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ORIGINAL ARTICLE

A molecular modeling study of complex formation and self-aggregation behavior of a porphyrin–β-cyclodextrin conjugate

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Abstract This paper reports a molecular modeling study of complex formation and aggregation behavior of a supramolecular system comprising three different moieties forming two distinct molecules. One molecule is a phenol derivative of porphyrin conjugated to a macrocyclic oligosaccharide, β-cyclodextrin (β-CD), and the other is 1-adamantanol (ADM). The inclusion complex of the latter molecule with the porphyrin–β-cyclodextrin (β-CD) conjugate, and the dimeric aggregates of the conjugate both in the presence and in the absence of the guest are investigated through molecular mechanics and molecular dynamics methods in vacuo, since the systems are scarcely soluble in polar solvents. In this way, we can find the most likely geometry of the complexes or aggregates and characterize the competitive inclusion behavior of ADM and of a porphyrin phenol within the β-CD cavity in terms of the various energy contributions stabilizing the resulting aggregates and/or inclusion complexes.

Keywords Molecular dynamics · β-Cyclodextrin · Porphyrin · Inclusion complexes · Supramolecular self-aggregates

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Introduction

Cyclodextrins (CD), i.e., cyclomaltohexaose, cyclomaltoheptaose and cyclomaltooctaose, usually denoted as α-CD, β-cyclodextrin (β-CD) and γ-CD, respectively, are macrocyclic oligosaccharides with a truncated-cone shape, having an inner hydrophobic cavity and an external hydrophilic surface. The narrow and the wide rims of the macrocycle comprise primary and secondary hydroxyl groups, respectively. Therefore, apolar moieties of a guest molecule can be hosted into the CD cavity and carried in solution in a polar solvent. Quite a large number of inclusion complexes of the natural α-, β- and γ-CDs and of their derivatives are reported in the literature: for instance, already in 1998 a systematic review [1] collected a huge number of inclusion complexes with their stability constants and thermodynamic data obtained experimentally by a variety of techniques (microcalorimetry, UV–Vis and NMR spectroscopy, circular dichroism, chromatography, solubility, and so on), and additional complexes were studied afterwards. Other papers reviewed the simulation studies carried out on CD and their inclusion complexes, focusing in some case on the predicted stoichiometry of the complex, in addition to the most common case of a 1:1 host to guest ratio, also considering a few examples of a 2:1 ratio and of 1:2 complexes [2]. Further examples of simulation studies, reporting also even smaller ratios that are possible with small molecules such as water or ammonia, were discussed more recently [3].

It is however more interesting here to point out that functionalization of the CD rims may affect the solubility in a given solvent and/or the stability of the inclusion complexes (see again for instance Ref. [1]), but it may also yield self-aggregation phenomena in the presence of pendant hydrophobic groups, in particular with aromatic moieties. As an

example, such aggregation behavior was reported for β-CD carrying phenolphthalein bound to the primary rim, even though the structural characterization of the aggregate was outside the scope of the paper [4]. More recently, the structure of self-aggregate complexes was investigated considering CDs functionalized with a small aromatic group: in one case, a pendant *o*-xylylene group was bound to two adjacent secondary hydroxyls of otherwise permethylated α- and β-CD [5] and in another case a (propynyloxy)phenoxy group was bound to a primary β-CD hydroxyl through a 1,2,3-triazole linker, as better discussed later [6]. A more complicated system showing both self-aggregation and the formation of an inclusion complex was investigated experimentally a few years ago. This system comprised 5,10,15,20-tetra(4-hydroxyphenyl) porphyrin (tetrahydroxyphenyl porphyrin derivative (THPP), in the following) bound to a primary β-CD hydroxyl through one phenoxy group, thus yielding the porphyrin–CD CD-THPP conjugate, while the included guest was 1-adamantanol (ADM) [7]. The rich potentialities offered by self-aggregation behavior and inclusion complex formation of similar systems have already been explored in many fields, including for instance biomimetic chemistry and photoinitiated processes, briefly summarized for instance in Ref. [7]. Here we only note as further and more recent examples that porphyrin- and adamantane–β-CD conjugates were shown to self-assemble in well-defined nanowires with interesting electronic and photodynamic properties [8]; other porphyrin–β-CD conjugates show promising anticancer properties for their combined drug-carrier features and electronic behavior, thus acting through the combined effect of chemotherapy and photodynamic therapy [9]; perylene-bridged β-CDs forming inclusion complexes with porphyrin derivatives showed interesting excitonic interactions relevant for molecular and electronic devices [10].

Molecular simulations carried out with molecular mechanics (MM) and molecular dynamics (MD) methods are a powerful theoretical tool to study molecular recognition phenomena and the formation of host–guest inclusion complexes. In fact, we have recently shown that using these techniques accurate and reliable predictions can be made about the formation, the geometry and the stoichiometry of the inclusion complexes of CD [2, 11] by following a general simulation protocol [12]. This protocol involves a preliminary energy minimization of the system in different starting geometries with an outer guest to mimic a random approach from solution. The initial adducts thus obtained are then subjected to separate MD runs at a constant temperature (typically at room T), and the instantaneous conformations periodically saved are then used for further analysis. The protocol was proposed to model non-covalent molecular recognition in inclusion complexes, and it was successfully adopted and fully validated by modeling a 1:1 complex of β-CD with an amphiphilic glycoconjugate guest [11] and the 2:1 complexes of γ- and δ-CD with C_{60} [13]. In the former case, an excellent agreement was found with the observed behavior in solution, in particular with the results of NMR experiments, successfully interpreted through the statistical distribution of the distances between diagnostic intermolecular pairs of atoms. The MD simulations were performed in water, but we also showed that the same results were obtained with an implicit solvent and with simulations performed in vacuo (mimicking a weakly interacting apolar solvent). In fact, it turned out that hydration did not affect the relative stability of the possible inclusion geometries, but only their energy differences, in particular for the higher-energy geometries exposing to water the hydrocarbon moiety [11]. Analogous results from the methodological viewpoint were also obtained for the inclusion complexes of γ- and δ-CD with the strongly hydrophobic C_{60}. In this case, we predicted the formation of a 2:1 complex between the CDs and C_{60} both in water and in vacuo, in good agreement with the observed stoichiometry [13]. The main reason for the satisfactory performance of using an implicit water environment relies on the strength of the van der Waals interactions between the guest and the hydrophobic cavity, and on the dipolar and hydrogen-bond interactions at the CD rims, which may involve the sugar hydroxyls in the case of the amphiphilic glycoconjugate or the hydroxyls of the other CD in the 2:1 complex with C_{60}.

In this paper, we report a new simulation study of the non-covalent interactions involving three unlike moieties forming two distinct molecules: the first one is the above-mentioned CD-THPP conjugate containing the THPP porphyrin derivative and β-CD, and the second one is ADM. Porphyrins and their metal complexes are molecules with remarkable spectroscopic, photochemical and electrochemical properties, but due to the well-known tendency of porphyrins to self-aggregate, in particular in water, (see for example Ref. [14]), in the CD-THPP/ADM system we can expect an array of possible behaviors comprising the formation of an inclusion complex and the self-aggregation of CD-THPP both in the absence and in the presence of an included ADM guest. These possibilities were explored through MM and MD simulations carried out for simplicity in vacuo, thus assuming a non-polar and non-coordinating solvent. However, in view of the above-mentioned results [11, 13] we expect that most results do also apply to a polar solvent. In this context, it should also be noted that CD-THPP turns out to be essentially insoluble in pure water and scarcely soluble in polar solvents, so that it could only be experimentally studied either in neat methanol, or in mixed solvents, such as a water/methanol mixture [7].

In the following, after the methodological section we report the results about the conformational behavior of the isolated CD-THPP and its inclusion complex with ADM, and then about its self-aggregation behavior both with and without the guest. In the final section, we also discuss some implications of the present results.

Methodology

The simulations were performed in vacuo with InsightII/Discover 2000 [15], using the consistent valence force field CVFF [16]. This force field, satisfactorily used also in previous work [11, 13], was originally designed to model proteins, but it was later augmented to include additional functional groups, and therefore it can be satisfactorily used to model CD [11, 17]. The starting geometries of CD-THPP and of ADM were generated with the available templates, and the complex or aggregate formation were obtained as described later. After an initial energy minimization up to an energy gradient lower than 4×10^{-3} kJ/mol/Å, the individual molecules, the complexes or the aggregates were then subjected to an MD run. The final energy minimizations of many instantaneous conformations obtained from the MD trajectories yielded the optimized geometries of the isolated molecules. The MD runs were performed in vacuo for 10 ns using a time step of 1 fs at a constant temperature (300 K) controlled through the Berendsen thermostat. Integration of the dynamical equations was carried out with the Verlet algorithm, and the instantaneous coordinates were periodically saved for further analysis. The most stable geometry of each system was found by energy minimization of a large number of instantaneous conformations (50 conformations sampled in the last half of the MD run at large time intervals after equilibration was achieved), adopting the same convergence criterion as before. In the MD runs the main changes took only place in the initial part of the simulation, and the system equilibration was monitored by the time change of the potential energy and of its components, and of relevant inter-molecular distances such as those between the centers of mass of the molecules involved in supramolecular interactions.

Results and discussion

The conformation of CD-THPP and its inclusion complex with ADM

The most stable geometry of the isolated CD-THPP molecule is shown in Fig. 1a. It is clearly seen that the molecule assumes a relatively compact conformation, with a

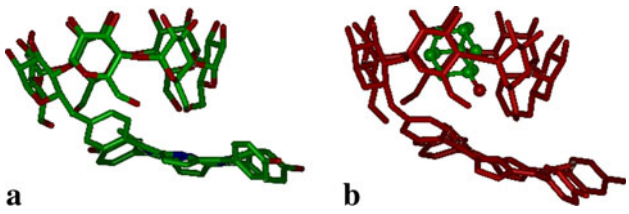

Fig. 1 The most stable geometry obtained for CD-THPP (**a**, *left*) and for its inclusion complex with ADM (**b**, *right*). Hydrogen atoms are not shown for clarity. At left, C atoms are shown in *green*, O atoms in *red* and N atoms in *blue*. At *right*, CD-THPP is shown in *red* and ADM in *balls* and *sticks* with the C atoms in *green* and the O atom in *red* for visual clarity. (Color figure online)

favorable interaction between the porphyrin moiety and the primary β-CD rim, leading to some intramolecular distortions due to the dipolar interactions between the porphyrin nitrogen atoms and some of the nearby β-CD hydroxyls.

The interaction of ADM and CD-THPP was then studied assuming four possible starting geometries, roughly representative of the interaction between independent molecules, without any a priori assumption about the possible geometry of the inclusion complex or even its presence. The unbiased starting geometries comprised an outer ADM approaching the primary or the secondary rim of β-CD with the hydroxyl group of ADM pointing either towards or away from the CD cavity. In all cases, an inclusion complex was easily formed upon simple energy minimization. The geometry of the most stable complex, eventually obtained by long MD runs and optimization of many independent instantaneous conformations, is shown in Fig. 1b. In this arrangement, the interaction energy turns out to be quite large in absolute value, amounting to $E_{\text{int}} = -124$ kJ/mol thanks to the favorable dispersion interactions within the cavity, and to the dipolar interactions of the ADM hydroxyl with the primary CD hydroxyls. Here we defined $E_{\text{int}} = E_{\text{complex}} - E_{\text{CD-THPP}} - E_{\text{ADM}}$, where $E_{\text{CD-THPP}}$ and E_{ADM} are the energies of the (optimized) isolated molecules. Therefore, ADM forms a stable inclusion complex with CD-THPP, in keeping with the well-known stability experimentally found with the simple β-CD host in water [18]. Still, the simulations indicate that in vacuo ADM undergoes a significant shuttling motion perpendicular to the main equatorial CD plane. Such excursions, expected to be obviously damped in water as already found in other complexes [11], are particularly significant in vacuo, and can be quantitatively gauged by monitoring the equilibrium fluctuations of the distance D between the centers of mass of ADM and of the CD macrocycle in CD-THPP, shown in Fig. 2. After equilibration, in the time range $2 \leq t \leq 10$ ns the average distance amounts to $1.27(1) \pm 0.44$ Å, where the SE on the last digit of the mean is shown in parentheses, and the

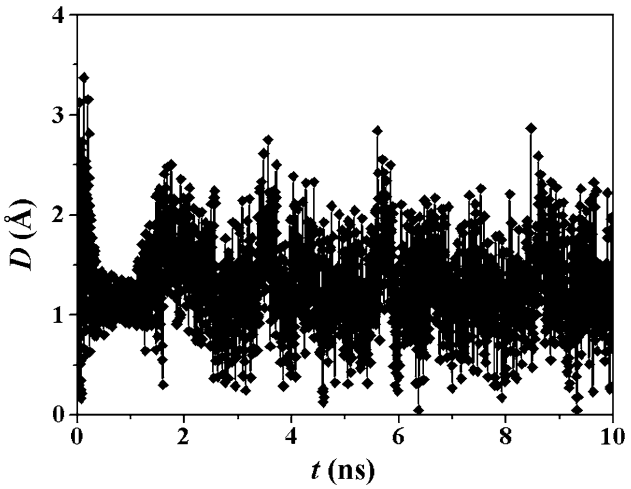

Fig. 2 The ADM fluctuations in the CD cavity of CD-THPP shown through the distance D between the centers of mass of ADM and of β-CD as a function of the simulation time

$\pm$ sign indicates the SD, hence the breadth of the distribution. It is noteworthy that ADM always keeps above the equatorial CD plane on the opposite side of the porphyrin moiety so as to optimize the dipolar interactions with the primary CD hydroxyls, forming also temporary H-bonds in the MD run. Interestingly, the instantaneous distances between the centers of mass of ADM and of the CD macrocycle can be quite large, amounting to up to 2.5 Å in a few cases, almost corresponding to a fleeting expulsion of ADM from the CD cavity. In these cases, the shuttling motion of ADM is accompanied by a significant, almost free rotation allowing its hydroxyl to temporarily interact also with the secondary CD hydroxyls through dipolar interactions and sometimes H-bonds.

The non-covalent CD-THPP dimer

Since porphyrins have a low solubility both in organic solvents and in water and tend to spontaneously form aggregates [14], the possibility of a non-covalent dimerization of CD-THPP was investigated. As anticipated, such behavior was for instance experimentally observed and theoretically found by MD simulations for the *o*-xylylene derivative of permethylated α- and β-CD [5] and the diphenoxy derivative of β-CD [6] mentioned before (see also later). In our case, two CD-THPP molecules were initially placed close to one another in different trial orientations as shown in Fig. 3. In these arrangements, the two porphyrin systems or the two CD macrocycles do roughly face one another while keeping distant the other moieties (cases a, b in Fig. 3), or the porphyrin system of one molecule does approach the CD macrocycle of the other one in two different orientations (cases c, d) and finally the two porphyrin systems do face each other while approaching the lateral CD surfaces of the other molecule (case e). In all cases, an "open" geometry of CD-THPP was arbitrarily chosen instead of the most stable one (Fig. 1a) so that the composite system could better explore its conformational space. In other words, in this way we better allow the system to separately optimize both the intra- and the intermolecular interactions and achieve more easily the thermodynamically most stable state after full equilibration.

The energy minimizations of the starting geometries produced in all cases a stable adduct, often with significant relative changes. For instance, starting from the geometries of Fig. 3a and e these optimizations led to a parallel porphyrin arrangement with the CD macrocycles at opposite sides, while from the positions of Fig. 3b and d the local energy minima respectively corresponded to the porphyrin interactions with the outer CD surfaces of the other molecule, or to a looser interactions between the primary and the secondary CD rims of the two molecules (the latter geometry is shown in Fig. 4a). All these adducts underwent significant rearrangements in the subsequent MD runs with a concomitant large decrease of the system energy. The final energy minimizations of selected instantaneous conformations yielded significantly better geometries.

The most stable complex, shown in Fig. 4b, was obtained starting from the loosest initial arrangement displayed in Fig. 4a having the highest local energy minimum. In this complex, the average potential energy at equilibrium is lower than in the other aggregates obtained in the MD runs starting from the other geometries (see also later), and the absolute energy minimum is found through the final energy minimizations of many instantaneous snapshots. For this complex (Fig. 4b) the interaction energy, defined as $E_{\text{int}} = E_{\text{complex}} - 2 \times E_{\text{CD-THPP}}$ as before, amounts to $E_{\text{int}} = -432$ kJ/mol. From the viewpoint of the simulation strategy, we stress that this dimeric aggregate was obtained starting from the initial adduct with the highest energy minimum. This fact emphasizes that in general different trial geometries must be considered in the simulations, in particular the loose adducts with poor interactions: in fact, for this very reason they can allow for easy (and often fast) large-scale rearrangements in the MD runs, and the system is not trapped in a deep, but local, energy minimum.

In the most stable arrangement of Fig. 4b the porphyrin rings are parallel with a strong non-covalent interaction, while the two CD macrocycles are quite apart, roughly facing the two primary rims in order to allow for a deep inclusion of the peripheral phenols and of part of the porphyrin system through these narrower CD openings. Interestingly, such inclusion geometry shows some analogies with that found for the dimers formed by β-CD carrying a (propynyloxy)phenoxy group bound to the primary rim through a 1,2,3-triazole linker: in this case, a stacking

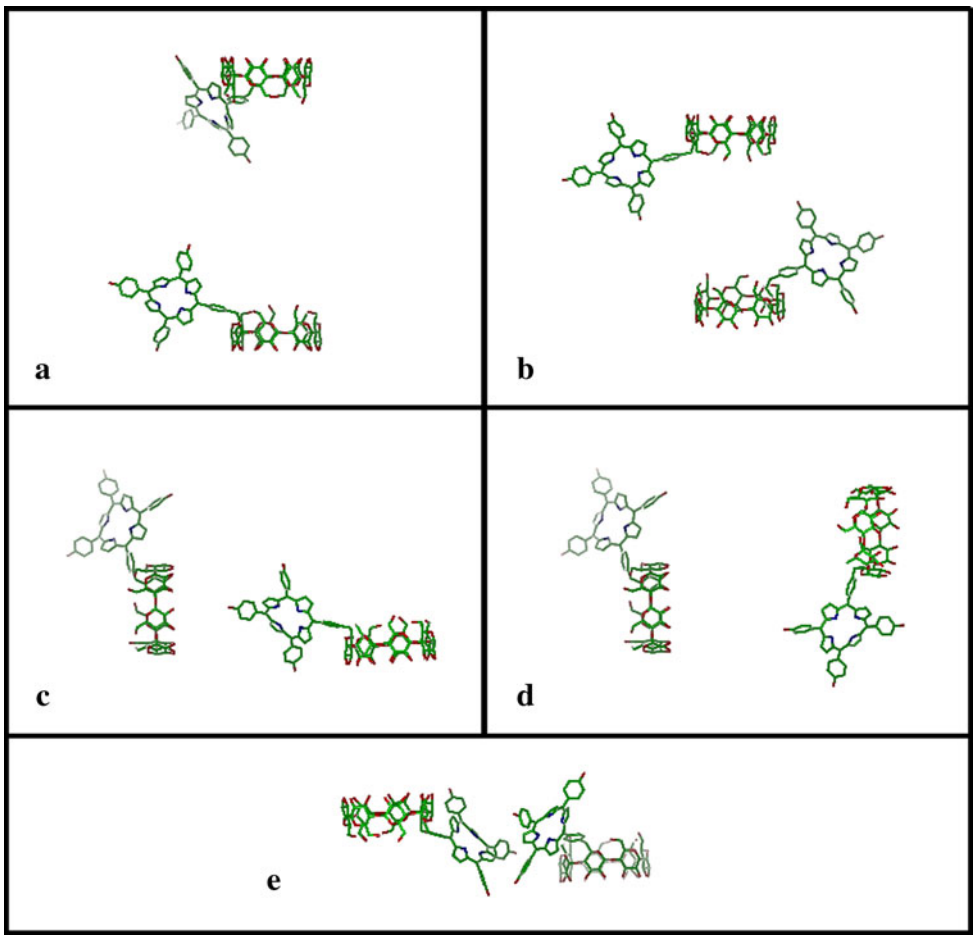

Fig. 3 The starting arrangements of two interacting CD-THPP molecules roughly facing their most relevant moieties. In all cases, an "*open*" starting geometry was adopted for the molecules (see text). Color codes are the same as in Fig. 1 at *left*. (Color figure online)

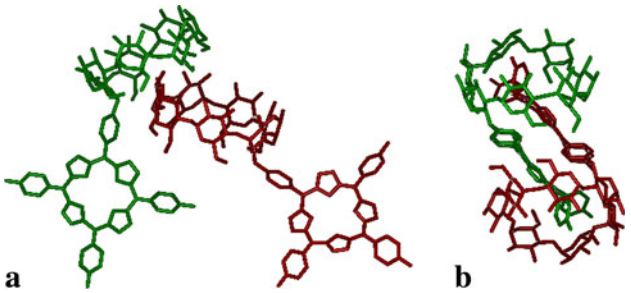

Fig. 4 The least stable initial arrangement (**a**, *left*), obtained by energy minimization starting from the geometry shown in Fig. 3d leads to the most stable arrangement achieved by the CD-THPP dimer after the MD runs and final energy minimizations (**b**, *right*). The two CD-THPP are shown in *green* and *red* for clarity. (Color figure online)

interaction between the triazole rings allowed for inclusion of each propynyloxy group into the CD cavity of the other molecule through the narrower primary rim [6]. Such inclusion geometry is quite unusual, since in general guest inclusion takes place at the wider secondary rim of CDs. However, in our case the other opening is still preferred as a compromise because of the link of the porphyrin moiety to the primary CD rim, since inclusion through the primary rim of the second CD imposes some energy penalty to this macrocycle, but a negligible strain to the link region joining the first CD and THPP. As a result, the latter moiety shows favorable dipolar interactions of the pyrrol nitrogens and their adjacent carbons with the primary hydroxyls of the CDs, while the two included phenol hydroxyls optimize the dipolar interactions with the secondary CD hydroxyls. Interestingly, these hydroxyls may temporarily form H-bonds with the phenol OH, but in the most stable state a more symmetric position is achieved that optimizes the dipolar interactions with no well-defined H-bond.

In view of the large rearrangement that the system shows in the MD run leading from the geometry of Fig. 4a to the complex shown in Fig. 4b, it is of interest to follow the kinetics of the process through the time changes of the potential energy E_{pot} and in particular of the intermolecular distance between the two molecules, expressed through the distances between the centers of mass (c.o.m.) of the CD macrocycles $D_{CD1–CD2}$, reported in Fig. 5. In particular, the inset of Fig. 5b better shows the extent of the large-scale rearrangements in the initial part of the MD run, involving a few close approaches and then a larger separation of the macrocycles before the final geometry is achieved.

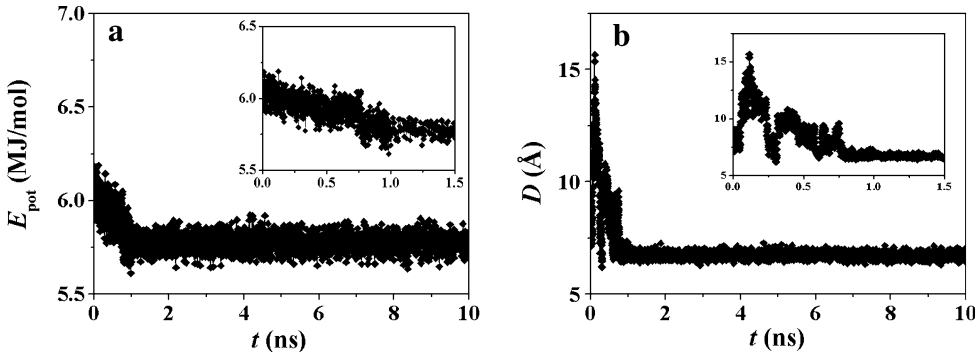

Fig. 5 The time changes of the potential energy E_{pot} (**a**, *left*) and of the distance $D_{CD1–CD2}$ between the centers of mass of the two CD (**b**, *right*). The *insets* show an expanded view of the initial part of the kinetics of the process to better show the large rearrangements undergone in the early stages of the MD runs

Furthermore, it should be noted that D settles to a constant value after about 760–770 ps of the MD run, while E_{pot} is still decreasing, achieving a constant value only after about 1,050 ps. In other words, when the final geometry has essentially been reached, further smaller-scale local rearrangements do further optimize the interaction geometry within the two cavities, enhancing the complex stability. Similar lengthier optimizations at the local scale of the inclusion complexes of CDs were also found in previous work [11], suggesting that this is a general feature of the non-covalent interactions involving flexible molecules: after the overall final geometry is basically achieved, final local rearrangements fully optimize the non-bonded interactions (H-bonds, dipolar and dispersion interactions).

Metastable arrangements were also found by the MD runs starting from the other initially optimized geometries. Their arrangements and the correspondingly less favorable interaction energies are useful to understand and to rank the various contributions to the stability of the preferred geometry. The first metastable complex, having an interaction energy of $E_{int} = -358$ kJ/mol, showed again a parallel arrangement of the porphyrin rings, but the inclusion of just one THPP phenol in the CD cavity of the second molecule, again through the narrower primary rim (Fig. 6a). Further less stable complexes were also found. One of these did show again the inclusion of two THPP phenols in the CD cavity of the other molecule (Fig. 6b), but the two macrocycles are facing the opposite rims (i.e., the primary rim of one molecule faces the secondary rim of the other one), while the porphyrin systems are bound to the primary rims. Therefore, a significant twist is imposed to a CD macrocycle (the lower one in Fig. 6b) to allow its porphyrin to interact with the other porphyrin and the other macrocycle through a sharp bend in the link region, producing a large strain that destabilizes the whole system (Fig. 6b). In a still higher-energy arrangement, the inclusion of only one THPP phenol is achieved, but the two porphyrin systems do not interact (Fig. 6c). Finally, in the least stable arrangement the inclusion of two phenols in the CD cavities takes place at the wider secondary rims,

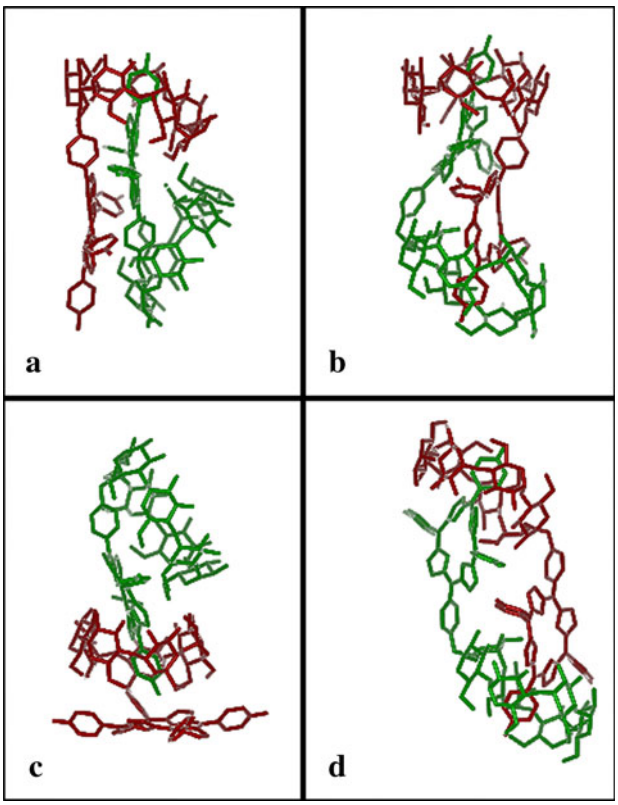

Fig. 6 Metastable arrangements of the CD-THPP dimer after the MD runs and final energy minimizations with an increasing energy from **a–d**

imposing again large distortions to the two CD macrocycles because of the sharp bends in the CD-to-THPP link regions, with no interaction between the porphyrin systems, which lie on roughly orthogonal planes (Fig. 6d). In view of the much larger energy of these complexes, only the most stable one (Fig. 4b) should actually be observed in practice.

In conclusion, the CD-THPP conjugate produces a very stable dimeric aggregate with a parallel arrangement of the porphyrin systems and inclusion of one THPP phenol of each molecule in the CD cavity of the other molecule, even

though this inclusion is accomplished through the narrower primary rim thanks to the much smaller strain imposed to the link region between CD and THPP. The large stability of the complex is due to a combined optimization of both the dipolar and the dispersive interactions among the involved moieties as described before, in spite of the above-mentioned local CD strain. Interestingly, the formation of a dimeric aggregate of CD-THPP in a water–methanol mixture (9:1 v/v) was experimentally reported, with a stacking interaction of the porphyrin system equal to what described before, while per se THPP would rather yield "polymeric" aggregates [7]. Moreover, the most stable geometry shown in Fig. 4b, involving phenol inclusion through the narrower primary rim is in good agreement with the arrangement proposed on the basis of the experimental data [7].

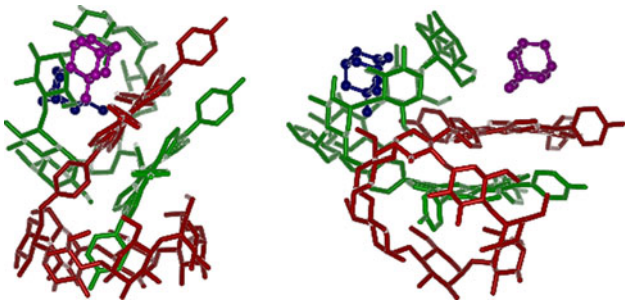

Fig. 7 Two views of the most stable dimeric aggregate of the CD-THPP inclusion complex with ADM. The *left* view best shows the parallel arrangement of the porphyrin rings and a phenol inclusion in a CD cavity, the right view best shows the position of the ADM molecules (shown in *balls* and *sticks* for clarity). The two CD-THPP are shown in *green* and *red*, and the ADM molecules in *blue* and *purple*. (Color figure online)

The non-covalent dimeric aggregate of the inclusion complexes of CD-THPP with ADM

The formation of this aggregate was studied assuming that the inclusion complexes with ADM in the CD cavity with its hydroxyl close to the primary rim is already present, in view of their easy and fast formation. However, as done before we assumed again as the starting geometry an "open" complex, so that the porphyrin systems are far from the CD hydroxyls. Two such inclusion complexes were placed close to one another in the same trial geometries previously considered for the CD-THPP dimers (see Fig. 3 and the previous section). The initial energy minimizations of these dimeric systems already suggested a favorable interaction, in particular when a parallel arrangement of the porphyrins was achieved. In these complexes, the ADM guest stayed in the CD cavity in all but one case, when it was replaced by a THPP phenol and remained outside the cavity through an H-bond with a primary CD hydroxyl.

The MD runs of these initial adducts led to large-scale rearrangements, analogous to those reported before for the CD-THPP dimer. In this system, ADM inclusion in the CD cavity is expected to compete with the inclusion of one phenol group bound to the porphyrin, since it has been reported that the inclusion complex of ADM with β-CD has a larger stability than the free phenol molecule [18]. The most stable complex was eventually achieved starting from a geometry similar to that shown in Fig. 3e. The complex, displayed in Fig. 7, keeps the parallel arrangement of the two porphyrins already realized after the initial optimization, but the CD cavities are differently occupied. In fact, one of them hosts a THPP phenol of the other molecule, deeply included at the primary rim and hydrogen-bonded to a secondary CD hydroxyl with an interaction pattern quite similar to that described before in the absence of the ADM guest. Conversely, the other CD cavity is still occupied by one ADM molecule, displaced towards the secondary rim by another THPP phenol hovering above the primary rim, hydrogen-bonded to a primary hydroxyl (as an H-bond donor) and to the included ADM (as an H-bond acceptor). The second ADM molecule is found close to, but outside, the latter CD, held in place by an H-bond with a primary CD hydroxyl and a dipolar interaction with a pyrrol nitrogen. The kinetics of the process is followed through the time changes of the total

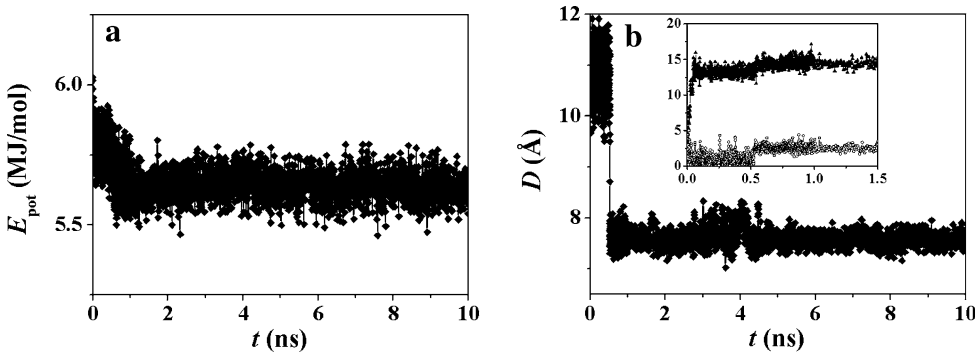

Fig. 8 The time change of the potential energy E_{pot} (**a**, *left*) and of the distance $D_{CD1–CD2}$ between the centers of mass of the two CD-THPP in the inclusion complexes with ADM (**b**, *right*). The *inset* in the right panel shows the distances $D_{ADM1–CD1}$ (*filled symbols*) and $D_{ADM2–CD2}$ (*open symbols*)

potential energy E_{pot} and of the intermolecular distance $D_{CD1–CD2}$ between the c.o.m. of the CD-THPP molecules, and through the distances between the c.o.m. of each ADM and of the CD macrocycles hosting it at the beginning of the MD run, $D_{ADM1–CD1}$ and $D_{ADM2–CD2}$. These plots, reported in Fig. 8, show that while E_{pot} smoothly decreases to a constant value in the initial part of the MD run (Fig. 8a), the two CDs change rather abruptly their relative position, as indicated by the distance $D_{CD1–CD2}$ (Fig. 8b). Conversely, the distances $D_{ADM1–CD1}$ and $D_{ADM2–CD2}$ show a continuous change (inset of Fig. 8b). However, the former distance sharply increases in the initial part of the MD run when inclusion of the phenol group leads to an expulsion of ADM1 from the CD1 cavity to its final position. On the other hand, the second distance shows a small increase, due to the slight displacement of ADM2 towards the secondary rim to accommodate the THPP phenol interacting with the primary rim of CD2.

A relatively similar assembly was also found starting from the arrangement of Fig. 3a. In either case, the initial energy minimum corresponds to a parallel arrangement of the porphyrin planes with the ADM guests in the CD cavities, but an unlike relative position of the two CD macrocycles was obtained. Large molecular rearrangements in the MD runs eventually led to a fully optimized geometry similar to what discussed above, apart from a single ADM outside the CD cavity forming only dipolar interactions with the pyrrol nitrogens. The system energy turns out to be appreciably larger than in the most stable state by about 23 kJ/mol, basically due to the lack of H-bonds formed by the outer ADM. Another higher-energy complex does not show a parallel arrangement of the porphyrin rings, which interact with the outer surface of one CD and with the secondary rim of the other CD. In this way, both ADM guests can remain in the macrocycle cavities, but with a much higher-energy (almost 70 kJ/mol above the most stable state). Increasingly less stable complexes were obtained with inclusion of a phenol group in a CD cavity through the wider secondary rim with ADM expulsion and little additional interactions, or else by inclusion of the ADM molecules in the CD cavities, and the porphyrin ring of each CD-THPP molecule close to the secondary rims of the other one. However, all these arrangements would not be observed in practice due to their much larger energy compared to the most stable state, even though they may form for kinetic reason and then remain kinetically trapped for a while at room temperature.

At the end of this section, we note that on the basis of some experimental data [7] it was also suggested that upon addition of ADM the dimeric complex initially formed by CD-THPP rearranges by keeping the parallelism between the porphyrin systems after inclusion of two molecules in the CD cavities and expulsion of the phenol groups, even though inclusion of just one molecule cannot be fully ruled out by the experimental data (G. Vecchio, personal communication).

Conclusions

We applied a general simulation methodology to study the non-covalent interactions leading to the complex formation and the self-aggregation behavior of a system involving three unlike moieties forming two distinct molecules, namely CD-THPP consisting of a THPP conjugated with a macrocyclic oligosaccharide, β-CD, and ADM that may be hosted within the CD macrocycle.

We employed MM and MD methods at a constant temperature to model the formation of the 1:1 inclusion complex between CD-THPP and ADM starting from an outer arrangement in different orientations. In this way, both the most stable complex and higher-energy metastable states were obtained. ADM inclusion in CD-THPP is stabilized both by the dispersion interactions with the hydrophobic cavity of the CD macrocycle and by the dipolar interactions of the guest OH with the primary CD hydroxyls that form the narrow CD opening. The non-covalent self-aggregation of CD-THPP either in the presence or in the absence of ADM was then investigated. The simulations, carried out in vacuo for simplicity in view of the scarce solubility in polar solvents of these molecules, indicate a strong dimeric self-association of CD-THPP, stabilized by stacking interactions between the porphyrin systems, and by inclusion of one phenol substituent of the THPP moiety of each molecule in the CD cavity of the other one through the narrower primary rims (in the absence of the ADM guest) in agreement with the experimental observations [7]. Even though this relatively unusual inclusion geometry imposes some strain to the CD macrocycle, still it is adopted quite easily in this case thanks to the non-bonded interactions among the different moieties (a combination of dispersion and dipolar interactions), while no major distortion is imposed to the linking region between CD and THPP. Finally, the non-covalent self-aggregation complex formed by two CD-THPP molecules, each containing an ADM guest, displays a behavior somewhat intermediate between those just described due to the competitive inclusion of ADM and of a phenol group bound to the porphyrin system in THPP. In fact, the most stable dimeric aggregate shows again a stacking interaction between the porphyrin systems, while the CD cavities are differently occupied: one of them still hosts an ADM molecule, while the other one contains one phenol substituent of THPP, after expulsion of the previously included ADM which is kept close to the complex by dipolar interactions with one porphyrin system and a H-bond with

a CD hydroxyl. Interestingly, inclusion of ADM is preferred over that of the free phenol molecule [18] because of its size that best fits in the CD cavity thus optimizing the dispersion interactions. In the present case, however, the porphyrin system bound to the phenol group significantly favors phenol inclusion because of the dipolar interactions of the pyrrol ring of THPP with the primary hydroxyls, making it roughly as favorable as ADM inclusion. We finally note that we do not expect that simulations in explicit water may largely affect such arrangement, since the hydrophobic cavities well interact with the hosted hydrophobic moieties, and the hydroxyls of the phenol groups and of β-CD are still largely exposed to the solvent.

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