Molecular Modeling Studies on the Chiral Separation of (±)-Catechins by Mono-succinyl-β-cyclodextrin

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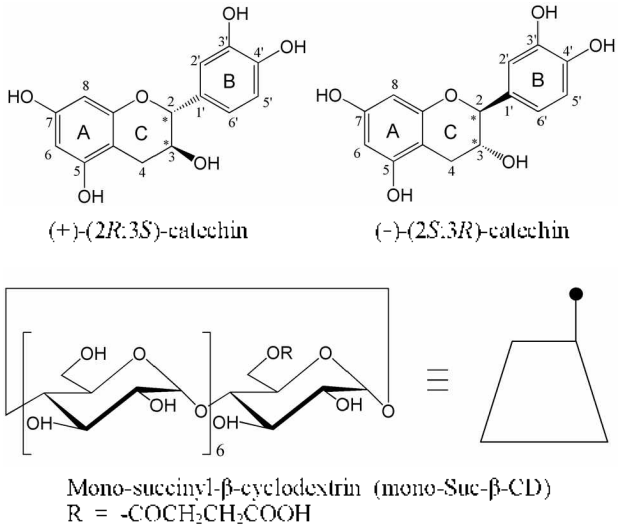
Chiral separation is a subject of great interest in the development, use, and action of pharmaceutical agents. Chiral pharmaceutical compounds typically have different pharmacological and toxicological properties, and therefore the quantitative chiral composition of these compounds should be determined.[1] The catechin discussed in this work belongs to the group of polyphenols, which are represented by the class of flavan-3-ols. The flavan-3-ols show notable physiological effects, including antioxidant, antimicrobial, and anticarcinogenic activities.[2] It was reported that (+)-(2*R*;3*S*)- and (−)-(2*S*;3*R*)-catechin had conflicting effects on glycogen metabolism in isolated rat hepatocytes.[3] A recent study also reported that (+)-(2*R*;3*S*)-catechin and (−)-(2*S*;3*R*)-catechin showed an allelopathic capability and antibacterial activity, respectively, and each respective counterpart, (−)-catechin and (+)-catechin, did not show any activity.[4] Many groups have thus tried to perform the separation of (±)-catechin by HPLC[5] and capillary electrophoresis (CE)[6] with various cyclodextrins and cyclic-oligosaccharide as chiral selectors.

An important method for separating chirals involves cyclodextrins.[7] Cyclodextrin (CD) is a macrocyclic molecule that forms α-(1→4) glycosidic linkages between D-glucose units, adopting a toroid shape. The resulting cavity of the cyclodextrins provides complexing properties with the appropriate guest molecules.[8] The inherent chirality of the cyclodextrins allows them to separate chiral compounds. Native CDs such as α-, β-, and γ-CDs, as well as their derivatives, have been used successfully in many applications.[9] As indicated in recent reviews, over the past decade, charged CDs have become the most widely used chiral selectors.[10] Succinyl-β-cyclodextrins (Suc-β-CDs) are a type of anionic-CDs. They have been used as chiral selectors in several types of chiral compounds.[11] Recently, our group separated (±)-catechin with Suc-β-CDs as chiral selectors in CE.[12]

In this study, the inclusion complexes between Suc-β-CD and both (+)- and (−)-catechin were modeled and refined by molecular modeling methods in order to investigate the binding mode and to predict the elution order for chiral separations. The most effective chiral selector is a mono-Suc-β-CD in the system (Scheme 1).[12] Without addition of mono-Suc-β-CDs, the migration time of (±)-catechin is 17.17 min at pH 9.8 and no separation of (±)-catechin occurred. The migration time of the first peak significantly reduced to 8.94 min at pH 9.8 with 5 mM mono-Suc-β-CD. These results clearly indicate the formation of an inclusion complex between Suc-β-CDs and (±)-catechin. Figure 1 show the partial electropherograms of the chiral separation of (±)-catechin with a mono-Suc-β-CD at different pH.[12] The pH of the buffer system had a strong influence on the separation compared to the nature of the buffer in the chiral separation of a flavonoid.[13] There is no chiral separation of (±)-catechin at either pH 8.5 or 11.0. However, at pH 9.8, (±)-catechin is separated with the baseline separation and (−)-catechin is eluted faster than (+)-catechin. The analyte that migrates earliest is considered to have relatively



Scheme 1. Schematic representation for the structure of (±)-catechin and mono-succinyl-β-cyclodextrin.

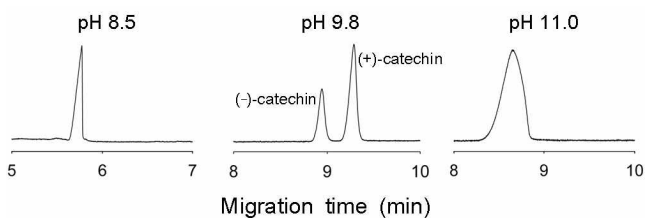
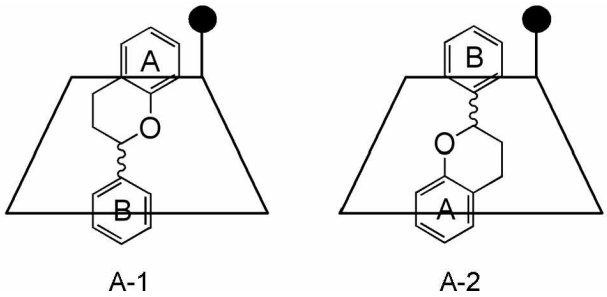


Figure 1. Dependence of the chiral separation of (±)-catechin with 5 mM mono-Suc-β-CD on the pH of background electrolyte.[12]



Scheme 2. Possible binding modes of catechin/mono-Suc-β-CD complex.

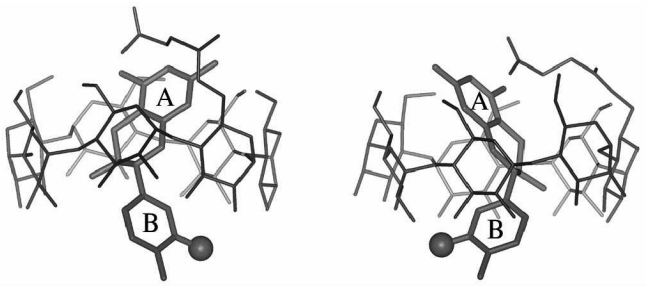


Figure 2. The structural model of (+)-catechin/mono-Suc-β-CD (left) and (−)-catechin/mono-Suc-β-CD (right) obtained from docking simulations. The ball model represents the deprotonated hydroxyl group and all hydrogen atoms are omitted for clarity. B-ring of catechin is found in the secondary hydroxyl side of cyclodextrin and A-ring of catechin is found in the primary hydroxyl side of cyclodextrin in both complexes. The upper and lower sides of CDs in the figure are the primary and secondary hydroxyl rims, respectively.

high affinity for the chiral selector in the system. Therefore, electropherograms are indicative of stronger interaction between (−)-catechin with mono-Suc-β-CDs as it migrates faster than (+)-catechin.

(±)-Catechin adopts different forms according to pH values. Neutral catechins are major forms at pH 8.5 and catechins with more than one ionized hydroxyl group are major forms at pH 9.8 and 11.0, because their pK_a values are 8.77, 9.97, and 11.99.[11] The first ionizable OH group occurs in the B-ring and the second ionizable group in A-ring in catechin. Increasing the pH value, catechin is firstly deprotonated in the B-ring and the 3′ hydroxyl group has a more tendency to deprotonate than the 4′ hydroxyl group.[14] The binding mode of mono-Suc-β-CD complexed with (±)-catechin with no, one (in B-ring), and two (in A- and B-ring) deprotonated hydroxyl groups were estimated by Monte Carlo (MC) docking simulations.

Figure 2 shows the lowest energy conformations of an inclusion complex between mono-Suc-β-CD and (+)- or (−)-catechin with one deprotonated hydroxyl group determined in docking simulations. Both (+)-and (−)-catechin penetrate completely into the cavity of CD, and deprotonated oxygen (O-3′) in the B-ring of catechin forms a hydrogen bond with the secondary hydroxyl group and the A-ring of catechin located in the primary hydroxyl rim of Suc-β-CD. There are basically two ways, *i.e.*, A-1 and A-2 for catechin to be located inside CDs (Scheme 2). However, in MC docking simulations (Fig. 2), we observed only A-1 mode in both complexes. These results might be due to the charge-charge repulsion between a deprotonated oxygen of catechin and a negatively charged carboxyl group of the succinyl moiety of mono-Suc-β-CD. The deprotonated hydroxyl group of catechin drives the direction in which it is oriented during the inclusion process. The deprotonated hydroxyl group remains in the outside of cyclodextrin cavity and the uncharged moiety is included in the cyclodextrin cavity. The deprotonated hydroxyl group of catechin in the complex can contact with the water molecules in aqueous environment. The interaction energy of (−)-catechin/mono-Suc-β-CD complex is lower than that of (+)-catechin/mono-Suc-β-CD complex. The interaction energies of (+)- and (−)-catechin/mono-Suc-β-CD complex are −40.91 and −45.63 kcal/mol, respectively. These results are consistent with experimental data of separation that (−)-catechin migrates faster than (+)-catechin at pH 9.8.

In the case of neutral catechin and catechin with two deprotonated hydroxyl groups in the A- and B-ring respectively, the binding mode of catechin with mono-Suc-β-CD is considerably different from the mode of one deprotonated catechin/mono-Suc-β-CD complex. Both A-1 and A-2 binding modes are observed in the lowest 10 conformations of all four MC docking studies (neutral (+)- and (−)-catechin/mono-Suc-β-CD complexes, and two deprotonated (+)- and (−)-catechin/mono-Suc-β-CD complexes). In A-1 mode, the A-ring of catechin is located at the primary hydroxyl rim of CD, as shown in Figure 2. The A-2 mode is the opposite direction of catechin in CD cavity where the A-ring of catechin is located at the secondary hydroxyl rim of CD. These results can explain why catechin did not separate in pH 8.5 and pH 11.0 by mono-Suc-β-CD. There is no definite conformation of each complex, which results in a insufficient specific (fixed) interaction such as three-point interaction between host and guest molecules to obtain the chiral discrimination.[15] Therefore, the biased catechin's orientation of the inclusion complex between mono-Suc-β-CD and one deprotonated catechin might be the one of the major factors for separation of (±)-catechin.

Mono-Suc-β-CDs were used as a chiral selector for separation of (±)-catechin in CE.[12] Efficient chiral separation was attained at optimum pH 9.8 and no chiral separation at both pH 8.5 and 11.0 in the system. The order of migration for (±)-catechin is indicative of stronger interaction between (−)-catechin with chiral selectors as it migrates faster than (+)-catechin at optimum pH. These results were supported by computational calculations with MC docking simulations. The mono-Suc-β-CD/(−)-catechin complex showed the lower interaction energy than mono-Suc-β-CD/(+)-catechin. The binding mode is also an important factor to explain the chiral separation of catechin by mono-Suc-β-CD. A single directed binding mode is observed only under optimum separation condition and mixed binding modes are observed in other conditions where no chiral separation occurred. These results demonstrate that the binding mode of the inclusion complex is an important factor as well as the interaction energy of the complex to predict the chiral separation by a computational approach. Detailed investigation of the Suc-β-CDs on other neutral and basic chiral analytes by experimental and computational methods will be necessary to understand the chiral separation mechanism by Suc-β-CDs.

Experimental Section

Molecular mechanics were performed with the Insight II/Discover program (version 2000. Accelrys. Inc. San Diego. U.S.A) using a consistent-valence force field (CVFF).[16] The β-CDs structure was obtained by the energy minimization of the crystallographic geometry. The molecular structure of β-CD was obtained from the crystal structure and mono-Suc-β-CD was built by adding a succinyl moiety to one of the primary hydroxyl groups of β-CD. The obtained models were optimized using a protocol of 300 steps of conjugated gradients so as to avoid steric hindrance. *Ab initio* calculations of deprotonated (±)-catechins were performed to determine the charges on each atom of deprotonated (±)-catechins using Gaussian 03.[17] Partial atomic charges of (±)-catechins were obtained from a Mulliken population analysis of the HF/6-31G wave function. All other parameters for the system used were default values from the CVFF potential function.

Docking studies were carried out using the Affinity module of Insight II and the CVFF force filed for docking and scoring. Monte Carlo docking simulations were performed on each complex. The detailed protocol for docking has been described elsewhere.[18] The (+)- or (-)-catechin were initially set above the center of the cavity of CD with a distance of ~15 Å. During the course of docking simulations, a catechin could make a maximum translational movement of 3 Å and a maximum rotation of 180° around the *x*, *y*, and *z* axes. Each cycle began with a random change of translation and rotation of the catechin. If the energy of the resulting configuration was within 1,000 kcal/mol of the last accepted one, it was subjected to 100 iterations of conjugated gradient energy minimization. After the energy minimization, the resulting structure was accepted based on energy and root-mean-squared displacement (RMSD), which compared the energy and RMSD of the new configuration against those accepted so far. Configurations above 1 kcal/mol energy and within 0.1 Å RMSD of pre-existing ones were discarded to obtain lower energy and to avoid accepting similar configurations. The docking simulations were performed until energy convertgence. No cutoff was imposed on the calculation of non-bonded interactions, and a distance-dependent dielectric of $4r$ was used to mimic solvent screening during the conformational searches.[19] The interaction energy of complexes has been calculated as the difference between the sum of the independently calculated energy of each molecule and the energy of the complex.

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