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Encapsulation of Thai Jasmine rice flavor

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Encapsulation of Thai Jasmine rice flavor

by

Miss Chanoknan Phongern

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บทคัดย่อ

ข้าวหอมมะลิเป็นข้าวหอมที่มีสารหอมระเหยคือ 2-acetyl-1-pyrroline (2AP) ซึ่งเป็นสารประกอบสำคัญที่ให้กลิ่นหอมเมื่อหุงสุก อย่างไรก็ตามสารหอมระเหยดังกล่าวจะสูญเสียได้ง่ายเมื่อให้ความร้อนหรือเก็บไว้เป็นเวลานาน บีตาไซโคลเดกซ์ทรินและอนุพันธ์ของบีตาไซโคลเดกซ์ทริน อาทิ เช่น เมทิล หรือ ไฮดรอกซีโพรพิล บีตาไซโคลเดกซ์ทริน ถูกนำมาใช้อย่างกว้างขวางเพื่อเพิ่มความเสถียรของสารระเหยต่าง ๆ ผ่านกระบวนการเอนแคปซูเลชันโดยให้สารระเหยเข้าไปยังโพรงที่ไม่มีขั้วของบีตาไซโคลเดกซ์ทริน ในการศึกษาครั้งนี้ ได้ทำการศึกษาโครงสร้าง พฤติกรรมพลวัต และความเสถียรของการเกิดสารประกอบเชิงซ้อนของสารหอมระเหย 2AP กับ บีตาไซโคลเดกซ์ทริน โดยใช้การจำลองพลวัตเชิงโมเลกุลของทุกอะตอมและจากการคำนวณพลังงานอิสระของกิบส์พบว่าแรงแวนเดอร์วาลส์เป็นตัวผลักดันการห่อหุ้มสารหอมระเหย 2AP สามารถปรับเปลี่ยนโครงสร้างของบีตาไซโคลเดกซ์ทรินและอนุพันธ์ของบีตาไซโคลเดกซ์ทรินให้มีความเสถียรมากขึ้น ข้อมูลด้านโครงสร้างและพลังงานอิสระแสดงให้เห็นว่า 2,6-DMβCD เป็นโครงสร้างที่เหมาะสมที่สุดสำหรับการห่อหุ้มสารหอมระเหย 2AP นอกจากนี้บีตาไซโคลเดกซ์ทรินที่ถูกดัดแปลง โดยเฉพาะ 6-HPβCD และ 2,6-DMβCD สามารถเพิ่มความเสถียรของสารหอมระเหย 2AP ได้ดีกว่าบีตาไซโคลเดกซ์ทรินแบบธรรมชาติ

คำสำคัญ: 2-acetyl-1-pyrroline, บีตาไซโคลเดกซ์ทริน, กระบวนการเอนแคปซูเลชัน

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Abstract

Thai jasmine rice is an aromatic rice with variable prices due to the odorant from volatile gas called 2-acetyl-1-pyrroline (2AP), which a key odor-active compound contributing to the pleasant smell of the cooked rice. However, the 2AP was rapidly gone after 6 months of storage or at high temperature condition. Beta-cyclodextrin (βCD) and its derivatives (e.g. methyl (M) and hydroxypropyl (HP) βCDs) have been extensively used to enhance the stability of many volatile compounds through an encapsulation process into the lipophilic inner cavity. In the present study, the dynamics behavior and stability of inclusion complexes of 2AP with βCDs were studied using all-atom molecular dynamics simulations and Gibbs free energy calculations. The obtained results showed that the van der Waals driven encapsulation of 2AP could adapt the structures of the βCDs to become more stable conformers. The structural data and free energy results demonstrated that the 2,6-DMβCD is the most suitable host for 2AP encapsulation. In addition, the modified βCDs, especially 6-HPβCD and 2,6-DMβCD, could enhance the stability of 2AP better than natural βCD.

Keywords: 2-acetyl-1-pyrroline, beta-cyclodextrin, encapsulation

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List of Abbreviations

2AP	2-acetyl-1-pyrroline
βCD	beta-cyclodextrin
2,6-DMβCD	2,6–dimethyl beta-cyclodextrin
2,6-DHPβCD	2,6–dihydroxypropyl beta-cyclodextrin
2HPβCD	2–hydroxypropyl beta-cyclodextrin
6HPβCD	6–hydroxypropyl beta-cyclodextrin
RMβCD	randomly methyl beta-cyclodextrin
Å	angstrom
ns	nanosecond
MD	molecular dynamic
^{O}C	degree Celsius
mg	milligram
ml	milliliter

Chapter 1

Introduction

1.1 Background and motivation of study

Nowadays, researchers are interested in a field of food's scent chemical. They have an aim to develop and increase food value. Thai jasmine rice is very famous in the world rice market for its dominant features, which are shinny white like jasmine flower color, soft and tender with aromatic fragrance when cooked[1]. These favourable properties generated higher consumer demand in 2016–2017 that considered from increase of the export value and export amount[2]. Several studies have investigated on the chemistry of aroma and found that 2-acetyl-1-pyrroline (2AP) was a key fragrance-active compound profoundly contributing to the pleasant odor of the cooked rice[3-5]. However, the smell was rapidly gone storage for a long time or at high temperature condition[6]. Therefore, the researchers have found a way to store and prevent the aroma by using encapsulation techniques. By this technique encapsulates unstable substances with a wrapper that makes the encapsulated compound more stable and volatile[7,8]. In the past, researchers developed molecular inclusion by cyclodextrins (CDs). CDs are comprised of 6, 7 and 8 glucose molecules called alpha - , beta - and gramma - CD respectively. Among the three different CDs, beta-cyclodextrin (βCD) has been the most studied CDs because of lowest-priced, suitable porous size and generally the most useful[9]. However, it has low water solubility (18.5 mg/mL at 25 ^{O}C) and nephrotoxicity[10-11]. On the other hand, βCD derivatives such as methyl (M)-CD and hydroxypropyl (HP)-CD have received much attention in an attempt to increase solubility and to reduce such limitations.

In this study, we studied the binding between 2AP from Thai jasmine rice and six βCD derivatives as follows: βCD, 2,6–dimethyl βCD (2,6-DMβCD), 2,6–dihydroxypropyl βCD (2,6-DHPβCD), 2–hydroxypropyl βCD (2HPβCD), 6–hydroxypropyl βCD (6HPβCD) and randomly methyl βCD (RMβCD). We simulated molecular docking and molecular dynamics to understand the binding between 2AP and βCD derivatives.

1.2 Literature review

Thai Jasmine rice is a fragrant rice with variable prices due to the odorant from volatile gas called 2-acetyl-1-pyrroline (2AP), which a key odor-active compound contributing to the pleasant smell of the cooked rice[3-5]. However, non-fragrant rice was rarely found. The IUPAC name of 2AP is 1-(3,4-dihydro-2H-pyrrol-5-yl)ethanone, its CAS number is 85213-22-5 and its FEMA (Flavour and Extract Manufacturers Association) number is 4249. 2AP was first identified in rice by Ron Buttery with his co-workers in 1982. (**Figure 1**)[3]. This compound also occurs naturally in some other plants such as pandan leaf and popcorn[12]. Among the complicated chemistry of volatile compounds of fragrant rice, the 2AP is regarded as the single most important compound that is responsible for rice aroma. Nevertheless, some other volatile compounds such as (*E*, *E*)-2,4-decadienal, hexanal, octanal, nonanal, decanal, 4-vinylphenol, 4-vinyl-guaiacol, 4,5-epoxy-(*E*)-2-decenal, 2-amino acetophenone, and 2-acetyl-*L*-pyrroline are also regraded as the important contributors of rice aroma[13]. 2AP is a substituted pyrroline in with the hydrogen position 2 is replaced by an acetyl group with a methyl ketone group. The pyrroline ring makes the compound highly unstable[14]. Pure 2AP is a viscous liquid which disintegrates in minutes under ambient condition. Consequently, this molecule is not commercially available and rarely used by the flavor industry[15]. The aroma of rice was rapidly gone storage for a long time or at high temperature condition. Several studies have shown that encapsulation technology is extensively applied in aroma and flavor industries to encapsulate desired compounds. It forms protective film against extreme conditions such as pH, oxygen, heat and moisture[16]. Duby & Huynh-Ba[17] studied processes for the preparation of 2AP by βCD in encapsulated form, it showed that the storage temperature decreased to result in encapsulation performed better. Thus, βCD have limit to storage to 2AP.

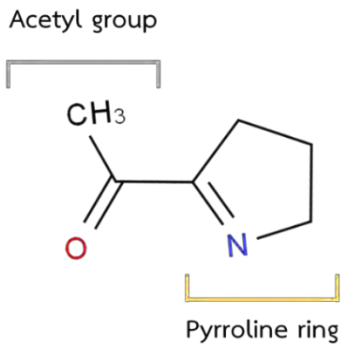
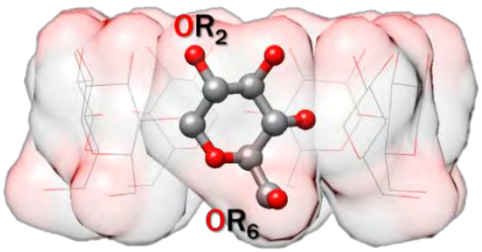


Figure 1. Chemical structure of 2-Acetyl-1-Pyrroline.

βCD is a cyclic oligosaccharide which consists of seven *D*-glucopyranose units linked with α-1,4 glycosidic bonds. The shape of βCD looks like trunked cone with hydroxyl groups which are orientated at the edges of the cavity. The secondary hydroxyl groups (at C2 and C3 atoms of the glucose units) situate on one edge of the ring, whereas all primary hydroxyl groups (at C6 atoms of the glucose units) place on the other edge[18]. At position C6, the primary hydroxyl groups of the glucose residues locate at the narrow rim. On the other hand, the secondary hydroxyl groups are at the wider rim of the trunked cone. This structural characteristic of βCD gives it to be hydrophilic exterior surface whereas the interior of the cavity is rather hydrophobic. Therefore, a poorly water-soluble molecule can be inserted into the βCD cavity leading to an increased solubility[18]. The complex formed is called "inclusion complex". Such inclusion compounds result from the energetically unfavorable interaction between the included water molecules in the hydrophobic βCD cavity on one hand, and between water and guest on the other, in comparison with the hydrophobic and van der Waals interactions between the guest and the host cavity. βCD is widely used in pharmaceutical and food industries because of low cost, easy synthetic accessibility, and suitable cavity size (0.60–0.65 nm) for the inclusion of small- and medium-sized guests[19]. Albeit, βCD shows a relatively lower water solubility (18.5 mg/mL) than other CDs[20, 21] because of the high number of intramolecular hydrogen bonds among secondary hydroxyl groups within the molecule. These interactions make the structure rigid and prevent hydration by water molecules[22]. Moreover, βCD still has some disadvantages, such as difficult *in vivo* metabolism, and nephrotoxicity[23]. Molecular encapsulation with βCD derivatives for example, hydroxypropyl and methyl βCD has

received much attention in an attempt to increase solubility and to reduce such limitations. **Figure 2**, the hydroxypropyl βCDs such as, 2-hydroxypropyl β-cyclodextrin (2-HPβCD), 6-hydroxypropyl β-cyclodextrin (6-HPβCD) and 2,6-hydroxypropyl β-cyclodextrin (2,6-DHPβCD), as well as the methyl βCD derivatives, show a greater water solubility and less toxicity than βCD[24, 25]. Thus, this project aimed to theoretically investigate the inclusion complexation efficiency of 2AP utilizing six βCD derivatives (e.g. βCD, 2-HPβCD, 6-HPβCD, 2,6-DMβCD, 2,6-DHPβCD and RMβCD) as the host molecules, as well as to study the dynamic behaviors of such inclusion complexes in aqueous solution.



βCD:	$-R_2 = -H, -R_6 = -H$
2-HPβCD:	$-R_2 = C_3H_7O, -R_6 = -H$
6-HPβCD:	$-R_2 = -H, -R_6 = C_3H_7O$
2,6-DHPβCD:	$-R_2 = C_3H_7O, -R_6 = C_3H_7O$
2,6-DMβCD:	$-R_2 = CH_3, -R_6 = CH_3$
RMβCD:	$-R_2 = CH_3$ (unit 1, 3 and 5) $-R_6 = CH_3$

Figure 2. The three-dimensional (3D) structure of βCD, where the representative glucopyranose subunit is highlighted in ball and stick, and the list of βCD and its derivatives showing the overall substituents is presented below.

In recent years, the computational approaches have been played an important role for monitoring inclusion complex between host cyclodextrin and guest molecules[26, 27] like volatile compound in molecular level. The molecular dynamic (MD) simulations were used to describe the molecular reasons of inclusion complex and to compare the data between experiment results and computational theoretical data. Engin Durgun et al. also reported the theoretical study based on MD simulation in order to understand the volatile compound/βCD derivatives complexes[28].

To understand the host-guest inclusion complex between 2AP and βCD derivatives in molecular level, MD simulations were compared the efficiency of

2AP/βCD derivatives complex. In order to obtain detailed insight into the inclusion complex, intermolecular interaction and the binding free energy were accurately analyzed.

1.3 Objectives of the study

1.3.1 To study and understand the host-guest inclusion complex between 2AP to the βCD and various derivative of βCD.

1.3.2 To compare the efficiency of 2AP to the βCD and various derivative of βCD.

1.4 Benefits of the study

1.4.1 gain better understanding on the molecular recognition of βCD and various derivative βCD toward 2AP.

1.4.2 obtain 3D structures of complexes between 2AP to the βCD and various derivative of βCD.

Chapter 2

Methods

2.1 Materials

2.1.1 High-performance computing

2.1.2 Programs and websites

2.1.2.1 Zinc database

2.1.2.2 Gaussview 5.0

2.1.2.3 Gaussian 09W

2.1.2.4 Accelrys Discovery Studio Visualizer 3.0

2.1.2.5 AMBER16

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2.1.2.7 Chimera 1.13.1

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2.1.2.9 WinSCP

2.1.2.10 MobaXterm

2.2 Computational details

2.1.1 System preparation and molecular docking

The starting structures of all βCD derivatives were taken from a previous study[29]. The geometry of 2AP was optimized at the HF/6-31(d) level of theory using the Gaussian09 program[30]. The inclusion complexes of 2AP with βCDs were constructed using the CDOCKER module implemented in the Accelrys Discovery Studio 3.0 program with 100 docking runs. Finally, the 2AP binding with the βCD derivatives with the lowest interaction energy of complex structure is used as the initial structure for molecular dynamics simulation (MD simulation).

Standard procedures, based on previous studies[31-33], were used to generate the required parameters of ligands. Briefly, the electrostatic potential (ESP) charges around the optimized geometry were calculated by the HF/6-31(d) level of theory using the Gaussian09 program. The antechamber module was used to evaluate the restrained electrostatic potential (RESP) charges. The Glycam06j-1 carbohydrate force field[34] was applied for all βCDs, whereas the parameters of ligands were taken from the parmchk module based on the general AMBER force field[35]. To relax the structures

prior to MD simulations, all hydrogen atoms of each docked complex were minimized with 1000 steps of steepest descents (SD) followed by 3000 steps of conjugated gradient (CG). Afterward, the TIP3P water model was used to solvate each inclusion complex. Subsequently, the added water molecules were minimized with 1,000 steps of SD and continued by 3,000 steps of CG. Finally, the whole system was optimized using the same procedures.

2.1.2 Molecular dynamics simulations and free energy calculations

All 18 models of inclusion complexation were performed by all-atom MD simulations using AMBER16[36] according to previous studies[37, 38]. The periodic boundary condition with isobaric-isothermal (NPT) ensemble was applied for all systems using a time step of 2 fs. The Berendsen weak coupling algorithm was used to control pressure and temperature[39]. The Particle Mesh Ewald (PME) summation approach[40] was used to treat long-range electrostatic interactions, while the cutoff for non-bonded interactions was set at 10 Å. All covalent bonds involving hydrogen atoms were constrained using the SHAKE algorithm[41]. The temperature of each inclusion complex was increased from 0 K to 298 K for 0.002 ps, and continuously held at this temperature and with a pressure of 1 atm until reaching 100 ns.

The equilibrium state of all simulated models was determined by calculating the root-mean-square displacement (RMSD). The conformational changes of βCDs upon complexation were characterized using the potential energy surface (PES). The ligand mobility inside βCDs cavities was determined by measuring the distance between the centers of gravity (C_g) of 2AP and βCDs. The effect of water accessibility on 2AP/βCDs inclusion complex formation was characterized by solvent accessible surface area (SASA) calculations and the number of surrounding atoms around 2AP of βCDs was characterized using by the criteria of any atom within the 3.0 Å sphere of the ligand. Additionally, the molecular mechanics/Poisson–Boltzmann and generalized Born surface area (MM/PB(GB)SA)-based binding free energy (ΔG_{bind}) of all inclusion complexes were estimated by the MMPBSA.py module implemented in AMBER16 using 35000 MD snapshots taken from the last 100 ns simulation.

The MM-PBSA/GBSA approach is the energetic calculation for estimating the binding free energies or calculating the free energies of molecules in solution. The binding free energy of system was obtained from the difference of the free energies

between complex (ΔG_{cpx}), βCD derivatives ($\Delta G_{\beta CDs}$) and ligand (ΔG_{lig}) as shown by:

$$\Delta G_{bind} = \Delta G_{cpx} - (\Delta G_{\beta CDs} + \Delta G_{lig}) \quad (1)$$

From the second law of thermodynamics, the total free energy calculates from the mathematical relationship Gibbs free energy (ΔG), enthalpy term (ΔH) and entropic contribution at a constant temperature ($T\Delta S$).

$$\Delta G = \Delta H - T\Delta S \quad (2)$$

The ΔH term of the system was obtained the summation of enthalpy changes in the gas phase upon complex formation (ΔE_{MM}) and the free energy of solvation (ΔG_{sol}). From Equation (2) can be rewritten as:

$$\Delta G = (\Delta E_{MM} + \Delta G_{sol}) - T\Delta S \quad (3)$$

where ΔE_{MM} corresponds to the molecular mechanical energy, including the bonded and nonbonded energy terms. The nonbonded terms are also divided into the electrostatic (ΔE^{ele}) and van der Waal interaction energies (ΔE^{vdw}). Furthermore, ΔG_{sol} accounts for the solvation energy which is divided into the electrostatic term (ΔG_{sol}^{ele}) and nonpolar term ($\Delta G_{sol}^{nonpolar}$).

$$\Delta G_{sol} = \Delta G_{sol}^{ele} + \Delta G_{sol}^{nonpolar} \quad (4)$$

The Possion-Boltman (PB) method was used to compute the electrostatic component while the nonpolar term in solvation free energy was evaluated according to equation (5).

$$\Delta G_{sol}^{nonpolar} = \gamma SASA + \beta \quad (5)$$

where SASA is the solvent accessible surface area ($Å^2$) of each given molecule. The

solvent probe radius is of 1.4 Å, and solvation parameters, γ and β are 0.0072 kcal/mol Å^2 and 0.00 kcal/mol, respectively.

The contribution of 2AP in βCD derivatives to the free energy of βCD derivatives/ligand binding in equation (1) is estimated by the free energy components. The electrostatic account for the solvation is evaluated by the Generalized Born (GB) model as expressed in equation (6).

$$\Delta G_{sol}^{ele} = -\frac{1}{2}\left(1 - \frac{e_{GB}^{-Kf}}{\varepsilon_{\omega}}\right)\sum_{ij}\frac{q_i q_j}{f_{GB}} \quad (6)$$

Where K is the Debye-Hückel parameter and ε_{ω} is dielectric constant of solvent which were set to 0 and 80 respectively. f_{GB} was obtained by:

$$f_{GB} = [r_{ij}^2 + \alpha_i \alpha_j \exp(\frac{-r_{ij}^2}{4\alpha_i \alpha_j})]^{\frac{1}{2}} \quad (7)$$

The Born radius of atoms i and j are defined in parameters of α_i and α_j consecutively, while r_{ij} is the distance between atom i and j. Furthermore, the contribution of atom i to the electrostatic free energy is evaluated by equation (8).

$$\Delta G_{sol}^{ele}(i) = -\frac{1}{2}\sum_j \left(1 - \frac{e^{-Kf}}{\varepsilon_{\omega}}\right)\frac{q_i q_j}{f_{GB_{ij}}(r_{ij})} + \frac{1}{2}\sum_{j \neq i}\frac{q_i q_j}{r_{ij}} \quad (8)$$

In order to estimate the nonpolar solvation energy per atom, the SASA is the key parameter as obtained by equation (9).

$$\Delta G_{nonpolar,sol}^{i} = \gamma \times (SASA^{i,cpx} - (SASA^{i,\beta CD} + SASA^{i,lig})) \quad (9)$$

Nevertheless, the total binding free energy can be calculated from the summation of the contributions, (ΔE_{ele}^{i}), (ΔE_{vdw}^{i}), ΔG_{sol}^{ele} and $\Delta G_{sol}^{nonpolar}$ under the atoms of complex without entropy term.

Chapter 3

Results and Discussion

3.1 Molecular docking

The Chemical structure of 2AP was created by Gaussian09W program, while βCD derivatives structure were extracted from Protein Data Bank. These structures were then formed complexes using molecular docking technique for 100 independent docking runs in Accelrys Discovery Studio Visualizer 3.0 program. After molecular docking procedure, there are two preferential binding modes of 2AP inclusion complexations, including pyrroline ring and acetyl group dipped into hydrophobic interior of βCDs (**Figure 3**).

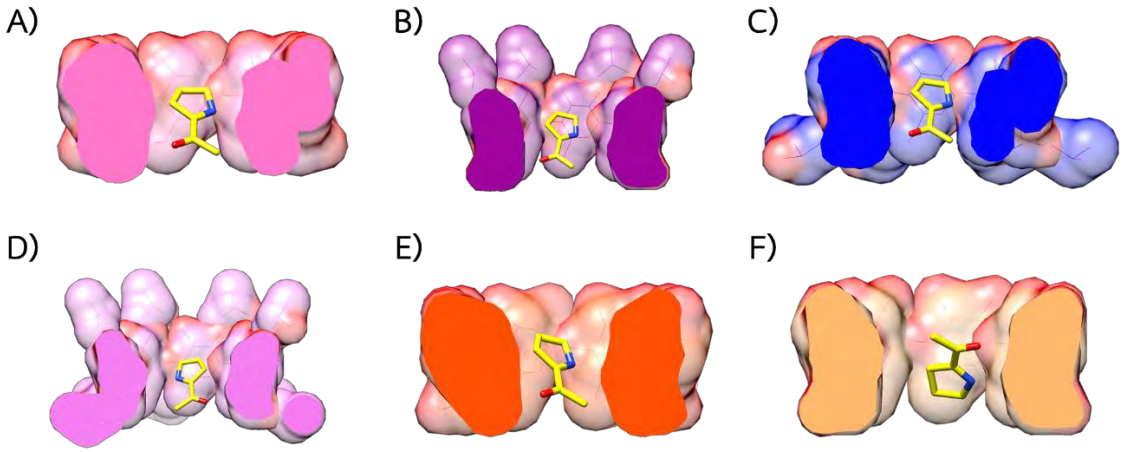

Figure 3. The orientations of 2AP/βCD (A), 2-HPβCD (B), 6-HPβCD (C), 2,6-DHPβCD (D), 2,6-DMβCD (E) and RMβCD (F) inclusion complexes from molecular docking.

The percentage docked conformation (%DC) of the pyrroline ring is found to be higher than that of acetyl group conformer for almost systems. The six complexes with top ranged lowest energy of the highest percent are chosen as the initial structure for MD simulations (shown in **Table 1** and **2**). However, the difference of the lowest interaction energy value in each form is not significant.

Table 1. The percentage docked conformation (%DC) of 2AP in complex with six different βCDs retrieved from 100 independent docking runs.

	% Docked conformation	
	Acetyl group insertion	**Pyrroline ring insertion**
βCD	40%	60%
2-HPβCD	8%	92%
6-HPβCD	28%	72%
2,6-DHPβCD	29%	71%
2,6-DMβCD	31%	69%
RMβCD	82%	18%

Table 2. The molecular docking energy of the six complexes with top ranged lowest energy.

	Interaction energy (kcal/mol)
βCD	-15.96
2-HPβCD	-16.97
6-HPβCD	-17.20
DHPβCD	-17.00
DMβCD	-16.80
RMβCD	-17.01

3.2 System stability

The system stability of all simulated inclusion complexes was calculated by Root mean square displacement or RMSD (**Figure 4**) by comparing the structure between starting structure that receive from docked structure and the configuration at simulation time. Consequently, the system stability was calculated by using cpptraj module of AMBER16 program. The obtained results show that the three independent simulations of 2AP (grey line) of all systems fluctuate in a similar manner to that of their starting structure. Interestingly, the fluctuation of 2-HPβCD, 2,6-DHPβCD and RMβCD are higher than that of native βCD, reflecting the structural transformation

occurred during simulation times. MD-1 of 2,6-DHPβCD shows a high level of fluctuation at 450 ns (5 Å) while the RMSD of βCD, 6-HPβCD and 2,6-DMβCD are relatively steady along the simulation times.

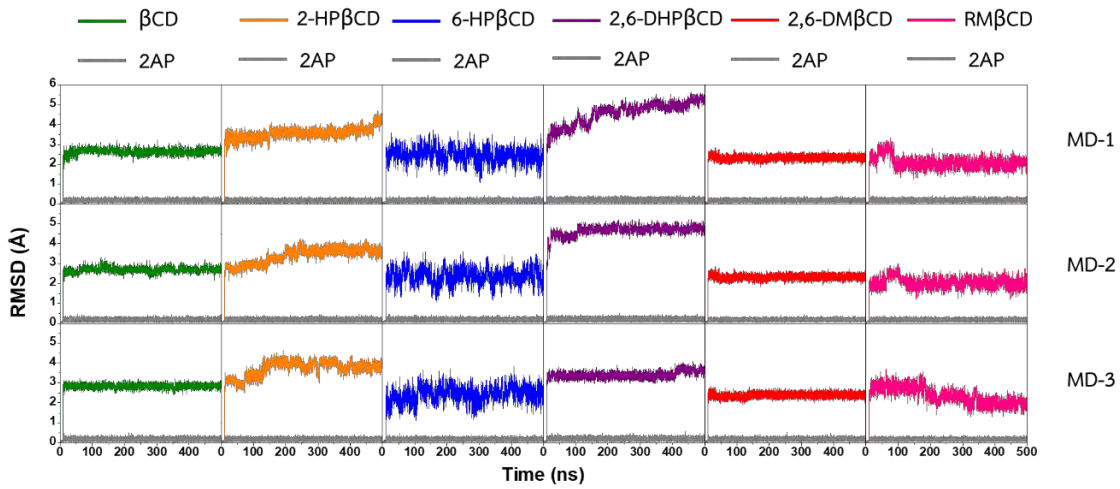

Figure 4. Plots of RMSD of 2AP dipped into the hydrophobic inner cavity of all studied βCDs.

3.3 Ligand mobility inside the βCDs cavity

The structural dynamics of 2AP inside the βCDs inner cavities were monitored by calculating the distance between C_g of ligand and βCD ($d[C_{g(ligand)} - C_{g(\beta CD)}]$) without considering the functional substituents at the simulation time interval from 0 ns to 500 ns. Note that, the horizontal light grey box ranging from -3.95 to 3.95 Å represents the positions of the primary (narrow) and secondary (wider) rims of unmodified βCD (~7.9 Å)[42].

As shown in **Figure 5**, it can be clearly seen that the 2AP can form inclusion complexes well with the 6-HPβCD and 2,6-DMβCD, indicating that the both βCDs are the suitable host for inclusion complexation. However, the 2AP inside the 2,6-DMβCD was found nearby wider rim of 2,6-DMβCD whereas the 2AP molecules moved out of hydrophobic cavity of βCD, 2-HPβCD, 2,6-DHPβCD and RMβCD. In addition, **Figure 6** show that the dynamic behavior of the acetyl group and pyrroline ring inside the hydrophobic interior of 6-HPβCD have a similar fluctuation pattern which mean two orientations of 2AP favorably dipped into 6-HPβCD. While pyrroline ring of 2AP is the preferred orientation in deeper position of (~1 Å) than in the acetyl group models (~4 Å) and the 3D structures (**Figure 8**) obtained from the last MD snapshot confirm that

the three independent simulations of 2AP are still in 6-HPβCD and 2,6-DMβCD along the simulation times.

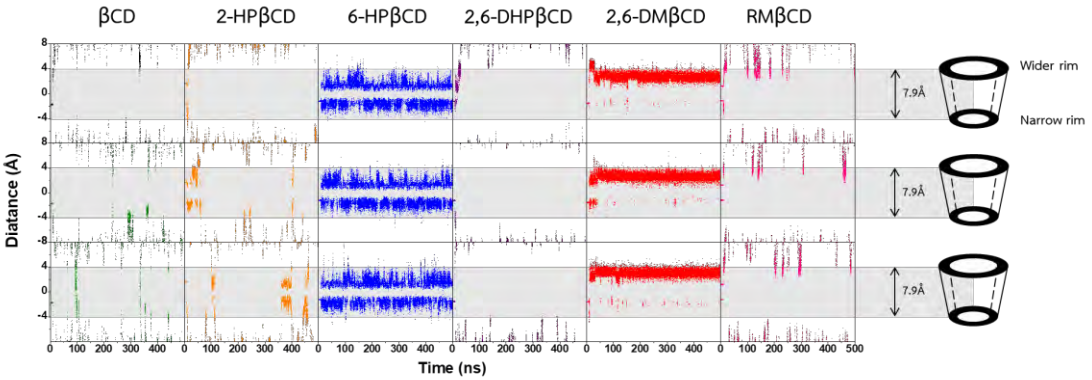

Figure 5. Distance plots between center of gravity of 2AP ligand and the βCDs. Light grey box depicts the height of βCD without considering the functional substituents.

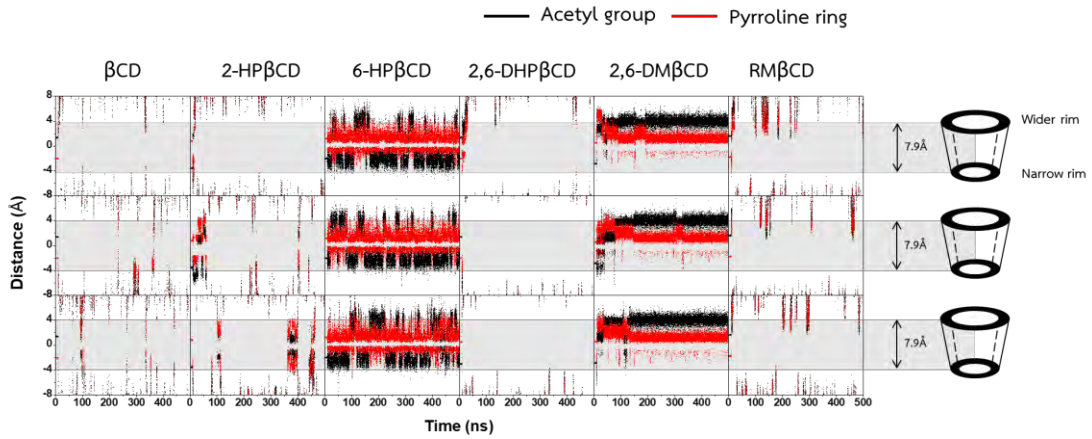

Figure 6. Distance plots between center of gravity of acetyl group and pyrroline ring ligand and the βCDs. Light grey box depicts the height of βCD without considering the functional substituents.

3.4 Solvent accessibility toward the inclusion complexes

The effect of water accessibility on 2AP/βCDs inclusion complex formation was characterized by solvent accessible surface area (SASA). It was calculated by using 2AP as the atomic radius for solvent-exposed area. The entire SASA results are depicted in **Figure 7**, Data in **Figure 7** indicate that the SASAs of 2AP/6-HPβCD and 2AP/2,6-DMβCD systems are more stable than those of 2AP/βCD, 2AP/2-HPβCD, 2AP/2,6-DHPβCD and 2AP/RMβCD complexes. In the case of 2AP/6-HPβCD and 2AP/2,6-DMβCD, the SASAs are relatively steady at ~25 $Å^2$ along the simulation times, whereas the SASAs of

2AP/βCD and 2AP/RM βCD are relatively steady at ~1400 $Å^2$ along the simulation times. By considering the 2AP/2,6-DHPβCD models, the SASA values considerably fluctuate ~1600-2000 $Å^2$. From three independent simulations reveal that the complexation of 2AP with 6-HPβCD and 2,6-DMβCD decrease the water accessibility toward the 2AP molecule inside hydrophobic inner cavity as compared to the 2AP/βCD, 2AP/2-HPβCD, 2AP/2,6-DHPβCD and 2AP/RMβCD, suggesting that 6-HPβCD and 2,6-DMβCD are the most preferred encapsulating agent for 2AP, which is in good correlation with MM/GBSA-based free energy calculations as discussed later.

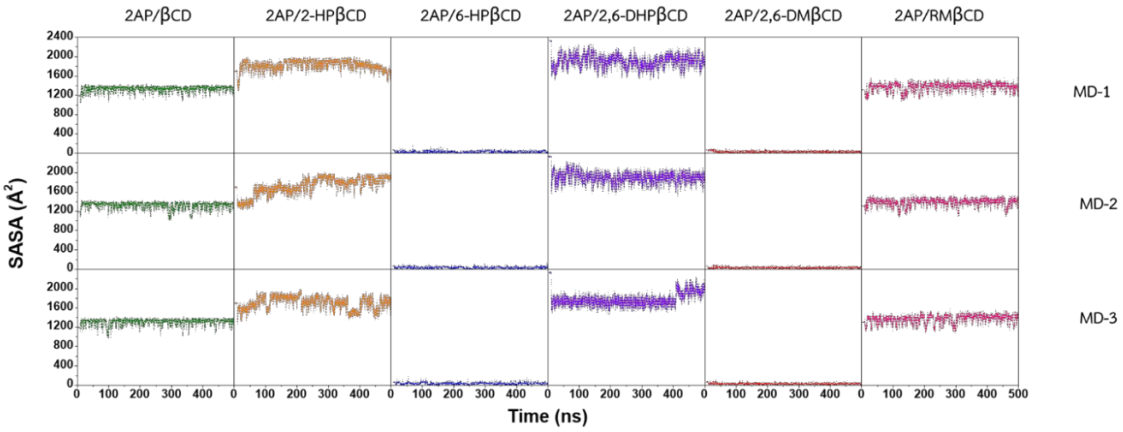

Figure 7. The SASA of 2AP/6-HPβCD and 2AP/2,6-DMβCD for three different MD runs.

3.5 The number of contact atoms

The number of surrounding atoms of 2AP (using the criteria of any atom within the 3.0 Å sphere of the ligand) were counted. The obtained results (**Table 3**) reveal that the number of surrounding atoms around 2AP of 2,6-DMβCD (~32 atoms) are rather than the 6-HPβCD systems (~29 atoms). Demonstrating that 2AP/2,6-DMβCD exhibit the most compact feature.

	2AP/6-HPβCD			2AP/2,6-DMβCD		
Number of contact of atom	MD-1	MD-2	MD-3	MD-1	MD-2	MD-3
	29.40±6.54	24.25±12.29	28.89±6.76	32.14±6.33	31.95±6.40	31.76±6.68

Table 3. The number of surrounding atoms within the 3.0 Å sphere of 2AP inside the 6-HPβCD and 2,6-DMβCD from the three independent simulations.

3.6 Binding free energy of inclusion complexes

The binding affinities of all studied inclusion complexes were predicted using 35,000 MD snapshots taken from the last 100 ns of three independent simulations. MM/GBSA-based free energies are summarized in **Tables 4** and **5**. According to MM energy, vdW interactions play a pivotal role in the encapsulation process of 2AP. The vdW energy of the 2,6-DMβCD (ΔE_{vdW} of ~-19 to -20 kcal/mol) is similar to the 6-HPβCD (ΔE_{vdW} of ~-14 to -20 kcal/mol), since the derivatized βCDs have lengthened internal hydrophobic cavities, which lead to more stable inclusion complexes. the $\Delta G_{binding}$ values of 2,6-DMβCD are greater than 6-HPβCD for 2AP. These findings suggest that 2AP preferentially embedded into 2,6-DMβCD rather than 6-HPβCD. Several studies have shown that the methyl and hydroxypropyl modifications on βCD can significantly enhance the stability of many lipophilic guest molecules[43,44]. Taken altogether, all structural and energetic analyses convince that 2,6-DMβCD is the most suitable host for 2AP encapsulation.

Table 4. The averaged MM/GBSA binding free energies ($\Delta G_{binding}$, kcal/mol) of 2AP with 6-HPβCD.

Energy (kcal/mol)	2AP/6-HPβCD		
	MD-1	MD-2	MD-3
	MMGBSA	MMGBSA	MMGBSA
ΔE_{vdw}	-20.35±0.03	-14.39±0.11	-20.58±0.03
ΔE_{elec}	-3.34±0.04	-1.80±0.03	-3.21±0.04
ΔE_{MM}	10.18±0.03	6.93±0.06	9.99±0.03
$\Delta E_{surface}$	-2.37±0.00	-1.68±0.01	-2.38±0.00
ΔG_{gas}	-23.69±0.05	-16.19±0.13	-23.79±0.05
ΔG_{solv}	7.81±0.03	5.26±0.05	7.61±0.03
ΔG_{total}	-15.88±0.03	-10.94±0.09	-16.18±0.03
$T\Delta S$	-13.31±0.03	-8.62±0.14	-13.37±0.03
$\Delta G_{binding}$	-2.57±0.04	-2.32±0.15	-2.81±0.04

Table 5. The averaged MM/GBSA binding free energies ($\Delta G_{binding}$, kcal/mol) of 2AP with 2,6-DMβCD.

Energy (kcal/mol)	2AP/2,6-DMβCD		
	MD-1	MD-2	MD-3
	MMGBSA	MMGBSA	MMGBSA
ΔE_{vdw}	-20.47±0.02	-20.47±0.02	-19.11±0.03
ΔE_{elec}	-3.93±0.02	-3.86±0.02	-5.62±0.03
ΔE_{MM}	8.32±0.01	8.25±0.01	10.12±0.02
$\Delta E_{surface}$	-2.43±0.00	-2.43±0.00	-2.35±0.00
ΔG_{gas}	-24.39±0.03	-24.33±0.03	-24.73±0.03
ΔG_{solv}	5.90±0.01	5.82±0.01	7.78±0.02
ΔG_{total}	-18.50±0.02	-18.51±0.02	-16.96±0.03
$T\Delta S$	-13.30±0.10	-13.22±0.02	-14.49±0.02
$\Delta G_{binding}$	-5.20±0.03	-5.30±0.03	-2.47±0.03

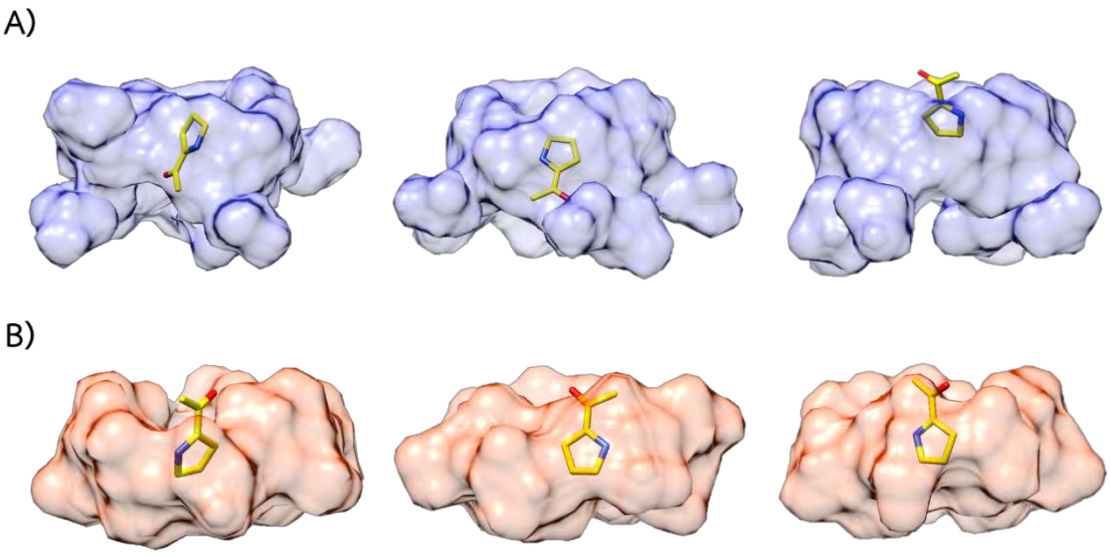

Figure 8. Binding orientations of 2AP inside the A) 6-HPβCD and B) 2,6-DMβCD drawn from the last snapshot of the three independent simulations.

Chapter 4

Conclusions

In this study, the structural dynamics and the suitable βCD host molecules for 2AP inclusion complexations were investigated using molecular modeling approaches. Molecular docking reveals that there are two possible modes of inclusion complex between 2AP and βCDs, namely the pyrroline ring and acetyl group dipped into the hydrophobic inner cavity of βCDs. From MM energy, we found that van der Waals interaction is the main force in the encapsulation process. The structure analyses indicated that pyrroline ring was the preferred binding mode for the 2AP/2,6-DMβCD. Moreover, the SASAs calculations suggested a lower water accessibility toward the 2AP encapsulated in the modified βCDs, especially 6-HPβCD and 2,6-DMβCD. Altogether, the obtained results demonstrated the good potentiality of βCD derivatives as suitable formulations of 2AP for further industrial.

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