o.m. of the first CD shows a slower increase only after more than 1.5 ns to a distance eventually similar to what found *in vacuo*. Interestingly, however, at equilibrium the C_{60} position within the cavity formed by the two δ -CD is perfectly symmetrical in water, within the statistical uncertainties. In fact, these distances between the c.o.m. of the two δ -CD and of C_{60} (averaged over the final 0.8 ns of the MD run) amount to 3.542 ± 0.017 Å, with a standard deviation $\sigma = 0.30$ Å showing a very broad distribution. Thus, the fullerene c.o.m. shows large fluctuations, even larger than *in vacuo* where its position is not perfectly symmetrical between the two macrocycles. Note that in water the C_{60} motion within the complex is not hampered by the solvent, since it is essentially isolated (see Figure 8c), and its large excursions are clearly allowed by the large size of the cavity and by the significant conformational flexibility of the two δ -CD even after the C_{60} inclusion.

The extent of the conformational fluctuations of the δ -CDs in the 2:1 complex with C_{60} can be visually appreciated by comparison of the most stable state of Figure 6d with the limiting case of an “open” conformation shown in Figure 6e as an example. Similar conformations are met within the MD runs, even though with a small probability, but are potentially important. In fact, they indicate the possibility that in the solid state the fullerene breaks free from the complex through the primary rim, so that it may not be stable against separation into two pure (micro)phases. Thus, such observation may reconcile the stability of the δ -CD complex with C_{60} predicted here in solution with the solid-state results mentioned in the Introduction.⁶

Concluding Remarks

The formation and stoichiometry of the complexes formed by the C_{60} fullerene and γ - or δ -CD have been studied by MD techniques *in vacuo* and in explicit water. The study is carried out without any *a priori* assumption about the complex stoichiometry or geometry, and the most stable arrangement in solution was obtained through the MD runs considering the stepwise addition of two CDs to C_{60} in different possible arrangements. The final results show that the stable host–guest

geometry in water is 2:1 in both cases, with the two CDs interacting at the secondary rims (the larger one) through intermolecular H bonds, with C_{60} hosted in the joint cavity. Because of the good size match between the cavity formed by the two γ -CD and C_{60} , in this complex the macrocycle show little deformations and a tight fitting interaction with the guest, so that the formation of the intermolecular H bonds at the CD rims in addition to the favorable dispersive interactions boost the complex stability. Conversely, the larger δ -CD rings must undergo significant conformational distortions to enhance the interaction with the guest, and the corresponding strain energy largely cancels the energy gain due to the intermolecular H bonds. Additionally, in the latter case the macrocycles retain a significant flexibility even in the 2:1 complex, so that the whole system shows large fluctuations with significant motion of C_{60} within the cavity. Moreover, the large conformational flexibility at the outer primary rims of the δ -CDs may provide a sufficient opening that might allow the included fullerene to escape from the cavity and segregate in the solid state. We suggest that this possibility may reconcile the relative stability of the complex in solution with the experimental results inferred by solid-state positron lifetime measurements, which suggest a sort of microphase separation in the absence of solvent, consistent with the pattern often observed with CD complexes observed in solution (for instance by NMR experiments) which however give rise to two distinct solid phases.

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