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Binding kinetics calculations of the inclusion of some
benzoic acid derivatives and paracetamol in β CD.

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ABSTRACT

This theoretical study is designed to identify the main driving forces of the inclusion of some benzoic acid (BA) derivatives as well as Paracetamol (ParCM) with β -cyclodextrin (β -CD) by computing the kinetics of binding. Unbiased molecular dynamics simulations were carried out. The guest molecules were used in ionized and non-ionized states. Based on the computed values of the dissociation rate constant k_{off} , the hydrophobic interactions were discussed. The results show that the more hydrophobic molecule in the non-ionized state give most stable complexes; whereas in the ionized state, less hydrophobic molecules give most stable complexes, meaning a clear charge effect. The low percentage of time of observed H-bonds during the simulations is an indication that ones may play only a secondary role in the stability of the non-ionized inclusion complexes, and they don't play any role in the stability of the ionized complexes. The comparison between the obtained results and those obtained experimentally gives a reasonably agreement.

1. INTRODUCTION

Cyclodextrin is an excellent model for the molecular recognition because of its structure, with a hydrophobic cavity, may include various small molecules. The guest molecules' chemical properties such as solubility, stability and biological activity can change due to its complexation with a cyclodextrin. This technique of encapsulation has become very popular and is being successfully used in different fields, chemistry, biology, nano chemistry, agro chemistry and industry [1-5].

The ease of the inclusion depends on various factors in particular the hydrophobicity and hydrogen bonding [6-8]. The ionized state of the guest compound has also a significant effect on the complexation and can considerably affect the stability of the inclusion complexes (ICs) [9-11].

The molecular binding of the ICs can be characterized through thermodynamic or kinetic properties. Thus, the thermodynamic properties are usually obtained by determining the energy profile of the complexation using biased molecular dynamic simulation method and the kinetic properties by unbiased molecular dynamics simulation method. These methods are becoming increasingly prominent for studying molecular binding as well as for determining the efficiency of a drug [12-13].

In this article we present the computed results on the kinetics of binding of the inclusion of some BA derivatives as well as ParCM in order to give a better understanding of the complexation and its binding mechanism. For this purpose, we computed the kinetic properties using unbiased molecular dynamic simulations. The choice of BA derivatives as guest molecules is justified by the fact that they have suitable structural features to be encapsulated in β -CD, and also by the number of the experimental results related to their ICs [6, 9-12, 14-19]. To note that ParCM is a more hydrophilic compound, and was also used as guest molecule in order to compare its kinetic behavior to those of BA derivatives.

METHODS

The initial structure of β -CD was built with the help of the graphical interface of ChemBioOffice (version 10, Cambridge software) [20], and the geometry of guest molecules was extracted from a PubChem Compound Database [21] (Figure 1).

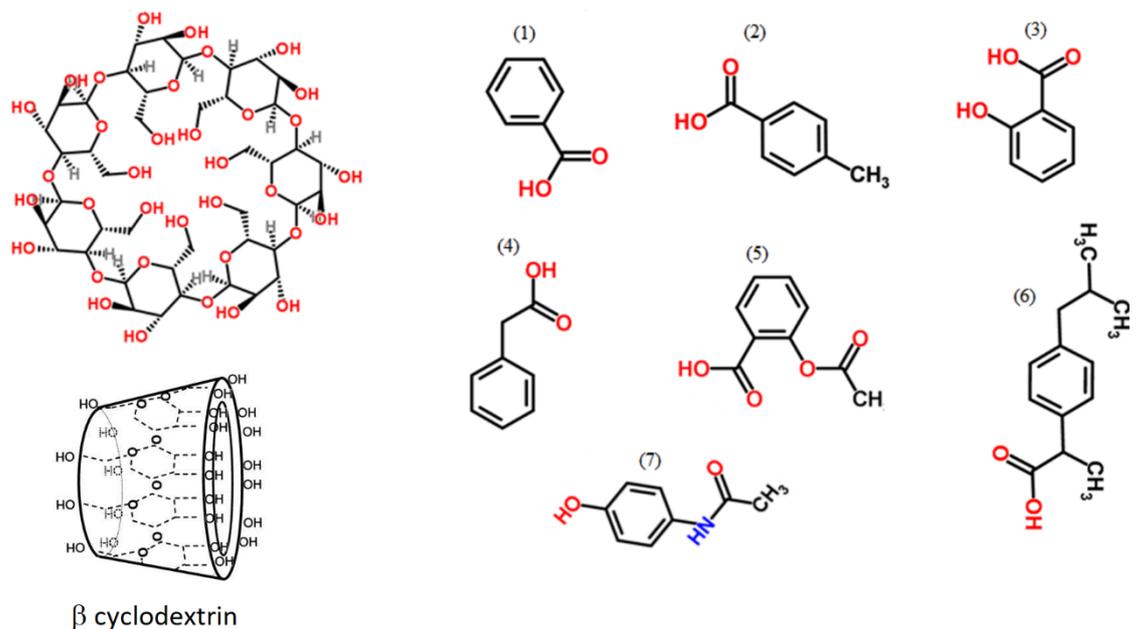


Figure 1. β -CD and the 7 guest molecular structures, (1) benzoic acid (BA), (2) toluic acid (TA), (3) salicylic acid (SA), (4) phenyl acetic acid (PAA), (5) aspirin (ASP), (6) ibuprofen (IBU) and (7) paracetamol (ParCM)

First, β -CD is fixed in the center of XYZ coordinate system. Then, the ionized or non-ionized guest molecule is placed to a distance greater than 10 Å from the center of β -CD (Figure 1).

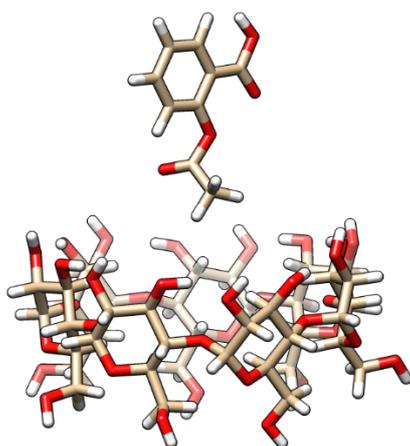


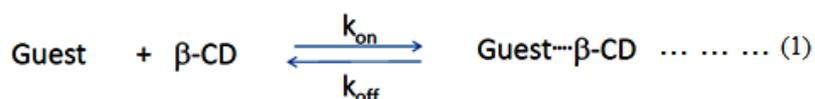
Figure 2. The initial geometry at the beginning of the simulation (ionized ASP with β -CD).

The force field parameters for both molecules were generated using the automated topology builder (ATB) [22] in the framework of GROMOS96 54A7 force field [23].

All simulations were carried out using the GROMACS package version 5.1.2 into an identical box containing 2380 water molecules represented by a simple point charge (SPC) model [24, 25]. The simulations were performed in the isobaric-isothermal ensemble (NPT) with a constant pressure of 1 bar and a temperature of 298 K controlled by the Berendsen thermostat. The electrostatic interactions were treated by the particle mesh Ewald (PME) method with a cutoff distance of 1.2 nm and the van der Waals interactions were modeled using a cutoff of 1.4 nm. The whole system was relaxed using the steepest descent algorithm. The position of the guest molecule is obviously altered after the equilibration steps. The classical Newton's equations of motion were integrated using the Leap-Frog algorithm and the Berendsen weak-coupling method. All simulations were carried out for 200 ns, which is long enough for the system to reach equilibrium. The trajectory coordinates were recorded every 500 steps with a time step of 1 fs. The lifetime inside β -CD cavity is quantified by inspecting the distance between the centroid of β -CD and that of guest molecule during the simulations.

To ensure that we have a correct canonical ensembles as well as correct structural and thermodynamic properties we used in a second step, the Langevin thermostat in addition of a more sophisticated water model, TIP3P.

The inclusion complex can be represented as a simple two-state model for guest reaction with β -CD.



The kinetic are assumed to be first-order with the association and dissociation rate constants k_{on} and k_{off} which can be evaluated as follows:

$$k_{\text{on}} = \frac{1}{\text{average unbound time} \times [\text{solute}]} \dots \dots \dots (2)$$

and,

$$k_{\text{off}} = \frac{1}{\text{average bound time}} \dots \dots \dots (3)$$

The thermodynamic and kinetic behaviors are related by the equation:

$$K = \frac{k_{on}}{k_{off}} \dots \dots \dots (4)$$

The binding free energy can be obtained from the constant K that:

$$\Delta G^\circ = -RT \ln K \dots \dots \dots (5)$$

RESULTS AND DISCUSSION

The values of the association and dissociation constant rate k_{on} and k_{off} , the equilibrium constant K and the binding standard free energy ΔG° of the ICs (ionized and non-ionized) were calculated using eq. 2, 3, 4 and 5. The results are shown in Table 1 and Table 2. Also, these tables include data extracted from the literature, obtained either by experimental methods or by molecular dynamics calculations. Note that all values in brackets correspond to those obtained for the simulations under the conditions of a TIP3P water model and a Langevin thermostat.

The more negative free standard energy gives the more thermodynamically favorable inclusion complex. The results summarized in Table 1 and 2 indicate clearly that all the molecules used, in both states, can form stable ICs with β -CD ($\Delta G^\circ < 0$).

Non-ionized state

Firstly, the MD simulations of the non-ionized ICs using SPC water model and the Berendsen thermostat were divided into three groups according to the guest molecule behavior during the simulation. The first group involves four BA derivatives: PAA, BA, ASP and IBU. These guest molecules once they penetrated inside the cavity, will stay there until the end of the simulation. The obtained complexes are stable giving the same computed values of the kinetics and thermodynamics parameters. Thus, ΔG° and K are respectively equal to -4.05 kcal/mol and $9.0 \cdot 10^2$ corresponding to a dissociation constant rate value, k_{off} , equal to $5.26 \cdot 10^6 \text{ s}^{-1}$. To note, that k_{off} values are the lowest ones, this is an indication that the hydrophobicity is larger. Accordingly, the guest molecules with a larger hydrophobicity give the more stable complexes.

Table 1. The rate association and dissociation constants k_{on} and k_{off} and thermodynamic parameters K , pK_a and ΔG° of the ICs in the non ionized states.

	$k_{off}(s^{-1})$ 10^6	$k_{on}(s^{-1}M^{-1})$ 10^9	$K(M^{-1})$ 10^2	ΔG° This study	ΔG° From literature
SA	5.43 [5.27]	2.97 [4.65]	5.47 [8.62]	-3.76 [-4.03]	-3.59 ⁽⁹⁾ ; -2.85 ⁽¹⁵⁾ ; -3.96 ⁽⁶⁾
TA	5.40 [5.19]	3.16 [6.32]	5.85 [12.18]	-3.79 [-4.23]	N/A
PAA	5.26 [5.18]	4.74 [6.78]	9.00 [13.09]	-4.05 [-4.27]	-2.82 ⁽⁹⁾
BA	5.26 [5.39]	4.74 [3.22]	9.00 [5.97]	-4.05 [-3.81]	-3.77 ⁽⁹⁾ ; -3.6 ⁽¹⁸⁾ ; -2.79 ⁽¹⁴⁾
ASP	5.26 [5.32]	4.74 [3.95]	9.00 [7.42]	-4.05 [-3.94]	-3.74 ⁽¹⁰⁾ ; -3.19 ⁽⁹⁾ ; -2.98 ⁽⁹⁾ ; -2.27 ⁽¹²⁾ ; GAFF6-CD; -4.11 ⁽¹²⁾ ; q4MD-CD
IBU	5.26 [5.15]	4.74 [8.01]	9.00 [15.55]	-4.05 [-4.38]	-4.28 ⁽⁹⁾ ; -6.45 ⁽¹⁵⁾
ParCM	6.00 [5.82]	1.35 [2.90]	2.25 [2.90]	-3.2 [-3.37]	-2.98 ⁽¹⁶⁾

The second group consists of SA and TA. During the simulation, these molecules are easily encapsulated inside the cavity staying until the end of the simulation. But sometimes, the guest molecules leave the cavity for short periods. A small difference is observed between the values of the free energies ΔG° of both complexes as well as between the values of the equilibrium constants K . The values of ΔG° are equal to -3.76 (SA- β -CD) and -3.79 (TA- β -CD) kcal/mol and the values of K are equal to $5.47 \cdot 10^2$ (SA- β -CD) and $5.85 \cdot 10^2$ (TA- β -CD).

However, the values of k_{off} reported in Table 1 shows a higher values, equal to $5.43 \cdot 10^6$ (SA- β -CD) and $5.40 \cdot 10^6$ (TA- β -CD) compared to those obtained in the first group unveils to a lower hydrophobicity, leading in consequence to a slightly less stable ICs.

Finally, the last group is composed of only one molecule, ParCM. In this case, ParCM gives also a complex with β -CD but sometimes leaves the cavity, staying much longer periods outside compared to the guest molecules of the second group. The computed value of k_{off} is much higher and equal to $6.0 \cdot 10^2 \text{ s}^{-1}$ indicating that ParCM is the least hydrophobic. This leads to the least stable IC characterized by a free energy ΔG° of -3.2 kcal/mol and a equilibrium constant of $2.25 \cdot 10^2$.

However a second time, when using the Langevin thermostat and the TIP3P water model, the behavior of the non-ionized ICs is becoming slightly different for TA and SA; this time once they have penetrated inside the cavity, they do not leave it anymore unlike previous simulations where they came out from time to time. In other words all benzoic acids, PAA,

BA, ASP, IBU, SA and TA behave the same way in these conditions. However, in the ParCM: β -CD complex, the ParCM behaves the same way, i.e. it goes in and out the cavity several times during the MD simulation.

Comparing the values of ΔG° in the first and second MD simulations, we observe an average difference of 0.25 kcal/mol corresponding certainly of the necessary fitting obtained by using the Langevin thermostat and the TIP3P water model, to make the results more accurate.

The ionized state

In this section we describe and discuss the MD simulations of the ICs of five BA derivatives in their ionized state TA, SA, PAA, BA and ASP with β -CD.

Table 2. The rate association and dissociation constants k_{on} and k_{off} and thermodynamic parameters K , pK_a and ΔG° of the ICs in the ionized states.

	$k_{off}(s^{-1})$ 10^6	$k_{on}(s^{-1}M^{-1})$ 10^9	$K(M^{-1})$ 10^2	ΔG° This study	ΔG° From literature
SA	6.71 [6.9]	0.93[0.86]	1.38 [1.24]	-2.93 [-2.86]	-2.77 ⁽⁹⁾ ; -2.68 ⁽⁶⁾
TA	5.71 [5.64]	1.90 [2.06]	3.32 [3.65]	-3.45 [-3.51]	N/A
PAA	7.76 [6.22]	0.67 [1.21]	0.86 [1.95]	-2.65 [-3.14]	N/A
BA	10.41 [8.1]	0.46 [0.61]	0.44 [0.75]	-2.25 [-2.53]	-1.4 ⁽¹⁸⁾ ; -2.37 ⁽⁹⁾
ASP	12.82 [8.6]	0.39 [0.56]	0.30 [0.56]	-2.02 [-2.39]	-2.34 ⁽¹⁰⁾

As it was expected, the equilibrium constant decreased significantly in the ICs of PAA, BA and ASP compared to the values of their corresponding non-ionized ICs; it is approximately 17, 21 and 31 times smaller respectively, while they are only 1.5 and 4 times smaller for TA and SA respectively.

Finally, it seemed interesting to establish a relationship between the hydrophobicity and the stability of the ionized ICs based on the values of k_{off} and K . Thus, from Table 1 the computed values K can be ordered according to: $K_{ASP} < K_{BA} < K_{PAA} < K_{SA} < K_{TA}$ and the computed values k_{off} according to: $k_{off_{ASP}} > k_{off_{BA}} > k_{off_{PAA}} > k_{off_{SA}} > k_{off_{TA}}$. This demonstrates that the less hydrophobic guest molecule gives the more stable complex on the contrary of the general rule of the formation of the ICs and with respect to our results obtained in the non-ionized state. This means that a clear charge effect in the guest molecules probably makes them less hydrophobic, and this is reflected in the process of their separation from the β -CD. Consequently, we can admit that the hydrophobic interaction is not the most

determining factor in the formation of these ICs, instead, the effect of charge which becomes the most important factor.

When using the Langevin thermostat and TIP3P water model in the simulations the behavior of the ionic ICs did not change, i.e. the invited molecules leaves the cavity several times during the simulation; deducing then the same relationship between the hydrophobicity and the stability of the ionized ICs based on the values of k_{off} and K : $K_{ASP} < K_{BA} < K_{PAA} < K_{SA} < K_{TA}$ and the computed values k_{off} according to: $k_{off_{ASP}} > k_{off_{BA}} > k_{off_{PAA}} > k_{off_{SA}} > k_{off_{TA}}$.

It should be noted that although, we observe an average difference of 0.25 kcal/mol in the calculated values of ΔG° between the two simulations, this has not affected the behavior of the guest molecules in this case.

The experimental values listed in Table 1 and Table 2 have been obtained using different technic, the potentiometric measurement [6], the simultaneous measurement of absorbance and circular dichroism [9], the ultrasonic relaxation [10], the low frequency ultrasonic relaxation [14] thermodynamics [15], UV-Visible [16] and the microcalorimetry [18]. Also two theoretical results obtained by Chang et al are cited, they use two different forces fields for MD simulation of Asp: β -CD (12). As it can be seen the data listed are scattered. They are, of because of the methods used using are different, or by the fact that these experiments were conducted in different measurement environments (pH, T, solvent). In addition, they are not complete and do not concern all of our examples. Hence, it is not straightforward to compare our results to those obtained experimentally in a fair and informative manner.

Taking into account these facts we can say that our results are overall more or less in line with those obtained experimentally in particular those obtained by Dehab et al [9] except in the ASP: β -CD and PAA: β -CD complexes which the difference in the ΔG° values was found slightly significant, equal respectively to 0.86 [0,75] and 1.23 [1.45] kcal/mol.

It also seemed to us that, despite its well-known limitations, using the Berendsen thermostat and the SPC water model in MD simulation may provide with a good approximation a satisfactory results, as shown the results on Table 1 and Table 2.

Hydrogen bonding

Here, we shall focus our main attention on the formation of the H-bonds between β -CD and guest molecules in both states throughout the simulation [26]. A hydrogen bond is considered

as formed during the simulation when the donor-acceptor distance is less than or equal to 3.0 Å and an angle between donor-hydrogen-acceptor is greater than or equal to 120°.

In Table 2 are displayed the distribution of the number of H-bonds observed between the guest molecules in both states and β CD during the simulation period.

Table 2. The distribution of the number of H bonds observed during the simulation

		Percentage of time (%)	
		Number of H-bond	
Compound	State	0	1
BA	non-ionized	96.5 [94]	3.5 [4]
	Ionized	96 [92]	3 [7]
TA	non-ionized	87 [94.5]	20.5 [3]
	Ionized	79 [83]	12 [15]
SA	non-ionized	88 [91]	11.5 [7]
	Ionized	88 [77]	11 [19]
PAA	non-ionized	92 [86]	8 [10]
	ionized	88 [81]	12 [17]
ASP	non-ionized	78 [72]	20 [25]
	ionized	98.5 [95]	1.5 [3]
IBU	non-ionized	88 [92]	11 [5]
ParCM	non-ionized	69 [61]	30 [35]

The distribution (in %) of the number of H bonds observed during the simulation given in Table 2, provides a general trend of non-affinity to the establishment of H-bonds except with ParCM which gives a moderate rate, about 30% [35 %]. In other words, during about 69 [61 %] to 98% [95 %] of the time of simulations the guest molecules do not establish any hydrogen bond with β CD. Thus, in the two states of the ICs of BA and SA, the same distribution of one H-bond were observed; they are around of 3% [4 %] for the β-CD-BA complex and of 11% [7 %] for the β-CD-SA complex. However, slightly different rates are detected in the ICs of PAA and TA in which the non-ionized complexes exhibit a larger proportion of one H-bond than in the ionized complexes of 4% [7 %] (β-CD-PAA complex) and of 7.5% [12 %] (β-CD-TA complex). This difference is more pronounced in the case of β-CD-ASP complex where the non-ionized complex gives a percentage of 20% [25 %] while the ionized state gives only 1.5% [3 %]. Lastly, IBU give 11% [5 %] of one H-bonds.

It is obvious that the small rate of the ionized ICs, can be explained by the fact that the ionized molecule spent much more time in aqueous medium away from the β-CD leading to less opportunity for establishing H-bonds. However, the hydrophobic interactions in the non-ionized ICs are more significant in the complexation; we suppose that likely the systems don't

need much more additional favorable interactions for their stabilities. In the non-ionized ICs, guest molecules establish more than one H-bond with β -CD; because of their freely moving inside the cavity, these H-bonds are formed and broken quickly giving them an intermittent and weakness character. Hence, it seems that H-bonds play only a secondary role in the non-ionized ICs.

Finally, note that a second hydrogen bond is significantly less detectable and its distribution would not exceed 2% in the best cases.

CONCLUSION

The kinetics of the association/dissociation inclusion process of some benzoic acid derivatives and paracetamol in the β CD was investigated using unbiased molecular dynamic simulations. The results show clearly a charge effect in the inclusion process of the ionized guest molecules which reduce considerably the stability of their corresponding inclusion complexes. We conclude that less hydrophobic compounds gives the more stable complex on contrary to the non-ionized guest molecules in which the more hydrophobic compound gives the more stable complex, and also H-bond interactions play only a secondary role in the stability of the non-ionized complexes, and do not play any role in the stability of the ionized complexes.

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