Computer simulation of reactions in β-cyclodextrin molecular reactors: transition state recognition†

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Cyclodextrins have attracted much interest in recent years because of their potential use as molecular reactors allowing organic reactions in aqueous solution. To better understand their effect on reaction mechanisms, we have carried out a computational study of a prototypical process (neutral ester hydrolysis) in a β-cyclodextrin (β-CD). Two models have been used for the reactor. The first and simpler one assumes that the medium can be described by a polarizable dielectric continuum. The second one takes into account the discrete nature of the β-CD and water molecules thanks to a computational approach that combines the use of Quantum Mechanics, Molecular Mechanics and Molecular Dynamics techniques. We focus on neutral pH processes for which either acceleration or inhibition has experimentally been observed depending on ester derivatives. Our calculations rationalize such observations by showing that the two reaction mechanisms usually invoked for hydrolysis, stepwise (involving two transitions states with formation of a –C(OH)$_2$OR tetrahedral intermediate) and concerted, undergo opposite effects in the β-CD environment. The results highlight the role played by molecular shape recognition. Thus, in spite of a higher polarity exhibited by the three transition states with respect to the reactants, the interactions with the β-CD cavity may either increase or decrease the activation barrier due to different 3D-arrangements of the chemical structures.

1. Introduction

Minimizing the use of volatile organic compounds (VOC) in the chemical industry and promoting the use of less polluting solvents are major objectives of Green Chemistry.[1] The development of reactions in aqueous media is particularly appealing although, obviously, solubility problems do represent a major drawback. One may overcome such a problem, at least in part, by the use of so-called "molecular vessels" or "molecular reactors", which are macromolecules displaying significant solubility in water and possessing a hydrophobic cavity of nanometric size. Cyclodextrins (CDs) are prototypical systems of this type[2] and not surprisingly they have attracted much attention in the literature during the last decades.[3,4] Many organic reactions have been carried out in the presence of CDs, but due to its biological and chemical relevance, ester hydrolysis has been one of the most studied ones.[5–18]

Bender *et al.* investigated the CD-induced hydrolysis of a variety of phenyl acetates[6,8] and phenyl benzoates[7,9] under alkaline conditions and discovered many characteristics similar to those observed in enzymatic processes. In accordance with these and other pioneering studies, it has been suggested that the neutral or ionized CD and the ester initially form an inclusion complex.[7,9] Then, an alkoxide ion from the CD secondary hydroxyl groups
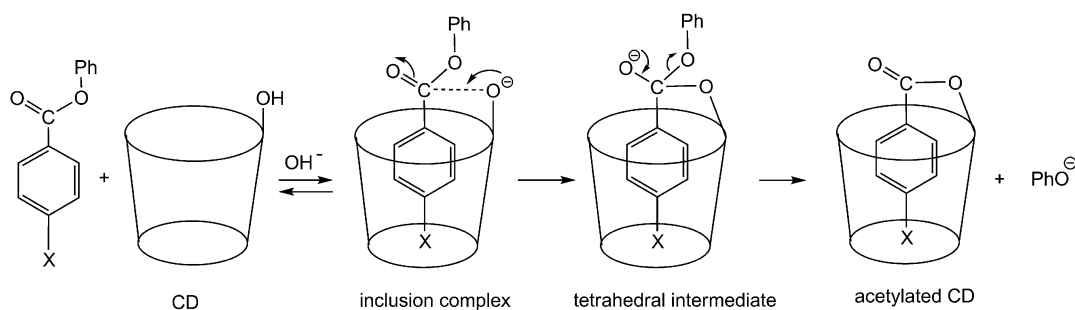
attacks the substrate to form a covalent tetrahedral intermediate, which subsequently decomposes into the acetylated CD and a phenoxide ion (Scheme 1). A rate increase has been observed for phenyl ester reactions (with respect to processes in bulk solvent) which is in sharp contrast with the inhibition reported for alkyl benzoates in basic medium.[5,9] The latter has been attributed to a non-productive binding of the complexed ester in which the carbonyl carbon remains far from the hydroxyl groups of CD or to an unfavourable partitioning of the tetrahedral intermediate.[7]

Posterior experimental studies on the CD-induced hydrolysis of various phenyl trifluoroacetate esters have shown that the reactivity of these processes can change depending on pH.[15–17] For instance, the hydrolysis of *p*-X phenyl trifluoroacetates (X = F, Cl, Br) is inhibited by CD at pH 6, but catalyzed in alkaline solution.[15] Interpretation of these findings has been made on the basis of a different nucleophilic reagent: water at pH ≤ 6 *vs.* the ionized –OH group of the CD in basic conditions. Direct attack by a free hydroxide ion, or by a water–hydroxide ion complex species (general base catalysis) has been invoked too.[17] Experimental studies of a related reaction, the benzoic anhydride hydrolysis in the presence of β-CD, have suggested the implication of at least two water molecules at pH between 3 and 6 (wherein a water molecule acts as nucleophile) and at pH 8 (wherein the general base and nucleophilic catalysis mechanisms should be operating).[19] Phenyl substituent[6,8,11,12,14] and chain length effects[11,13,17] of the substrate on the reactivity of ester hydrolysis mediated by CDs have also been addressed. For instance, the cleavage of *p*-nitrophenyl acetate is less efficient than that of *m*-nitrophenyl acetate due to a poorer transition state binding.[8] Hydrolysis of CF$_3$CF$_2$COOPh and CF$_3$(CF$_2$)$_2$COOPh esters is strongly inhibited both in acidic and basic media, while that of CF$_3$(CF$_2$)$_6$COOPh is strongly accelerated.[17] The replacement of

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† Electronic supplementary information (ESI) available: Cartesian coordinates of the optimized species in bulk water using the implicit model. Energetics using the implicit model in bulk water and in the water–CD environment. MM data for head-first (H-F) and tail-first (T-F) inclusion modes of the ester. See DOI: 10.1039/c0ob00058b

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Scheme 1 Suggested mechanism for ester hydrolysis in a CD at alkaline pH.

the water solvent by mixtures of water with other solvents like dimethylsulfoxide, acetonitrile or dimethylformamide can also change the reaction rate by modifying the binding strength of the substrate *vs.* that of the transition state with the CD.[13] The variation of the stability of the host–guest complex upon reaching the transition state has also been invoked to explain why phenyl acetates present a stronger catalysis than phenyl trifluoroacetates.[16]

Changes in hydrolysis rates have also been reported by varying the CD size.[11,13,14,18] A recent combined experimental and theoretical study on the hydrolysis of various S-phenyl thiobenzoates in the presence of α-, β- and γ-CDs has revealed that the largest catalytic effect is observed for γ-CD, which is probably due to hydrogen bond formation between the carboxyl oxygen of the ester with the secondary hydroxyl group of CD.[18]

From the theoretical point of view, much effort has been devoted to study inclusion complexes of CDs with a variety of compounds but only a few calculations, at empirical or semiempirical levels, have been done on chemical reactions. Some authors have tried to interpret the experimentally observed rate accelerations based on Molecular Mechanics[16,20] and Molecular Dynamics calculations[18] for CD–ester complexes and the corresponding acetylated CD derivatives. The semiempirical AM1 method[21] and the empirical valence bond approach,[22] have also been used to investigate alkaline hydrolysis and have provided interesting interpretations based on solvation and binding energy changes. It is also interesting to note that Molecular Mechanics calculations have been done to investigate the interaction modes between α-, β-, or γ-CD with several S-phenyl thiobenzoates, suggesting that the inclusion of the phenyl group bound to the carboxylate group is more favorable than that of the phenyl group attached to the sulfur atom.[18]

These theoretical analyses have been done using classical forcefields and/or simple quantum mechanical methods and therefore, the approximations assumed for the reaction paths and reaction energetics are probably too crude. In order to go beyond such qualitative considerations, achieve more precise interpretations of experimental facts and clarify the role of CD environments, high-level quantum mechanical calculations combined with statistical simulations are required. Moreover, previous works have focused on CD catalysis in alkaline media. However, as recently discussed for the hydrolysis of amides in bulk water,[23] a preliminary description of neutral hydrolysis mechanisms is an essential step towards the elucidation of catalytic effects, independently of the fact that even at neutral pH, acidic or basic catalysis cannot be excluded (see discussion in ref. 24 and references cited therein). Both remarks have motivated the present investigation to get a deeper insight into the role of β-CDs on ester hydrolysis considered

as a "chemical environment" rather than as an "active catalyst" and to analyze the differences exhibited by this environment with respect to bulk water. For this purpose, we report a detailed computational study, combining Quantum Chemistry, Molecular Mechanics and Molecular Dynamics methods, on the neutral hydrolysis of methyl *p*-nitrobenzoate ester inside an unsubstituted β-CD.

2.   Methodology

The hydrolysis of methyl *p*-nitrobenzoate has been studied in bulk water and in a β-CD macrocycle. For the reasons explained above, we assume neutral pH and we neglect the possible catalytic role of dissociated water or CD. Water and CD environments are modeled classically using either implicit or explicit methods described below. Based on literature studies,[25–27] two different hydrolysis reaction mechanisms have been envisaged, concerted and stepwise (addition-elimination mechanism). Moreover, non-assisted or water-assisted pathways can be postulated, depending on whether a single water molecule or a water dimer does react with the ester. Some experimental studies on neutral hydrolysis of esters in condensed phase have concluded that there is the participation of at least two water molecules,[28] and therefore we have assumed water-assisted hydrolysis in the present work. This choice is also supported by the comparison between theoretical and experimental data in the case of amides.[23] A graphic representation of the reaction mechanisms studied is shown in Scheme 2, which also presents the notation used below for relevant chemical structures.

2.1   Implicit model

In this case, the environment (water or CD) is assimilated to a polarizable dielectric continuum characterized by its static dielectric constant ε. The medium is polarized by the interaction with the solute's charge distribution and the resulting electric potential is included into the solute's Hamiltonian. This leads to the so-called self-consistent reaction field equations that allows to obtain the wave function of a solvated molecule.[29] Since their first application to the study of reactions in solution[30,31] continuum models have been much improved, and nowadays they represent a standard tool in theoretical chemistry. Applications to mimic host–guest interactions in a CD environment have been envisaged too. For instance, reported experimental[32] and theoretical[33] works have interpreted the modification of the reactivity of the guest in terms of the effective dielectric constant (ε_{eff}) of the medium surrounding the hydrophobic CD cavity. Values of ε_{eff} have been
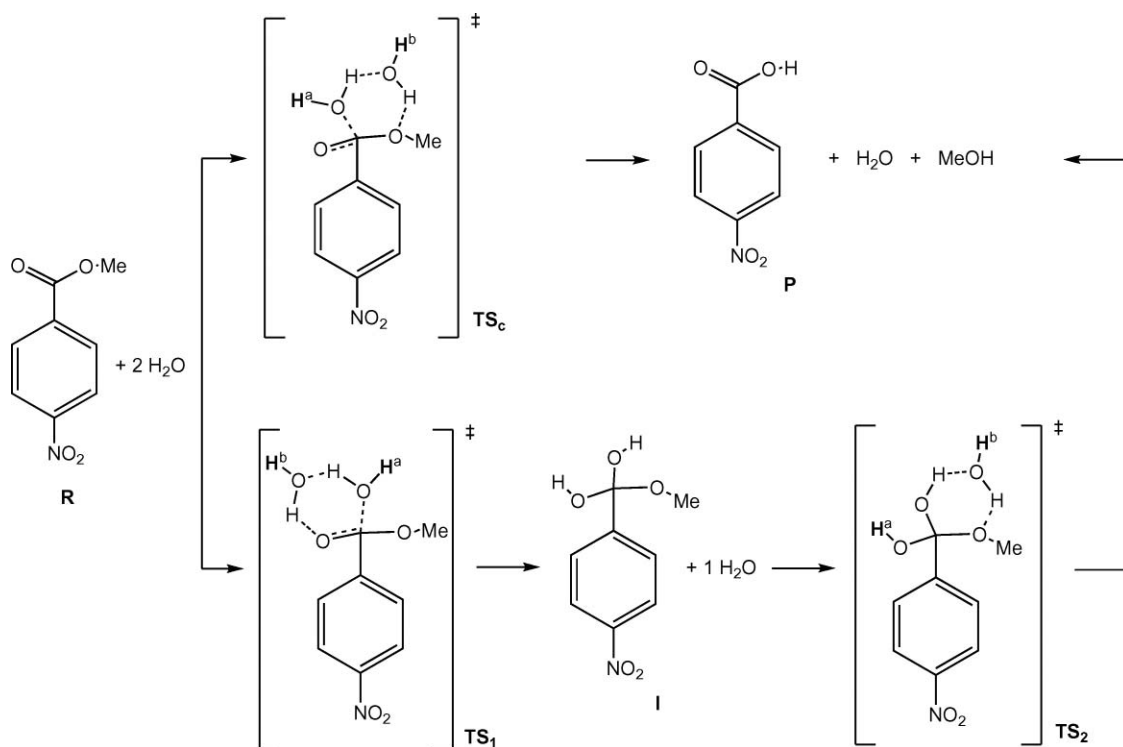
Scheme 2 Concerted (top) and stepwise (bottom) reaction mechanisms for water-assisted neutral hydrolysis of methyl *p*-nitrobenzoate. Notation used in this paper. The reaction is assumed to take place in bulk water or in the β-CD. In the latter case, the CD molecule does not form covalent bonds with the reactants and is not drawn for simplicity.

reported and vary in a wide range owing to the potential presence of solvent molecules in the cavity, which in turn depends on the difference between the inner diameter of the CD and the guest size.[34] In the case of β-CDs, values between 50 using fluorescence measurements[35] and 5[32] (or even 2.9[36]) have been proposed.

The Polarizable Continuum Model (PCM) approach of Tomasi and co-workers[37,38] together with the integral equation formalism model[38,39] and the united atom Hartree–Fock (UAHF) parametrization[40] have been used in the present work. Bulk water is characterized by its static dielectric constant of 78.39 while for the β-CD we have chosen low values of ε_{eff} (2 and 10) in order to get an upper bound of the effect. Following previous proposals,[23,27,28] the chemical system is initially composed of the ester interacting with two explicit water molecules. Gas phase optimizations are first carried out for the initial complex and for the stationary points along the reaction coordinate using standard algorithms.[41] The nature of these stationary points has been verified by analytical computations of harmonic vibrational frequencies. Further geometry relaxation has been done for the solvated systems although it leads to very small structural modifications. Frequency calculations in solution were attempted but in some cases two imaginary frequencies with small absolute values have been obtained (both for minima and transition structures). This problem seems to be connected with shortcomings in the computational algorithm for the second energy derivatives in the continuum and therefore we have preferred to use gas phase frequencies for determining thermal contributions to the free energy. Calculations have been carried out with the Gaussian03[42] program using the hybrid density functional method B3LYP[43] and the 6-31G(d) basis set.[44] The choice of this simple computational

level requires a few comments. First, it has been successfully used in similar hydrolysis reaction studies and differences with respect to higher computational levels have been analyzed before.[23,27] While it is not expected to provide very accurate energetics, it gives a good picture of the reaction mechanism thanks to cancellation of errors. Besides, in the case of the explicit model (see below) a large number of quantum mechanical calculations have to be done, so the computational level cannot be too high. Since we want to compare results obtained with both models, the same level has to be used. B3LYP/6-31G(d) calculations, therefore, represent a good compromise between accuracy and cost.

2.2 Explicit model

Another more realistic model has been employed to account for the microscopic structure of the medium. Water molecules and the β-CD macrocycle are described using a force-field from Molecular Mechanics (MM) and statistical averages are obtained through Molecular Dynamics (MD) simulations. The calculations are conducted as follows. First, MD simulations with a classical MM force-field are carried out for the chemical system at 300 K in a box of water molecules containing or not a β-CD molecule. Specific MM parameters have been developed for transition structures. Details on simulations are provided as ESI.†

From the MD simulations, 500 snapshot configurations are saved and for each saved configuration, a combined QM/MM calculation is carried out. In the QM/MM method, the chemical system is described quantum mechanically (B3LYP method[43]) while the environment is described classically (MM force field). In our case, the environment consists of all water molecules
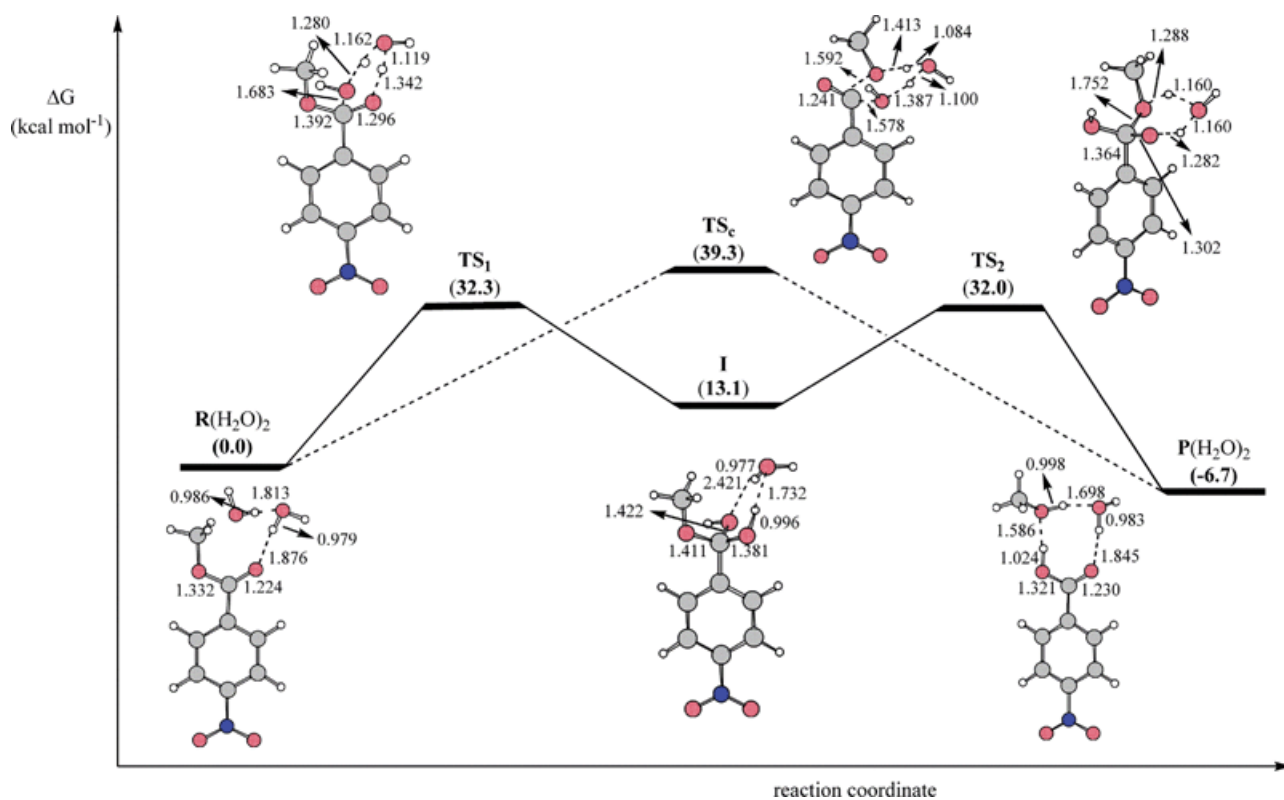
Fig. 1 B3LYP/6-31G(d) free energy profiles corresponding to the reaction mechanisms for the assisted neutral hydrolysis of methyl *p*-nitrobenzoate in water solution (implicit model) and optimized geometries of the species. Only the most relevant distances are given (Å).

within 9 Å of the centre of mass of the chemical system and, when present, the β-CD molecule. A QM calculation is carried out for the chemical system using a Hamiltonian that includes the electrostatic potential created by the point charges of the environment. Non-electrostatic interactions between the QM and MM subsystems are calculated by a Lennard-Jones potential using parameters from the classical force-field. Further details can be found in the original references.[45]

QM calculations have been done at the B3LYP/6-31G(d) level (see above). The Tinker[46] program has been used for MM and MD calculations and Gaussian 03[42] for the QM/MM calculations.

3. Results and discussion

The calculations for the hydrolysis reaction in bulk water are discussed first. We use in that case the implicit mode which, according to previous work for related reactions,[23] provides satisfactory values for activation free energies. Afterwards, we consider the reaction in a β-CD environment. Modification of the reactivity in going from bulk water to the β-CD is interpreted after the variation of the interaction energy of the chemical system with its environment. In this case, we use both the implicit and explicit models.

3.1 Reaction mechanism in bulk water

Ester hydrolysis mechanisms in water have already been discussed in the literature[26,27,47] and share common features with amide hydrolysis (see ref. 23,31 and references cited therein). Therefore, only a summary of main results will be presented below.

Fig. 1 displays the free energy profiles obtained for the concerted and stepwise mechanisms and a scheme of optimized structures. Table 1S and 2S in the ESI provide Cartesian coordinates and further details on energetics.† Energies are given relative to the ester–water reactant complex **R**(H₂O)₂, which is the appropriate choice in solution.[23] In this complex, the ester forms a hydrogen-bond with a water dimer (O···H distance 1.876 Å).

The concerted route in water has an energy barrier of 39.3 kcal mol⁻¹. In the associated TS (**TS$_c$**), the new C–O bond is almost formed (1.578 Å) and a proton has almost been completely transferred from the nucleophilic to the ancillary water molecule (1.099 Å). Since, in contrast, the ancillary water molecule has not yet transferred its proton to the leaving –OCH₃ group, **TS$_c$** exhibits an important charge separation with a positive charge located on a hydronium-like unit.

The energy barrier for the addition-step of the stepwise route, leading to the formation of the diol tetrahedral intermediate **I**, is 32.3 kcal mol⁻¹. In **TS$_1$**, the formation of the new C–O bond is just at the beginning (1.683 Å) and protons have been incompletely transferred so that charge separation is smaller in this case. The elimination step corresponds to the cleavage of the ester C–O bond with simultaneous formation of the methanol and *p*-nitrobenzoic acid moieties through **TS$_2$**, which lies 32.0 kcal mol⁻¹ above the reactant. In this TS, the original C–OMe bond is practically broken (distance 1.752 Å) and again, protons are less localized than in **TS$_c$**.

In summary, according to our results, the neutral ester hydrolysis in bulk water should proceed through a water-assisted stepwise mechanism involving an activation barrier of the order of 32.3 kcal mol⁻¹, which corresponds to a pseudo first-order kinetic constant of 3.2 × 10⁻⁸ s⁻¹ ($k' = k\cdot[H_2O]^2$ see ref. 23). These results are in line

Table 1 Relative free energies for relevant chemical species (with respect to the hydrated ester reactant, see Fig. 1) in the hydrolysis reaction using an implicit model. QM calculations at B3LYP/6-31G(d) level. For comparison purposes, relative free energies in water are also included. Units in kcal mol^{-1}

|	β-CD		Water
	$\varepsilon = 2$	$\varepsilon = 10$	$\varepsilon = 78.39$
TS$_c$	42.0	39.9	39.3
TS$_1$	32.4	32.4	32.3
I	14.8	13.8	13.1
TS$_2$	32.9	32.3	32.0
P(H$_2$O)$_2$	−8.6	−7.1	−6.7

with recent theoretical investigations on the neutral hydrolysis of ethyl acetate by Yamabe *et al.*[27] (these authors have shown that further water molecules might contribute to lower the activation energy, although the evaluation of the entropy using the harmonic approximation becomes less and less accurate). Besides this, our estimated kinetic constant is very close to the experimental value for phenyl acetate reported by Bowden *et al.*[48] (9.0×10^{-8} s^{-1} at 27 °C) although, as mentioned above, such an excellent agreement has to be considered as partially fortuitous.

3.2 Reaction mechanism in a β-CD

The chemical property changes of a solute in going from aqueous solution to β-CD environments have sometimes been attributed to the variation of the effective dielectric constant of the medium. To check the influence of polarization effects on ester hydrolysis, the energetics of the reaction have been calculated in a medium with dielectric constants $\varepsilon = 2,10$ and the relative free energies for the chemical species are summarized in Table 1. For the sake of clarity, we use the same geometries (those optimized in water) but we have checked that geometry relaxation produces marginal effects on the reported values. Details on solvation energies (for $\varepsilon = 10$) are given in Table 3S of the ESI.† As shown in Table 1, the free energy profiles change moderately with respect to the calculations in water (Fig. 1). The activation free energies increase a little while the reaction becomes slightly more exergonic. Hence, according to these results, the decrease of the dielectric constant of the environment should produce a moderate diminution of the ester hydrolysis rate.

In order to get a more realistic picture of the process within the β-CD, further calculations have been carried out using the explicit model. For comparison, the calculations have been carried out in aqueous solution and β-CD for the two mechanisms, concerted and stepwise. We shall focus on how interaction energies change from one medium to the other. Free energies of solvation have not been computed in this case since the required computational cost would be too large. For the same reason, we have not considered the reaction intermediate **I**, nor the product **P**, at this level. Since the reaction is supposed to be controlled kinetically, medium effects will mainly be connected to energy differences between the transition states and the reactants.

A preliminary question concerns the substrate inclusion mode, the two possible orientations head-first (**H-F**) and tail-first (**T-F**) being depicted in Scheme 3. Preference for the **H-F** mode in β-CD inclusion complexes has been reported for *m*- and *p*-nitrophenyl alkanoates using MM and MD calculations[49] and in

head-first (**H-F**)   *tail-first* (**T-F**)
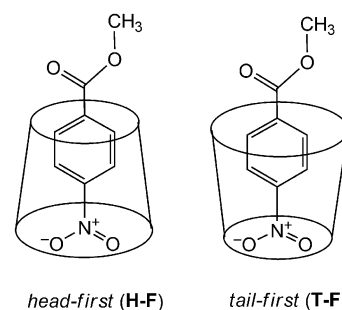
Scheme 3 Schematic representation of ester CD inclusion modes.

α-CD inclusion complexes for several benzoic acids using the AM1 method.[50] Calculations in the present work for the ester reactant do also support a slight preference for the **H-F** mode (ESI, Table 4S†). Therefore, we have only considered **H-F** complexes below.

Average interaction energies between the chemical system and the medium using the explicit model are summarized in Table 2. In going from water to β-CD, the electrostatic energy decreases in absolute value, while the non-electrostatic energy increases, consistent with the fact that the medium becomes partially hydrophobic. The loss of electrostatic stabilization is in all cases overcompensated by the increase in van der Waals stabilization. Hence, for all systems (reactant and TSs), the total interaction energies are larger in β-CD than in bulk water. Overall, the interaction energy modification Δ(β-CD − water) displays a similar order of magnitude for the reactant and for two of the TSs, namely **TS$_c$** and **TS$_1$**: it amounts about +3 to +5 kcal mol^{-1} for the electrostatic term, and roughly −12 to −13 kcal mol^{-1} for the non-electrostatic one. The situation is quite different for **TS$_2$**, which undergoes much larger modifications: the electrostatic energy lost under formation of the host–guest complex reaches +13.4 kcal mol^{-1} while the gain in van der Waals energy amounts −17.3 kcal mol^{-1}. The reasons explaining this specific behavior of **TS$_2$** will be analyzed below. Values in Table 2 do also show that, under encapsulation, **TS$_2$** is less stabilized than the reactant **R** by about 4.0 kcal mol^{-1}, whereas **TS$_c$** is more stabilized than **R** by about 2.6 kcal mol^{-1}. Thus, in contrast to the implicit model results, the explicit model calculations predict that in going from water to β-CD, the stepwise mechanism should be penalized, whereas the concerted one should be favored. This result provides a plausible explanation for the experimental observations at neutral pH. In general, moderate inhibition should be observed as also predicted with the implicit model calculations above. Nevertheless, acceleration of the ester hydrolysis can happen in the case of a derivative for which the magnitude of the medium effect would be particularly marked. In such a case, a change of mechanism should be in play, from stepwise in water to concerted in β-CD. Clearly, this last result cannot be predicted in terms of dielectric effects only and it has to be ascribed to molecular shape recognition by the CD cavity, as we are now going to discuss.

3.3 β-CD-recognition: non-specific interactions *vs.* molecular shape effects

According to the preceding results, it appears that accounting for the anisotropy of the molecular surrounding (or more precisely, its microscopic structure) may be fundamental to correctly describe reactions in molecular reactors such as CDs. To further investigate

Table 2 Average electrostatic (Elec) and van der Waals (vdW) interaction energies from QM/MM calculations for the studied hydrolysis reaction in water solution and in the β-CD using the explicit model. QM calculations at B3LYP/6-31G(d) theory level. Units in kcal mol^{-1}. Standard deviations are given in parentheses

	Water			β-CD			Δ(β-CD − water)		
	Elec	vdW	Tot	Elec	vdW	Tot	Elec	vdW	Tot
R	−19.9 (±4.1)	−15.9 (±2.2)	−35.8 (±4.1)	−15.5 (± 4.0)	−28.3 (± 2.2)	−43.7 (± 3.9)	4.4	−12.4	−7.9
TS$_c$	−48.0 (±6.5)	−12.2 (±3.5)	−60.2 (±5.3)	−45.3 (±6.9)	−25.4 (± 3.4)	−70.7 (± 6.1)	2.7	−13.2	−10.5
TS$_1$	−39.8 (±6.0)	−14.2 (±3.2)	−54.0 (±4.8)	−35.2 (±7.9)	−27.6 (±3.1)	−62.8 (±6.8)	4.6	−13.4	−8.8
TS$_2$	−44.5 (±6.8)	−12.8 (±3.3)	−57.3 (±5.6)	−31.1 (±6.8)	−30.1 (± 3.2)	−61.2 (± 5.3)	13.4	−17.3	−3.9

Table 3 Change in average interaction energies with the environment in going from bulk water to aqueous β-CD. MM force-field calculations using the same structures as those used for QM/MM calculations in Table 2. Units in kcal mol^{-1}

	Bulk water → aqueous β-CD					
	Electrostatic term			van der Waals term		
	With water	With CD	Total	With water	With CD	Total
R	+7.4	−2.8	+4.6	+9.3	−21.7	−12.4
TS$_c$	+12.0	−10.8	+1.2	+8.7	−21.9	−13.2
TS$_1$	+13.5	−8.6	+4.9	+9.5	−22.9	−13.4
TS$_2$	+19.2	−4.9	+14.3	+6.7	−24.0	−17.3

the role of the macrocavity, it is interesting to analyze the interaction energy differences Δ(β-CD − water) by extracting the contributions that come from the macromolecule and the solvent. Formally, within the QM/MM approach, this is possible for the van der Waals component (additive energies) but not for the electrostatic one (non-additive character). Hence, for this qualitative analysis we shall make use of the MM energies calculated along the MD simulation. The values are summarized in Table 3 (note that total electrostatic contributions are close to QM/MM values in Table 2; obviously, total non-electrostatic contributions are the same as the QM/MM quantities in Table 2). The following remarks can be made:

- All the species undergo a noticeable loss of interaction energy with water solvent (both electrostatic and van der Waals) after encapsulation in CD.

- The electrostatic energy loss is particularly high for **TS$_2$** (19.2 kcal mol^{-1}).

- The guest–CD electrostatic interactions do not compensate the loss in guest–water interactions; among the three TSs, **TS$_2$** exhibits the lowest guest–CD electrostatic interaction (−4.9 kcal mol^{-1}).

- The guest–CD van der Waals interactions are large and overcompensate the decrease of guest–water van der Waals interactions.

The most striking result in the above analysis is probably the huge difference of the electrostatic solvation energy for **TS$_2$** when one compares the reaction in bulk water or in the β-CD. Since this cannot be explained in terms of the effective dielectric constant, one would be tempted to invoke the modification of the hydrogen-bond network. The relevant radial distributions functions (RDFs) for the three TSs are plotted in Fig. 2. We focus on RDFs for the strongest hydrogen-bonds, *i.e.* H(TS)–O(water), where H

represents one of the two hydrogen atoms of the reactive water molecules not directly involved in the reaction coordinate and therefore available for H-bonding (H$_a$ and H$_b$ in Scheme 2). The RDFs exhibit a well-defined peak slightly below 2 Å with a higher intensity for H. When one compares the RDF first peaks in bulk water and aqueous β-CD, the main differences are found for the H$_a$(TS)–O(water) interaction in **TS$_1$** and to a lower extent for the H$_b$(TS)–O(water) one in **TS$_c$**. Analysis of the CD simulations shows that in both cases, the corresponding H atom is involved in a hydrogen-bond with hydroxyl groups in the upper CD rim. For **TS$_2$**, no significant differences are apparent for the RDF first peak but one observes a strong modification of the RDF shape beyond that peak, *i.e.* in the region 3–6 Å. The curves are significantly less intense in the β-CD suggesting that an effective desolvation happens beyond the first shell.

The analysis of the MD trajectory provides further clarifications. In Fig. 3, we represent the position of the TSs with respect to the center of mass of the macrocycle as a function of time, and in Fig. 4 typical snapshots are drawn. The upper and lower points in Fig. 3 represent, respectively, the position of the C(carbonyl) and N(nitro) atoms directly attached to the phenyl group. In the case of **TS$_c$** and **TS$_1$**, these atoms occupy a more or less symmetric position with respect to the center of mass of the cavity. Therefore, both the ester and nitro groups are able to interact with water but also with the hydroxyl groups of the cavity. **TS$_2$**, instead, occupies the bottom part of the macrocavity. In this case, the nitro group lies clearly outside the cavity and is quite mobile, while the reactive center is buried in the cavity. In spite of this position (see snapshot in Fig. 4), two water molecules included in the cavity solvate the reactive center and explain the results for the RDFs discussed above.
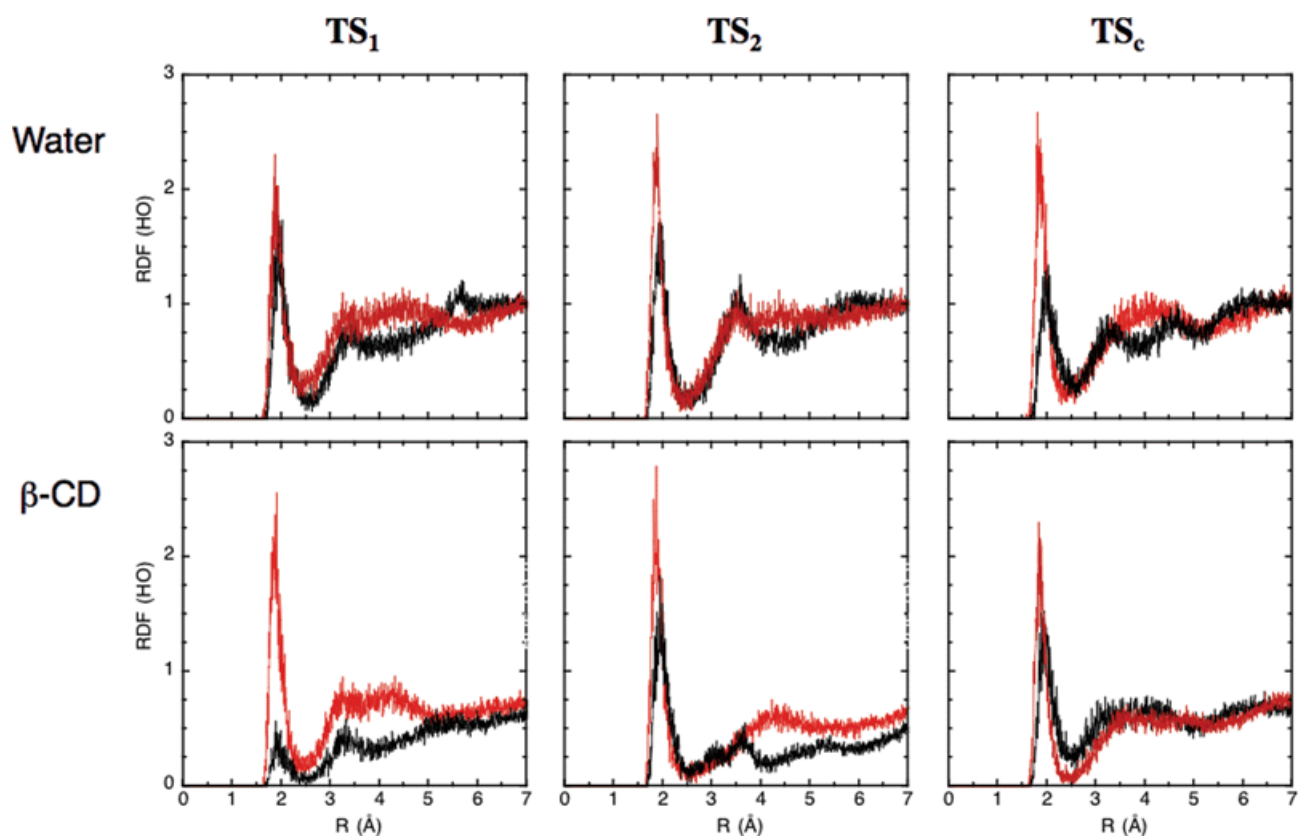
Fig. 2 HO radial distribution functions for hydrogen-bonds from MD simulations. O is the oxygen atom of solvent water molecules. H represents one of the two hydrogen atoms of the reactive water molecules not directly involved in the reaction coordinate, *i.e.* H_a (black graph) or H_b (red graph) in Scheme 2.
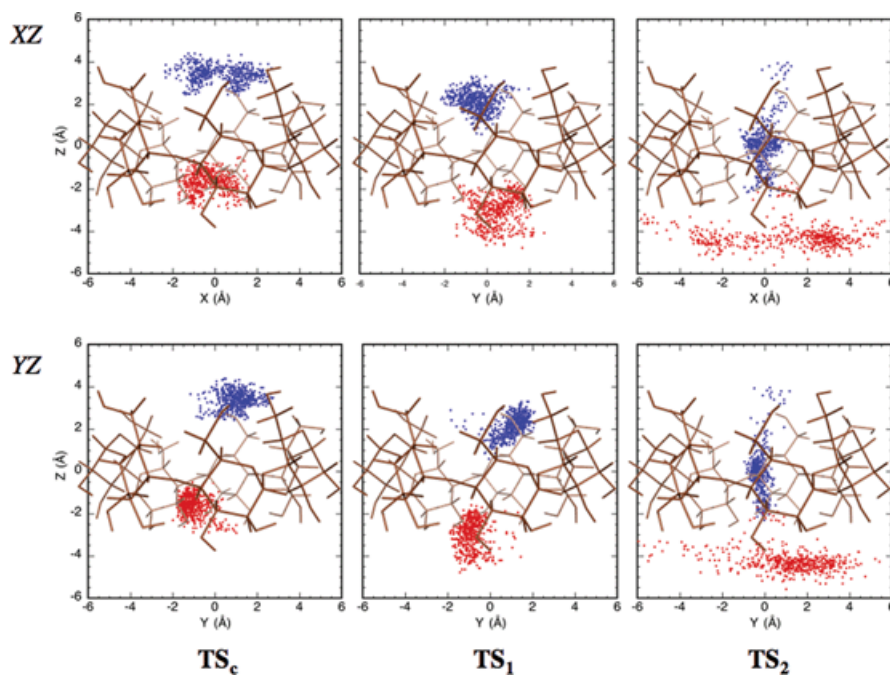

Fig. 3 Analysis of MD trajectories for the TSs in aqueous β-CD. The points represent the instantaneous position of C(carbonyl) and N(nitro) atoms directly bonded to the phenyl ring with respect to the center of mass of the macrocavity. XZ and YZ planes are represented. One arbitrary CD structure is drawn for clarity.
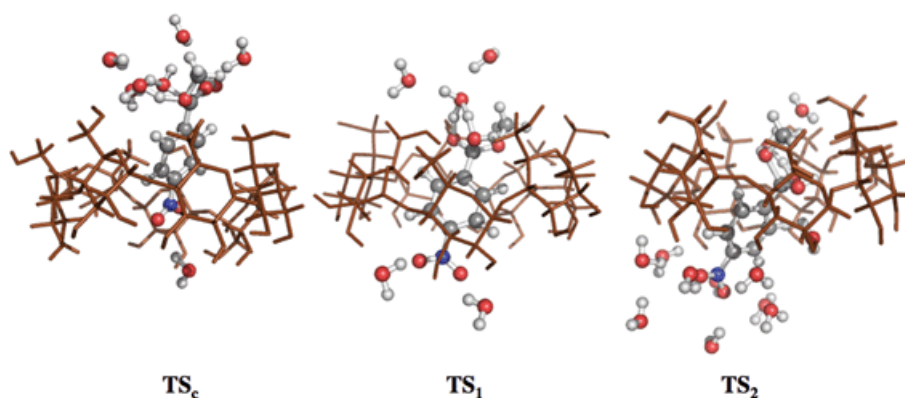
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Fig. 4 Typical snapshots from the MD simulations of the TSs in aqueous β-CD. The cavity, the substrate and some interacting water molecules in the solvation shell are represented.
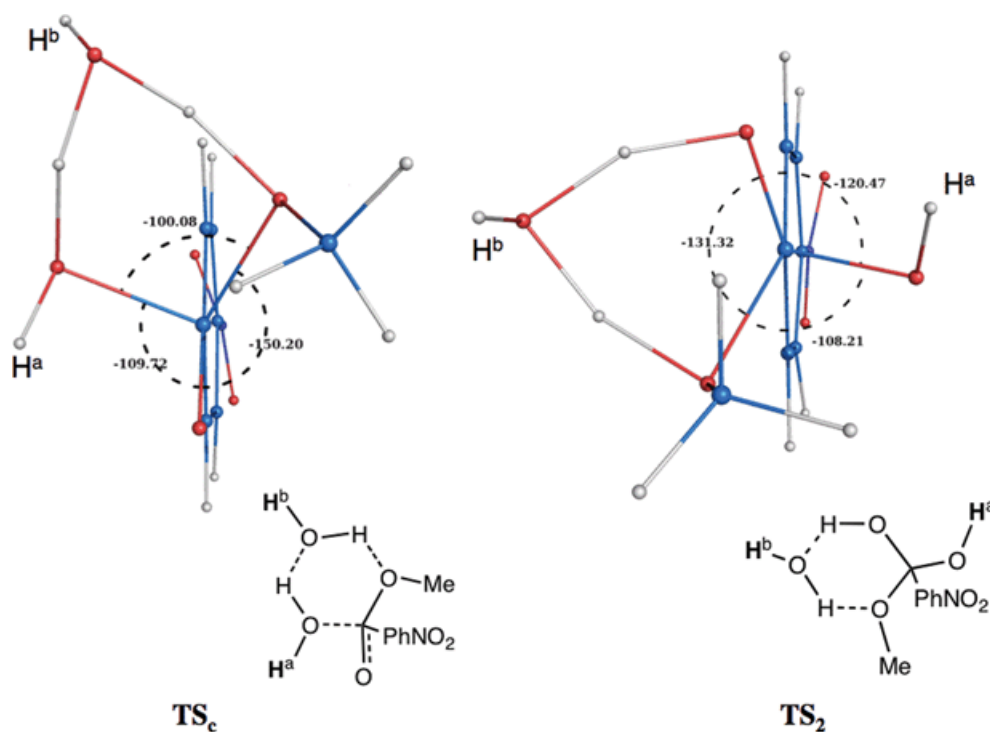

Fig. 5 Typical 3D structures of **TS$_c$** and **TS$_2$** from MD simulation snapshots (angles in degrees).

In summary, the hydrogen-bonding ability of the three transition structures does not appear to be a main factor explaining the noticeable structural differences found for the complexes with the β-CD. Such differences can only be explained by the specific 3D arrangement of the three TSs. To illustrate this point, Fig. 5 compares typical geometries of **TS$_c$** and **TS$_2$** from the simulation. The relative position of H_a and H_b, the orientation of the six-membered ring that defines the reaction coordinate with respect to the phenyl ring, as well as the tetrahedral character of the C(carbonyl) atom (see dihedral angles) exhibit clear disparities. They influence the interaction mode between the guest and the host, and *in fine* the degree of stabilization by the macrocavity of the transition structures.

4. Conclusions

In this work, we have carried out a theoretical investigation of neutral ester hydrolysis in the presence of β-CD as a representative case of reactions occurring in chemical reactors. A computational approach based on the combination of several techniques, QM, MM and MD, has been proposed. The results demonstrate that the change in chemical environment, from bulk water to the macrocycle, may produce significant modifications on reaction rates and mechanisms. Part of the predicted effects can be ascribed to modifications of the effective dielectric constant felt by the chemical system, but analysis of the calculations also shows that host–guest molecular recognition plays a major role. In fact, the

interaction in the host–guest complex strongly depends on the spatial arrangement of the guest, not only on its global polarity and/or polarizability. In other words, the role of the macrocavity can be compared to that played by the active site of an enzyme.

For the specific reaction studied here (the neutral hydrolysis of *p*-nitromethylbenzoate), we predict a rate constant of about 10^{-8} s^{-1} in bulk water, very close to other theoretical results and experimental data for similar systems. The reaction proceeds through a stepwise water-assisted mechanism. In going from water to β-CD, provided one assumes the same reaction mechanism, inhibition is predicted. This is consistent with most experimental findings for ester hydrolysis at neutral or closely neutral pH. Nevertheless, acceleration has been experimentally observed for some esters in similar pH conditions. Our calculations suggest that in this case a change of mechanism comes into play. Actually, in contrast to the stepwise mechanism of the hydrolysis process, the concerted one is favored by β-CD encapsulation of the ester reactant.

Acknowledgements

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