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Molecular recognition by β-cyclodextrin derivatives: molecular dynamics, free-energy perturbation and molecular mechanics/ Poisson–Boltzmann surface area goals and problems

Ivan Beà[1], **Carlos Jaime**[1], **Peter Kollman**[2]

[1] Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[2] Department of Pharmaceutical Chemistry, University of California San Francisco, USA

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Abstract. The complexation of *p-tert*-butylphenyl *p-tert*-butylbenzoate and *N*-(*p-tert*-butylphenyl)-*p-tert*-butylbenzamide with a β-cyclodextrin derivative formed by two cyclodextrin units linked by a disulfide bridge on one of the C6 atoms has been studied by computational methods. The better amide solubility and the better internal interactions of the ester complex explain the experimentally observed better association constant for the ester. The free-energy perturbation methodology and molecular mechanics/Poisson–Boltzmann surface area analysis have been used to explain the problem and to compare the results.

Key words: Cyclodextrin inclusion complexes – Molecular dynamics simulations – Free-energy perturbation analysis – Molecular recognition

1 Introduction

Molecular recognition is the driving force for many biomolecular processes [1]. Researchers are interested in studying the basis for molecular recognition, but usually biomolecules are too large and complex to be studied and model systems are used. Cyclodextrins (CyDs) are considered good models for enzymes owing to their ability to form inclusion complexes with many organic molecules, which resemble enzyme–substrate interactions [2, 3]. CyDs are cyclic oligomers of α-D-(+)-glucopyranose normally formed by 6–8 glucose units (thus called α-, β- and γ-CyD, respectively). The size of the CyDs is quite large for studies by computational methods based on ab initio molecular orbital calculations and is of moderate size for force-field calculations [4], although recently some semiempirical molecular orbital (MO) calculations for CyDs and their inclusion complexes appeared in the literature [5, 6]. Molecular dynamics (MD) simulations and the free-energy perturbation (FEP) methodology have been applied to the study of CyD inclusion complexes for obtaining relative binding energies for different substrates [7] and for studying different transition-state energies in the hydrolysis of phenyl esters catalyzed by β-CyD [8].

In this work, inclusion complexes were used to determine the interactions responsible for experimentally observed differences in association constants of very similar substrates. Specifically, the complexation of *p-tert*-butylphenyl *p-tert*-butylbenzoate and *N*-(*p-tert*-butylphenyl)-*p-tert*-butylbenzamide (hereinafter called the ester and the amide, respectively) with two β-CyDs linked through an S–S bond over two C6 atoms (hereinafter called the β-CyD dimer), Fig. 1, were studied by MD, FEPs [9] and molecular mechanics/Poisson–Boltzmann surface area (MM/PBSA) [10] using the parm94 force field [11].

These complexes were studied experimentally by Breslow et al. [12], who found that the complexation constant of the ester was much larger than that of the the amide (1×10^8 and 2.4×10^4 M^{-1}, respectively) in spite of their great structural similarity. The better complexation of the ester is, in part, explained by its worse solvation in water in comparison with the amide. The ester thus has a greater tendency to form a complex than the amide. The solvation difference could partly explain (2–3 $kcalmol^{-1}$) the complexation difference of 4.9 $kcalmol^{-1}$. However, other interactions must be taking place, and the purpose of this study is to find and describe them.

A very long FEP (20 ns) was found to be necessary to reproduce the conformational movement and consequently to match the experimental results. With such a long FEP, the difference in association constants was reproduced [13], but the geometry of complexation was found to be very different with respect to the initial geometries. Consequently, other complex geometries were studied with the MM/PBSA methodology (not with FEP, which is computationally too expensive).

Correspondence to: C. Jaime
e-mail: carlos.jaime@uab.es

These findings are now presented to demonstrate that all three methods used (MD, FEP and MM/PBSA) lead to the same conclusions and are mutually supported.

2 Computational methodology

The parm94 force field [11] and the AMBER program [14] were used throughout this work. Atomic charges for all molecules were obtained by the restrained electrostatic potential (RESP) methodology [15, 16].

Ab initio single-point calculations with the STO-3G basis set on one conformation for each molecule (ester and amide) were performed. The geometry obtained was used to generate the molecular electrostatic potential at the HF/6-31G* level. Atomic charges reproducing these electrostatic potentials were obtained using the RESP program after consideration of the corresponding atom equivalencies due to molecular symmetry.

The β-CyD dimer is too large to be studied as a single unit by this methodology; thus, the molecule was divided into pieces (residues). The model (shown in Fig. 2, with three units of glucose and considering three conformations for the central glucose) was employed to generate the electrostatic potential and to obtain the atomic charges. Those atomic charges for the central glucose unit, obtained after applying the RESP program, were used in the AMBER calculations as atomic charges for the glucoses of the β-CyD-dimer. Atomic charges for glucose rings forming the S–S bond were obtained by a similar approach. This time a fragment of three glucose units was also used. The central one contained a (C6)–S–S–$(CH)_3$ group, and three conformations of the central unit were also considered to derive the electrostatic potential and the RESP charges.

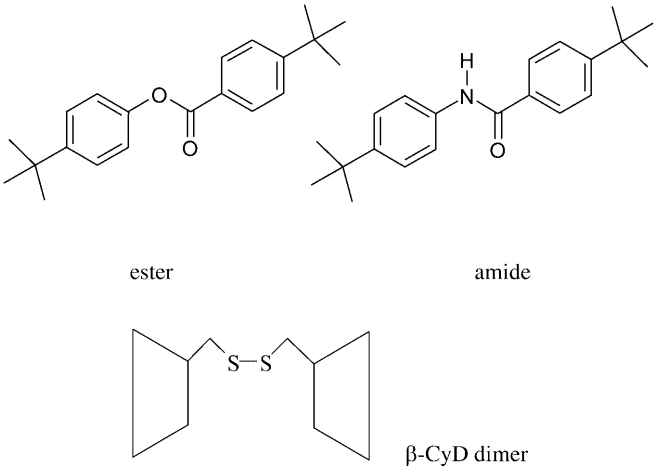


Fig. 1. Molecules studied (ester and amide) and the β-cyclodextrin (*CyD*) dimer

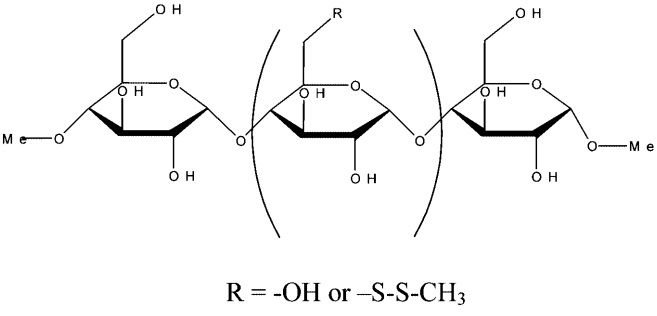


Fig. 2. Model, composed by three glucose units, used to obtain atomic charges. Only those for the central glucose unit (between *parentheses*) were used in this work

Additional parameters (bond lengths, bond angles and torsion angles) for these molecules were derived as follows.The set of added parameters are as follows (see AMBER file parm94.dat for a better understanding of the atom types): CA-OS, 570.0, 1.229; OS-C, 570.0, 1.229; CA-CA-OS, 70.0, 120.4; OS-C-O, 80.0, 126.0; C-OS-CA, 70.0, 115.0; OS-C-CA, 70.0, 112.0; O-C-CA, 70.0, 122.4; OS-CT-OS, 80.0, 126.0; C-OS-CA-CA, 4, 6.0, 180.0, 2.0; CA-OS-C-O, 2, 1.8, 180.0, 2.0;CA-OS-C-CA, 2, 1.80, 180.0, 2.0. All missing parameters for evaluating improper torsions were set to 1.10, 180.0, 2.0

1. The force constants were deduced by comparison with other similar parameters in the parm94 force field.
2. The natural bond lengths and angles were set so as to properly reproduce the molecular geometry obtained from the STO-3G ab initio calculations. Missing torsion angles were always related to ester and amide fragments that are always planar according to the MO calculations; consequently, they do not introduce any difficulty for parameterization.

In the MD simulations, all the molecules were solvated by a rectilinear box (34.4 Å × 33.5 Å × 32.5 Å) of a total of 953 TIP3P waters [17]. Periodic boundary conditions, 8 Å for the primary cutoff and 13 Å for a secondary cutoff for nonbonded interactions were applied.All nonbonded interactions were calculated between atoms separated by less than 8 Å (short range); interactions for those atoms separated between 8 and 13 Å (long range) are only calculated every NSNB step (100 in our case) All the systems were initially minimized, heated to 300 K in three intervals of 50 ps, followed by 50 ps more for achieving equilibration. Production runs of 500 ps were carried out with structures saved every 1 ps. A time step of 2 fs was used together with constant temperature and pressure, using separate temperature scaling factors for solvent and solute atoms, and molecule scaling for pressure.

FEPs were carried out by the windows method. The mutation procedure involves gradually changing the force field describing the system from one state (molecule) to another. Each mutation was performed in 201 windows using $\Delta\lambda$ of 0.005. In a first approach, equilibration was allowed for 1 ps and data were collected for 1.5 ps in each window, making a total of 500 ps for each FEP. FEPs with different lengths were carried out with the aim of obtaining better results. The FEP lengths were of 5 ns (5-ps equilibration and 20-ps collection in each window), 10 ns (10-ps equilibration and 40-ps collection in each window), and finally 20 ns (20 ps equilibration and 80 ps collection in each window).

The MM/PBSA methodology was also applied, in all the MD simulations with water, to estimate relative binding free energies, $\Delta G_{\text{binding}}$, from absolute energies in the gas phase, E_{gas}. Solvation free energies, $G_{\text{PB}} + G_{\text{nonpolar}}$, for the complexes, for the guests (ester or amide) and for the host (β-CyD dimer) were also computed. The entropy term, S, was estimated with the NMODE module of AMBER, based on structures of the complex, guest and host extracted from the MD simulations (six structures from each simulation) and with water molecules removed. The normal-mode analysis was carried out for the energy-minimized structures using a dielectric constant of $4r_{ij}$ (where r_{ij} is the distance between ith and jth atoms). The final entropy value for each system (complex and molecules) was obtained by averaging those coming from the six snapshots.

$$\Delta G_{\text{binding}} = \Delta G_{\text{water}}(\text{complex}) - [\Delta G_{\text{water}}(\text{guest}) + \Delta G_{\text{water}}(\text{host})]$$

Free energies, ΔG_{water}, for each species were evaluated by the following scheme:

$$\Delta G_{\text{water}} = E_{\text{gas}} + G_{\text{solvation}} - TS,$$

$$G_{\text{solvation}} = G_{\text{PB}} + G_{\text{nonpolar}},$$

$$E_{\text{gas}} = E_{\text{internal}}(\text{bond}, \text{angle}, \text{torsion}) + E_{\text{electrostatic}} + E_{\text{vdW}},$$

3 Results

3.1 MD simulations

MD simulations were run for each guest molecule and for each complex, both in vacuum and in water solution. Three different orientations of the guest inside the host were considered: ''central'', ''up'', and ''down'' – the names denote the position of the carbonyl group with respect to the S–S linkage (Fig. 3). The amide molecule is always planar but the ester is not (neither of the phenyl rings is in the same plane). The complexes remain formed in all the MD runs, the energy rapidly achieves equilibrium and the structures of the complexes do not substantially change from their initial values. In a first approach, only the central complexation was considered for the ester/β-CyD dimer and amide/β-CyD dimer complexes, but orientations up and down were also studied in the light of the results from the 20-ns FEP (vide infra), likewise showing stable complexes. The average structures obtained for these three orientations are presented in Fig. 3.

A hydrogen-bond analysis was done with the CARNAL module of AMBER. The solvated amide forms hydrogen bonds between the amide proton and the oxygen of water molecules, and between the carbonyl oxygen with the protons of water. As expected, the N atom of the amide does not form any hydrogen bond. The solvated ester creates hydrogen bonds between the water hydrogens and both ester oxygens.

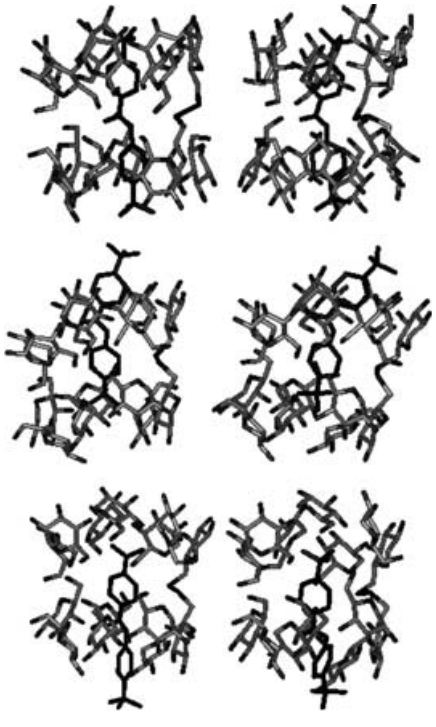


Fig. 3. Average structures of the solvated amide/β-CyD dimer (*left*) and ester/β-CyD dimer (*right*) complexes (from *top* to *bottom*: ''central'', ''up'' and ''down'' orientations) obtained from the molecular dynamics simulations. Water molecules and hydrogen atoms have been removed for the sake of clarity

The results of the hydrogen-bond analysis for the amide/β-CyD dimer and ester/β-CyD dimer complexes are presented in Table 1. The values in Table 1 are obtained by the addition of the number of short contacts between specified atoms divided by the number of structures (snapshots) considered (usually 500).

The amide/β-CyD dimer complex in the central orientation presents hydrogen bonds between the amide carbonyl oxygen and both the primary hydroxyl protons of the β-CyD dimer and the water protons (CDh-G and G-hw). The amide proton creates hydrogen bonds with primary hydroxyl oxygens and sulfur atoms (CD-Gh) but not with water (Gh-w). The ester/β-CyD dimer complex in the central orientation forms hydrogen bonds between the two ester oxygens and both primary hydroxyl protons of the β-CyD dimer and water protons (CDh-G and G-hw). In fact, the ester/β-CyD dimer complex exhibits fewer guest–host hydrogen bonds than the amide/β-CyD dimer complex, the latter being more stabilized. However, the ester/β-CyD dimer complex forms more intermolecular host hydrogen bonds (between the two CyDs forming the dimer, inter- and intramolecular, CDint) as well as more hydrogen bonds between host and water (CDh-w and CD-hw). Consequently, the ester/β-CyD dimer complex is better solvated than the amide/β-CyD dimer. These effects are also observed in the MM/PBSA results (vide infra).

The amide/β-CyD dimer complex in the up orientation presents hydrogen bonds between the amide carbonyl oxygen and both the secondary hydroxyl protons and water protons (CDh-G and G-hw). The amide proton only creates hydrogen bonds with the glucosidic oxygens (CD-Gh). The ester/β-CyD dimer complex in the up orientation presents hydrogen bonds between the ester carbonyl oxygen and both the secondary hydroxyl protons and the water protons (CDh-G and G-hw). The amide/β-CyD dimer complex shows more host–guest (CD-Gh and CDh-G) and host–water (CD-hw and CDh-w) hydrogen bonds than the ester/β-CyD dimer complex, but fewer host–host hydrogen bonds (CDint).

The amide/β-CyD dimer complex in the down orientation presents hydrogen bonds between the amide carbonyl oxygen and all (secondary and primary) hydroxyl protons and water protons (CDh-G and G-hw). The amide proton only creates hydrogen bonds with the glucosidic oxygens. The ester/β-CyD dimer complex in the down orientation presents hydrogen bonds between its two ester oxygens and the secondary and primary hydroxyl protons, as well as between the carbonyl oxygen and water protons (CDh-G and G-hw).

The amide/β-CyD dimer complex has more host–guest hydrogen bonds than the ester/β-CyD dimer complex (CDh-G and CD-Gh), but fewer host–host ones (CDint); the guest–water (G-hw) and the host–water (CDh-w and CD-hw) hydrogen bonds are similar in both complexes.

The ester/β-CyD dimer complex exhibits a larger total number of hydrogen bonds than the amide/β-CyD dimer complex only in the central orientation. Other orientations have very similar total numbers pof hydrogen bonds but the amide figures are slightly larger. A previous work exclusively considered the central

Table 1. Hydrogen-bond analysis for the amide and ester complexes in the three orientations ("central", "up" and "down"). The *numbers* are the addition of the number of close contacts between specific atoms divided by the number of snapshots of each molecular dynamics simulation. The hydrogen bonds are grouped in classes: intramolecular (*CDint*), host oxygens with guest protons (*CD-Gh*), host protons with guest oxygens (*CDh-G*), host protons with water oxygens (*CDh-w*), host oxygens with water protons (*CD-hw*), guest protons with water (*Gh-w*), guest oxygens with water protons (*G-hw*), and the addition of all the terms in each case (*HB total*)

	Amide complex								Ester complex					
	CDint	CD-Gh	CDh-G	CDh-w	CD-hw	Gh-w	G-hw	HB-total	CDint	CDh-G	CDh-w	CD-hw	G-hw	HB total
Central	2,372	135	99	4,775	9,335	0	42	16,758	2,696	86	4,797	9,459	21	17,060
Up	2,539	38	28	4,774	9,302	0	95	16,776	2,679	20	4,587	9,279	97	16,662
Down	2,279	71	70	4,796	9,429	0	52	16,697	2,337	76	4,783	9,379	59	16,634

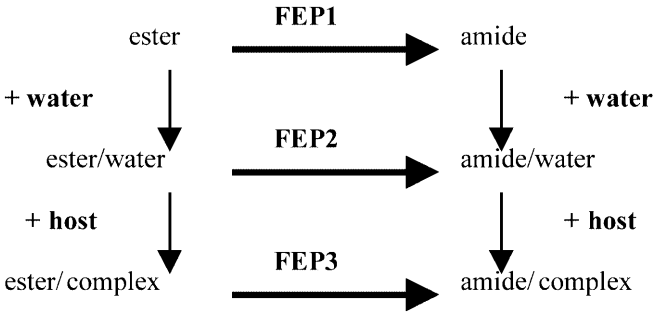
orientation [13], and the ester/β-CyD dimer was overstabilized by those computations.

Guest–host and host–host hydrogen bonds stabilize the complex and guest–water and host–water ones make it better solvated (thus stabilizing it too). These terms should have some role in the complexation, but obviously these are not the total extent of their contributions. In Sect. 3.3, the importance of other terms (internal, van der Waals) is also discussed, and the electrostatic and solvation terms correlate with the number of intramolecular (complex) and intermolecular (with solvent) hydrogen bonds, respectively. It may be noted here that the amide proton does not form hydrogen bonds with water. This can be explained because the β-CyD dimer orients the water molecules with the oxygen pointing out and the hydrogens pointing in; thus, is more probable for the guest to interact with the water hydrogens than with the oxygens. Moreover, the guest is deeply included into the cavity of the CyD and water encounters severe steric hindrance to approach the amide hydrogen.

3.2 Free-energy perturbations

The FEP methodology was used to evaluate the free-energy differences in both the complexation and solvation processes for both ester and amide. The three FEPs considered to close the thermodynamic cycles are shown in Fig. 4. All ester atoms (and their corresponding atomic charge and parameters) were gradually mutated to those of the amide. FEP1, FEP2 and FEP3 mutate the ester to the amide in vacuum, in water solution and in the solvated β-CyD dimer complex. The energy differences obtained from these computational processes should be comparable with experimental free-energy differences, such as the solvation free-energy difference between the amide and ester, and the free-energy difference between the complexation of amide and ester.

When the three FEPs were carried out in simulations of 500-ps length each [13], the results were not totally satisfactory [$\Delta\Delta G$(complexation) was almost zero but favoring the amide complexation] and to improve them, FEPs were performed in much longer simulations. Reverse runs for the FEPs of 500 ps, 5 and 10 ns were performed. The results were totally coincident, and no reverse runs were performed for the 20-ns FEP The results of all the simulations performed are contained in Table 2. FEP1 and FEP2 were run with 10-ns-length



$$FEP2 - FEP1 = \Delta G_{amide} - \Delta G_{ester}\ \textbf{(solvation)}$$

$$FEP3 - FEP2 = \Delta G_{amide} - \Delta G_{ester}\ \textbf{(complexation)}$$

Fig. 4. Thermodynamic cycles used to computationally evaluate the free-energy differences existing in the solvation and complexation of the amide and the ester

simulations. No significant differences with those previous values were obtained; consequently, they can be considered as converged. However, when FEP3 was performed with a simulation of 5-ns length, the final result changed substantially (from almost zero to 1.05 kcalmol^{-1}, favoring the ester complexation, see Table 2). Consequently, another simulation of 20-ns length was performed for FEP3.

A better solvation (FEP2–FEP1) for the isolated amide than for the isolated ester has been obtained (in agreement with what is experimentally known) [18], with a computed $\Delta\Delta G$(solvation) of -2 kcalmol^{-1}. The major contribution comes from the electrostatic term where the interactions with the solvent are counted. The free-energy difference of complexation, $\Delta\Delta G$(complexation), between the ester/β-CyD dimer and amide/β-CyD dimer solvated complexes was almost zero with the 500-ps FEPs, but using the longest perturbations (FEP3 of 20 ns and FEP2 of 10 ns) the difference reached 3.31 kcalmol^{-1}, favoring the ester complexation. The major contributions (Table 2) came from internal (bond, angle and dihedral, BADH) and electrostatic terms. The experimental difference, 4.9 kcalmol^{-1} favoring the ester complexation, is not fully achieved, but now the preference is correct.

Initially, when perturbations of 500 ps were performed [13], it was supposed that FEP3 did not properly reproduce the interactions of the complex with water,

Table 2. Free-energy perturbation (*FEP*) results (*total*, kcalmol^{-1}) and the different energy contributions (kcalmol^{-1}) for the ester to amide mutations (FEP1, FEP2 and FEP3) as a function of different simulation times (*length*). FEP2–FEP1 represents the difference in solvation, while FEP3–FEP2 is the difference in complexation energy

	FEP1	FEP2	FEP3	FEP3	FEP3	FEP2–FEP1	FEP3–FEP2	
Length	10 ns	10 ns	500 ps	5 ns	20 ns		FEP3 = 5 ns	FEP3 = 20 ns
Electrostatic	−2.47	−5.61	−4.71	−3.73	−3.88	−3.14	1.88	1.73
Nonbonding	0.32	0.14	−0.13	−0.13	−0.19	−0.18	−0.27	−0.33
14NB	0.88	0.97	0.86	1.00	1.02	0.09	0.03	0.05
14EL	−21.60	−21.68	−21.57	−21.69	−21.85	−0.08	−0.01	−0.17
Bond, angle and dihedral	−5.85	−4.54	−5.42	−5.11	−2.50	1.32	−0.57	2.04
Total	−28.72	−30.71	−30.97	−29.66	−27.40	−2.00	1.05	3.31

especially the water flux going in and out of the complex cavity. With the FEP3 of 5-ns length, the largest energy change was observed in the electrostatic term, proving the former assumption. Nevertheless, the complexation difference did not agree with the experimental value, indicating that some other reason may still exist. With the 20-ns FEP3, the improvement in the electrostatic term is maintained, but the major improvement is observed to be in the internal forces (BADH). The interactions of the complex with water molecules are thus already correctly reproduced in the 5- and 20-ns FEP3, but the conformational equilibrium (considering the conformation of guest and host and their relative orientation) is much better reproduced in the 20-ns FEP3.

The evolution of the complex geometry during this simulation is presented in Fig. 5. The guest starts from the central orientation and ends in the up orientation. This does not mean that the ester/β-CyD dimer complex exhibits central orientation (starting point) and the amide/β-CyD-dimer complex has up orientation (final structure), but notes the importance of sampling in these complexes where different orientations are possible. A much longer FEP3 would be needed, long enough to reproduce the central–up–down orientations in every window, but this escapes our computation limitations. It is worth noting here that the 20-ns FEP took as long as 50 days even using an HP V2500 supercomputer

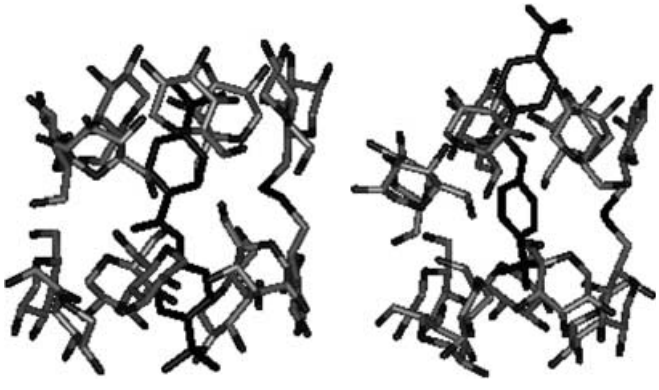


Fig. 5. Starting structure for the 20-ns FEP3 simulation (*left*, ester/β-CyD dimer complex in the central orientation) and the final structure obtained (*right*, ester/β-CyD dimer complex in the up orientation)

To cover all these orientations, MD simulations were done for all situations (vide supra), and MM/PBSA analyses were carried out to compare the ester and amide complexations (vide infra).

3.3 MM/PBSA approach

This methodology was applied to analyze the 500 snapshots from the MD simulations of the solvated ester/β-CyD dimer and amide/β-CyD dimer complexes, and of the isolated guest and host in water solution.

The solvation terms for the isolated amide and ester are shown in Table 3. The amide shows better solvation than the ester, 3.8 kcalmol^{-1}, as expected. The results of $\Delta G_{\text{binding}}$ for the complexes are contained in Table 4, and it shows that the ester complexation is more favored than the amide one in all cases by 5–6 kcalmol^{-1}. Interestingly, what makes the complexation more favorable is the change in the host and guest structures, E_{gas}, while solvation disfavors it. Both central orientations present the highest E_{binding}; it is thus reasonable that the previous work did not agree with the experimental results [13]. The complexation of both ester and amide is occurs preferably in the up orientation, a result that is consistent with the final orientation obtained in the 20-ns FEP3.

The differences between ester and amide complexation in each orientation ($\Delta\Delta G_{\text{binding}}$) are presented in Table 5. The central orientation favors the ester complexation by 4.8 kcalmol^{-1}. The electrostatic term favors the amide complexation (it possesses more hydrogen bonds with the host) but the polar solvation term, E_{PB}, favors the ester complexation (it has more host–water hydrogen bonds) compensating the first term; the ester complexation is also favored by van der Waals interactions.

Table 3. Energy terms (kcalmol^{-1}) due to solvation (E_{nonpolar}, E_{PB} and $E_{\text{solvation}}$) for the isolated amide and ester molecules, as well as their differences [kcalmol^{-1}, Δ(amide−ester)]

	Isolated amide	Isolated ester	Δ(amide−ester)
E_{nonpolar}	4.3	4.2	0.1
E_{PB}	−18.9	−15.1	−3.8
$E_{\text{solvation}}$	−14.6	−10.8	−3.8

Table 4. Binding energy (kcalmol^{-1}, $E_{total,PB}$) resulting from molecular mechanics/Poisson–Boltzmann surface area analysis for the three orientations (central, up and down) of the amide/β-CyD dimer and ester/β-CyD dimer complexes. The components of the total binding energy, $E_{total,PB}$, are also shown (kcalmol^{-1}): electrostatic (E_{elec}), van der Waals (E_{vdW}), internal ($E_{internal}$), gas (E_{gas} = the sum of the previous three terms), nonpolar solvation ($E_{nonpolar}$), polar solvation (E_{PB}), solvation ($E_{solvation}$ = the sum of the last two terms), and the sum of polar solvation and electrostatics ($E_{PB + elec}$)

	Amide/β-CyD dimer complex			Ester/β-CyD dimer complex		
	Central	Up	Down	Central	Up	Down
E_{elec}	−24.0	−24.9	−26.0	−16.1	−29.8	−26.3
E_{vdW}	−32.0	−33.2	−26.6	−34.6	−33.5	−27.6
$E_{internal}$	14.6	11.3	8.1	15.2	5.4	5.8
E_{gas}	−41.3	−46.8	−44.5	−35.5	−57.9	−48.1
$E_{nonpolar}$	−4.7	−4.5	−4.2	−4.6	−4.5	−4.2
E_{PB}	38.8	41.6	39.8	27.9	45.3	37.5
$E_{solvation}$	34.1	37.1	35.6	23.4	40.8	33.3
$E_{PB + elec}$	14.7	16.8	13.7	11.9	15.5	11.2
$E_{total,PB}$	−7.3	−9.7	−8.9	−12.1	−17.1	−14.8
Standard deviation	2.7	2.6	2.7	2.9	2.7	2.7

Table 5. Differences in the binding energies (kcalmol^{-1}) between the amide/β-CyD dimer and ester/β-CyD dimer complexes in all three orientations considered (central, up and down), as well as their average

	Central	Up	Down	Average
E_{elec}	−8.0	4.9	0.3	−0.9
E_{vdW}	2.7	0.2	1.0	1.3
$E_{internal}$	−0.6	5.9	2.3	2.6
E_{gas}	−5.9	11.1	3.6	2.9
$E_{nonpolar}$	−0.1	0.0	0.1	0.0
E_{PB}	10.8	−3.7	2.2	3.1
$E_{solvation}$	10.7	−3.7	2.3	3.1
$E_{PB + elec}$	2.9	1.3	2.5	2.2
$E_{total,PB}$	4.8	7.4	5.9	6.1

The up orientation favors the ester complexation by 7.4 kcalmol^{-1}; the electrostatic and internal terms favor the ester complexation (the amide has more guest–host hydrogen bonds and fewer host–host). The CyD dimer is deformed, trying to interact with the amide, resulting in a worse internal energy. In the ester, this does not occur, the β-CyD dimer is not deformed and it exhibits better host–host interactions (the electrostatic term is more favorable); the polar solvation term favors the amide as it exhibits better host–water hydrogen-bond interactions.

The down orientation favors the ester complexation by 5.9 kcalmol^{-1}. The internal and polar solvation terms favor the ester complexation, although to a lesser extent than in other orientations. As in the up complexation, the β-CyD dimer is deformed owing to its interactions with the amide (it shows a worse internal energy). The host–water hydrogen-bond interactions are similar in both complexes, and the polar contribution comes from the difference of solvation of the ester and amide molecules.

All the complex orientations are presumably possible and must consequently be taken into account by averaging them (see Table 5). Results considering all the orientations (average) indicate that the ester complexation is favored by 6 kcalmol^{-1}. The ester exhibits worse electrostatics (in summary, the amide has more guest–host interactions), better van der Waals interactions, better internal energy (the host is not deformed to interact with the guest) and better polar solvation (as the amide is more soluble than the ester molecule).

Our first assumption was that the amide was more soluble than the ester, but that this fact alone could not explain the better complexation of the ester. This work indicates that the ester complexation is favored by the better solubility of the isolated amide (3.1 kcalmol^{-1} in the polar solvation term) and also by a better internal energy of the ester/β-CyD dimer (2.5 kcalmol^{-1} in the internal term). The worse electrostatic interactions for the ester/β-CyD dimer are compensated by its better van der Waals interactions. The role of the internal energy is also noted in the perturbation methodology (vide supra) making this hypothesis plausible.

The entropy contribution was estimated for the central complexation of the ester and the amide (Table 6). It favors the ester complexation by 1 kcalmol^{-1} over the amide complexation. The ester in the complex moves more freely than the amide because the amide undergoes stronger interactions with the CyD dimer. It is worth noting here that the entropy is calculated exclusively from the complex, guest and CyD dimer structures by removing all water molecules. Thus, the disorder of waters is not considered and will be different in the ester/β-CyD dimer and amide/β-CyD dimer complexes. As the entropy is an approximate

Table 6. Binding energy (kcalmol^{-1}, $E_{total,PB}$), entropy contribution (kcalmol^{-1}, $-T\Delta S$), and free energy (kcalmol^{-1}, ΔG_{total}) for the amide/β-CyD dimer and ester/β-CyD dimer complexes in the central orientation

	Amide complex	Ester complex	Difference (amide−ester)
$E_{total,PB}$	−7.3	−12.1	4.8
$-T\Delta S$	21.3	20.1	1.2
ΔG_{total}	14.0	8.0	6.0

term and makes a minor contribution in the complexation difference, it was only estimated for the central complexation.

4 Conclusions

Both the better solvation of the isolated amide and the worse internal energy of the amide complex, as a consequence of its electrostatic interactions, could explain the energy difference observed (4.9 kcalmol^{-1}) in the complexation of an ester and an amide with a β-CyD dimer. Three methods were used to demonstrate these interactions. MD simulations show the complex structures and the hydrogen-bond interactions, FEPs show the energy differences in complexation and solvation and MM/PBSA analysis allows the comparison between complexations and the contribution of the different terms.

The FEP methodology is very computationally demanding and has to continue long enough to reproduce all the molecular movements. On the other hand, the MM/PBSA methodology provides good qualitative results, despite being an approximate method, and MM/PBSA has the advantage of being much faster. All the methods used lead to the same conclusions and are mutually supported.

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