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## Discovery of a non classic host guest complexation mode in a $\beta$ -cyclodextrin/propionic acid model†

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**A non-classic complexation mode was discovered upon spectroscopic, thermodynamic, crystallographic and computational studies of a  $\beta$ -cyclodextrin/propionic acid complex. A “fully immersed” complexation phenomenon, where both the guest’s hydrophobic and polar moieties are located inside the host and are stabilized by it, was found and calculated as the most favorable configuration.**

Host–guest chemistry is utilized for the accumulation, delivery and release of active agents. Cyclodextrins (CDs) are natural or artificially synthesized oligosaccharides and are important players in this field.<sup>1,2</sup>  $\beta$ -CD is the most utilized member of this family;<sup>3</sup> it is natural, widely available, generally recognized as safe and non-costly.<sup>4,5</sup> Its complexing abilities have been comprehensively studied and are generally used to incorporate or dissolve large hydrophobic guest molecules. This is implemented in a variety of fields such as pharmaceuticals, agriculture, environmental protection, organic synthesis, food industry, and more.<sup>6</sup>

Amphiphilic guest molecules in this study refer to polar chain molecules such as monocarboxylic acids and primary aliphatic amines or alcohols. Such guests’ polar heads tend to protrude beyond the CD rim and interact with the bulk solvent *via* H-bonds. This is a classic CD complexation mode and has been described extensively in the literature thus far for large amphiphilic guests.<sup>7</sup> It was generally believed in the past that  $\beta$ -CD cannot form the necessary interactions with small amphiphilic guest molecules due to geometry and size considerations, and that the smaller  $\alpha$ -CD should be implemented instead.<sup>8,9</sup>

Therefore,  $\beta$ -CD’s complexes with such molecules have been scarcely studied.<sup>10,11</sup> In our previous studies we have found that adding  $\beta$ -CD to a biopolymer matrix significantly increased the polymer’s capacity for propionic acid (PA) uptake, used for delivery to agricultural products.<sup>12</sup> These results prompted us to further investigate the interactions between  $\beta$ -CD and PA and the possible formation of an inclusion complex (IC) more thoroughly. To the best of our knowledge, the only related work was made by Nishikawa *et al.*, who reported an interaction between these two molecules using the ultrasonic relaxation method.<sup>11</sup>

In this work we report spectroscopic, thermodynamic, crystallographic and computational studies of the  $\beta$ -CD–PA IC. During the study, a new non-classic complexation mode was observed, in which the guest’s polar moiety interacts with the host as well, allowing an unusual “fully immersed” configuration. Molecular dynamics (MD) calculations revealed that for the present system, this “fully immersed” configuration is the most favorable. A CD:alkanoic acid system is not mandatory for this complexation mode. The model may be implemented in the future with similar small guests with suitable stereochemical and constitutional characteristics for both academic and applicative potentials. PA, utilized in this study, is a naturally occurring carboxylic acid with antimicrobial properties. It is used in various applications such as the manufacture of herbicides, cosmetics, artificial fruit flavors and preservatives for food and grain.<sup>13</sup>

<sup>1</sup>H and <sup>13</sup>C NMR measurements have demonstrated shift displacements for  $\beta$ -CD’s and PA’s peaks upon complex formation in water.  $\beta$ -CD’s H3 (the proton located on carbon C3) was found to be the most displaced, whereas its H5 was the least displaced proton (−0.0216 vs. −0.0059 ppm, respectively). This suggests that PA mainly oscillates near  $\beta$ -CD’s O-2, O-3 side (Fig. 1).

Upon complexation, PA’s methyl group undergoes a heavier shift displacement than its methylene group (0.1396 and 0.0055 ppm vs. 0.1029 and −0.0009 ppm, for <sup>1</sup>H and <sup>13</sup>C NMR, respectively). This observation indicates that PA’s methyl group is embedded deeper in  $\beta$ -CD’s hydrophobic cavity. This alignment is driven by hydrophobic interactions between PA’s carbohydrate

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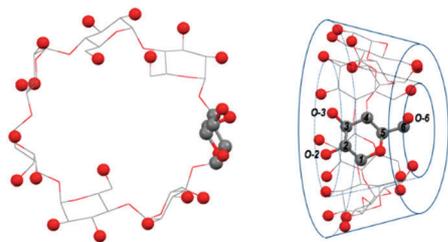


Fig. 1 Front and side view of  $\beta$ -CD. A single pyranose ring is highlighted in grey; hydroxyl groups are shown in red.

tail with  $\beta$ -CD's hydrophobic cavity. The registered shift displacement values correspond to spectroscopic data from other comparable complexes.<sup>14,15</sup> Notably, PA's carboxyl demonstrated a  $^{13}\text{C}$ -NMR shift displacement by 0.0139 ppm, suggesting an "immersed" guest configuration.

A significant reduction in PA's carboxyl peak intensity (over 90%) upon complexation was observed by FTIR. The peak intensity decrease approves interactions within  $\beta$ -CD's ring.<sup>16</sup> PA's carboxyl group undergoes a shift from  $1719\text{ cm}^{-1}$  to  $1716\text{ cm}^{-1}$  upon complexation. Similar carboxyl group shifts were reported for non-amphiphilic guests that interact within  $\beta$ -CD's ring with its hydroxyls.<sup>17,18</sup>

Diffusion NMR measurements enabled determining PA's and  $\beta$ -CD's diffusion coefficients' relative differences. These were then utilized to calculate the complex's thermodynamic parameters. The complexation process was found to have a negative free Gibbs energy ( $\Delta G$ ) of  $-4.2 \pm 0.5\text{ kJ mol}^{-1}$  supporting a spontaneous IC formation and equilibrium association constant ( $K_a$ ) of  $5.5 \pm 1.3\text{ M}^{-1}$ . Thermodynamic parameters for the  $\beta$ -CD-PA IC were also independently obtained using Van't Hoff plots in a temperature-reliant experiment.<sup>19,20</sup> The obtained  $\Delta G$  and  $K_a$  ( $-4.1 \pm 0.1\text{ kJ mol}^{-1}$  and  $5.2 \pm 0.3\text{ M}^{-1}$ , respectively) were in accordance with results from the diffusion NMR experiment. Rekharsky *et al.* plotted thermodynamic parameters as a function of the number of carbons in short carboxylic acids in their review of complexation thermodynamics in CD complexes.<sup>7e,21</sup> Our results successfully align with this trend as the plot's new shortest acid (ESI<sup>†</sup>). Furthermore, our  $K_a$  values matched those obtained by Nishikawa *et al.* ( $5.3\text{ M}^{-1}$ ).<sup>11</sup>

A single-crystal X-ray analysis revealed formation of a  $\beta$ -CD-PA IC. The crystal structure indicated that in addition to hydrophobic interactions in the  $\beta$ -CD cavity, host-guest H-bond interactions also play part in determining the orientation of the guest molecule.<sup>8</sup> PA molecules occupy two positions with an undetected order inside of  $\beta$ -CD's cavity and with an approximate ratio of 60/40 (Fig. 2A and B, accordingly). PA is fully immersed in the host molecule practically perpendicular to the median plane of  $\beta$ -CD's ring. Two PA positions differ only by rotating around the ring's axis. A competition between the shortest H-bonds between PA's carboxyl moiety and the host ( $\text{O6} \cdots \text{H15-C15}$ ,  $\text{O7} \cdots \text{H25-C25}$  and  $\text{O7A} \cdots \text{H15-C15}$ ;  $\text{O} \cdots \text{H}$  distances: 2.692, 2.630 and 2.678 Å accordingly) plays an important role in determining the orientation of the guest molecule and in a ratio of the two PA orientations. Unlike  $\beta$ -CD complexes with larger carboxylic

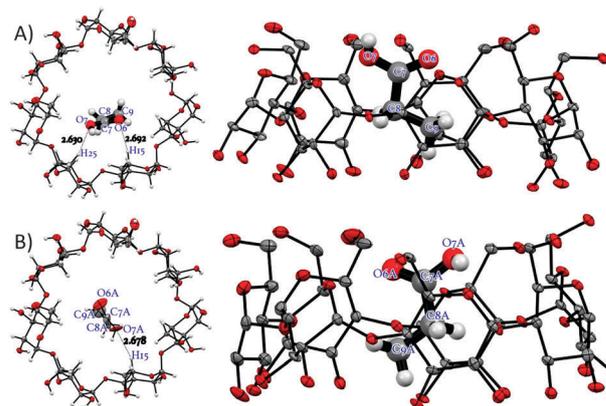


Fig. 2 Top (left) and side (right) views of two PA orientations (A and B) in the  $\beta$ -CD-PA IC.  $\beta$ -CD's hydrogen atoms in the side views are omitted for clarity. The shortest interatomic distances between PA and  $\beta$ -CD are  $\text{O7-H25}$  (2.630 Å),  $\text{O6-H15}$  (2.692 Å) and  $\text{O7A-H15}$  (2.678 Å).

acids whose heads protrude beyond the CD rim or form dimers,<sup>22</sup> PA is seen as fully embedded inside the  $\beta$ -CD cavity with its carboxyl group facing the narrower O-6 side (Fig. 2).

The presented  $\beta$ -CD-PA IC crystal structure was found to have a tight herringbone style packing. The crystal's symmetry is characterized by a  $P2_1$  space group. Neighboring complexes are linked by H-bonds both directly and through water molecules.  $\alpha$ -CD ICs with small amphiphilic guests such as short acids and alcohols (2–4 carbons long) all share the same crystal packing and crystallize in the same  $P2_1$  space group, forming a cage structure.<sup>23</sup>  $P2_1$  is also  $\beta$ -CD's natural crystal space group when no guests are present. However, complexes with longer guests such as valeric acid and 1-octanol were found to crystallize in a hexagonal space group and form a channel structure. The change in complex space group is affected by the guest's size. If the guest molecules are small enough they do not alter  $\beta$ -CD's natural space group and packing.<sup>10,21,24</sup> Other relevant  $\beta$ -CD complexes with the same space group include guests such as ethanol,<sup>25</sup> DMSO,<sup>26</sup> and formic and acetic acids.<sup>10,27</sup> These complexes were studied by crystallographic methods only; however, it can be suggested that they also belong to the non-classic IC formation with a "fully immersed" configuration.

In order to conduct quantitative analyses of the  $\beta$ -CD-PA IC formation and obtain atomic insights into the different binding modes, computational MD simulations for different host-guest configurations generated in docking analyses were performed. For comparison purposes, MD trajectories for identical host-guest docking configurations with the same initial PA position (in neutral and anionic forms) were also analyzed. The host and guest molecules were assembled *in silico* to create different orientations for PA within the host. Complex MD simulations were conducted in an explicit water solvent for  $\sim 50\text{ ns}$  to achieve a full equilibration for each system. PA's alkyl tail was always found to face  $\beta$ -CD's cavity, in accordance with our spectroscopic and crystallographic results. The computational MD study indicated that both PA's neutral and anionic forms induce changes in  $\beta$ -CD's conformational dynamics.

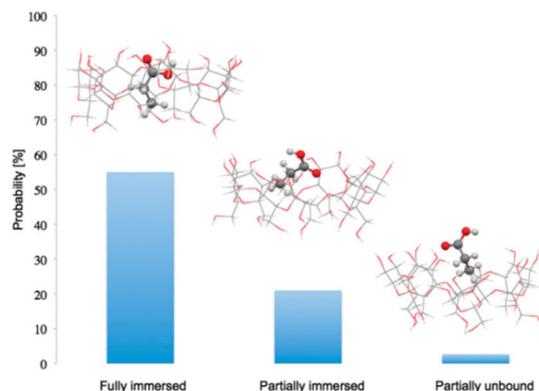


Fig. 3 Calculated probabilities for  $\beta$ -CD-PA configurations in the O-2, O-3 orientation with neutral PA.

The cone-shaped  $\beta$ -CD was found to be in a state of conformational equilibrium prior to PA involvement in accordance with previous studies.<sup>28</sup> However, once bound, the  $\beta$ -CD region interacting with PA's alkyl seemed to be less flexible and more compact.

During the simulation, PA's neutral form visited many transient conformations that fall into three main configurations (Fig. 3). The 1st configuration shows PA to be "fully immersed" inside  $\beta$ -CD's cavity. PA's fit enables its carboxyl head to be stabilized by interactions with the O-2, O-3 hydroxyl groups around it and with oxygen atoms in the internal cavity surface. In this orientation specific stereochemical conditions that allow the guest to be fully encompassed inside the host cavity are met. Larger guests (butyric or pentanoic fatty acids) may have a larger affinity due to their increasing hydrophobicity, but their polar head will not experience hydrophilic stabilization from the host. PA's neutral form was found to exist mostly in this "fully immersed" configuration. The 2nd configuration depicts PA as "partially immersed", with its carboxyl head outside the cavity, stabilized by hydrogen-bond interactions with the bulk solvent. Such a configuration would be expected from larger organic acid guests and in PA's case it was less visited. Previous MD publications have identified such a classic complexation mode for larger guest molecules.<sup>29</sup> The 3rd configuration is termed "partially unbound" and was scarcely found. In this configuration PA's hydrocarbon tail is partially immersed in  $\beta$ -CD's cavity, and its carboxyl head is completely solvated. Despite this flexibility in configurations, the average number of intermolecular contacts in the " $\beta$ -CD-PA-water" system remained mostly constant during the simulation time, which indicates an equilibration was achieved within the MD simulation 50 ns time frame. PA's anionic form was also simulated and led to a less stable IC than its neutral form (ESI<sup>†</sup>). Thus, analysis of the configuration probabilities led to the conclusion that PA is likely to form an IC in its neutral and "fully immersed" configuration.

In summary, the  $\beta$ -CD-PA IC was comprehensively studied utilizing spectroscopic, crystallographic, thermodynamic and computational analyses. A notable non-classic complexation mode was observed, in which the guest's polar head interacts with the host molecule, allowing an unusual "fully immersed" configuration. MD studies showed that for the present system a

"fully immersed" configuration provides the most favourable complexation mode. Unlike the case with large guests, where hydrophobic interactions provide the main driving force for complexation, in this IC the role of hydrophilic interactions is also significant. Hydrogen bonds supplied by the host allow the guest's polar head to be stabilized, thus forming an amphiphilic complementary pair between the guest and host.

We hope the presented model will promote future investigations of the physical and chemical processes that occur upon formation of other ICs with small amphiphilic guest molecules. The discovery of a non-classic "fully immersed" complexation mode may contribute to the exploration of new directions in host-guest chemistry and lead to revealing new scientific and applicative potentials.

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