Interaction Energy Analysis for Drug-Cyclodextrin Inclusion Complexes in Aqueous Solutions

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Abstract: It is vital to elucidate the role of asymmetric intermolecular interactions resulting from the stereospecific structures of molecules in order to understand the mechanisms of chemical and biochemical reactions such as enzyme-substrate reactions, antigen-antibody reactions, etc. In order to reveal the mechanism of the inclusion phenomenon for β-cyclodextrin (CD)-ampicillin complexes and β-CD-ibuprofen complexes, binding free energies were determined using molecular mechanics/Poisson–Boltzmann surface area (MM/PBSA) analysis. To clarify the details of the interaction energies of these complexes, pair interaction energy decomposition analysis (PIEDA) was carried out. The direction of inclusion of drugs into β-CD cavities was clarified on the basis of results obtained using the above-mentioned methods.

Keywords: Binding free energy, energy decomposition, drug-cyclodextrin complex, dispersion force, interaction energy.

1. INTRODUCTION

Molecular recognition and discrimination may be caused by the interactions among containing surfaces of colliding molecules in solutions and mixtures. In particular, stereospecific interactions due to neighboring surfaces may play the leading role in, for example, enzyme-substrate reactions, antigen-antibody reactions, some types of mechanisms of the senses of smell and taste, etc. Therefore, it is vital to elucidate the role of asymmetric intermolecular interactions due to the stereospecific structure of a molecule in order to understand the mechanisms of chemical and biochemical reactions. In previous research, thermodynamic functions for the molecular inclusion of simple molecules into α- and β-cyclodextrin (CD) cavities in dilute aqueous solutions were systematically determined using microcalorimetry in order to clarify the mechanism of molecular recognition and discrimination [1-10]. Improvements in the stability and solubility of drugs can be expected from formation of CD-drug inclusion complexes. Moreover, complexation of CD with drugs can prevent aggregation of drugs. Therefore, consideration of the interaction energy using energy decomposition analysis is required as basic information for understanding the mechanisms of CD-drug complexation. For the CD system of a pharmaceutical drug, theoretical calculations of molecular interactions of the complex (β-CD + chlorambucil) were carried out using the molecular orbital method. The correlation between energy changes and molecular structures were examined. The large interaction energies calculated by the molecular orbital method showed the inclusion phenomenon [11]. However, the major contributions to the interaction energy were not discussed in detail. In addition, the direction from which ampicillin enters the CD cavity could not be clearly concluded using the semiempirical molecular orbital method and the NMR spectrum [12].

In this study, we were interested in clearly detailing the major contribution to the interaction energy of CDs and guest molecules. The interaction energies for the inclusion complexation of the β-lactam antibiotic ampicillin and the nonsteroidal anti-inflammatory drug ibuprofen with β-CD in aqueous solutions were analyzed by molecular mechanics/Poisson–Boltzmann surface area (MM/PBSA) analysis [13] and pair interaction energy decomposition analysis (PIEDA) [14]. Predictions regarding the direction of inclusion of drugs into the cavity of β-CD were made on the basis of the computational results.

2. COMPUTATIONAL METHODS

2.1. MM/PBSA Analysis

A conformation search was performed with the MMFF94s force field using CONFLEX 6 [15] to determine the direction from which drugs enter the β-CD cavity. Ampicillin has a lactam ring and a phenyl

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ring, whereas ibuprofen has an isobutyl group and propanoic acid as shown in Figure **1**. It was clear that these functional groups cannot insert from the primary hydroxyl side of β-CD and it was confirmed that they can insert from the secondary side of β-CD. We examined two conformations for each CD-drug complex as follows: (3a) the lactam ring of ampicillin exists inside the β-CD cavity while the phenyl ring exists outside on the secondary hydroxyl side of β-CD (Lact_BCDAmp); (3b) the phenyl ring of ampicillin exists inside the β-CD cavity while the lactam ring exists outside on the secondary hydroxyl side β-CD (Phen_BCDAmp); (4a) the isobutyl group of ibuprofen exists inside the β-CD cavity while the propanoic acid exists outside on the secondary hydroxyl side of β-CD (Isob_BCDIbup); and (4b) the propanoic acid of ibuprofen exists inside the β-CD cavity while the isobutyl group exists outside on the secondary hydroxyl side of β-CD (Prop_BCDIbup). The optimization and electrostatic potential calculations of these conformations were conducted using Gaussian 09 Revision C.01 [16]. The partial charges of all molecules were fitted with HF/6-31G(d) calculations and the RESP module in the AMBER package [17], and were generated automatically using the antechamber program in AMBER 12.0.

In molecular mechanics (MM) minimizations and molecular dynamics (MD) simulations, the general AMBER force field (GAFF) [18] was used for CD and the guest molecules. The solvated systems were equilibrated by carrying out 1000 short steps of minimizations, and 10 ps of heating and constant pressure equilibration at 300 K. All simulations were run with shake on hydrogen atoms, a 2 fs time step, and Langevin dynamics for temperature control. We ran a total of 20 ns or production recorded the coordinates every 10 ps. This was run 4 times to obtain 20 ns of simulation time. An average structure of each conformation was created over our entire trajectory of the last 5 ns.

In MM/PBSA, the binding free energy (ΔG_{bind}) between CD and a drug to form a complex was calculated as:

$$\Delta G_{bind} = G(\text{complex}) - G(\beta\text{-CD}) - G(\text{drug}) \quad (1)$$

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{solv} - T\Delta S \quad (2)$$

$$\Delta E_{MM} = \Delta E_{internal} + \Delta E_{vdw} + \Delta E_{els} \quad (3)$$

where ΔE_{MM}, ΔG_{solv}, and $-T\Delta S$ denote the changes in the gas phase MM energy, solvation free energy, and entropy term upon binding, respectively. The term ΔE_{MM} includes $\Delta E_{internal}$ (bond, angle, and dihedral), ΔE_{els} (electrostatic), and ΔE_{vdw} (van der Waals) energies.

The entropy change upon guest binding, $-T\Delta S$, was computed by normal-mode analysis on a set of conformational snapshots taken from MD simulations. The term ΔG_{solv} was calculated as:

$$\Delta G_{solv} = \Delta G_{PB} + \Delta G_{SA} \quad (4)$$

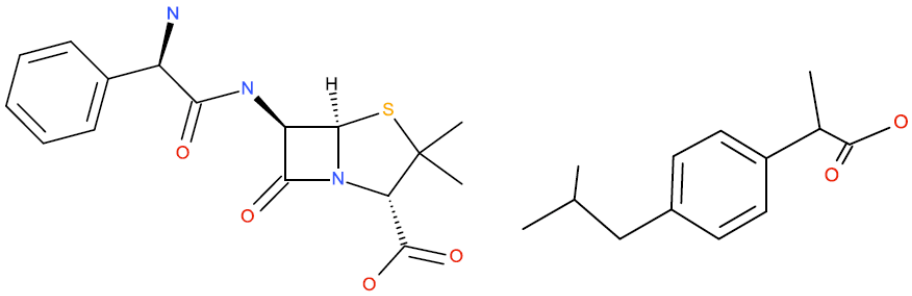
where ΔG_{PB} was calculated with the Poisson–Boltzmann (PB) method and ΔG_{SA} was calculated from the solvent-accessible surface area.

The interaction energy, ΔG_{gas}, between the complex, β-CD, and the drug in the gas phase was calculated as [19]:

$$\Delta G_{gas} = G_{gas}(\text{complex}) - G_{gas}(\beta\text{-CD}) - G_{gas}(\text{drug}) \quad (5)$$

$$\Delta G_{solv} = \Delta G_{solv}(\text{complex}) - \Delta G_{solv}(\beta\text{-CD}) - \Delta G_{solv}(\text{drug}) \quad (6)$$

$$\Delta G_{bind} = \Delta G_{gas} + \Delta G_{solv} \quad (7)$$



Ampicillin Ibuprofen

Figure 1: Structures of ampicillin and ibuprofen.

where ΔG_{solv}(drug), ΔG_{solv}(β-CD), and ΔG_{solv}(complex) are the solvation free energies of the drug, β-CD, and complex, respectively. The solvated monomer molecules mentioned in equation (6) are shown in Figure **2**. In aqueous solution, the water molecules remaining in the β-CD cavity are released into the bulk water upon inclusion of the drug.

2.2. PIEDA

PIEDA of the fragment molecular orbital method (FMO) was performed using the Gamess package [20] in order to evaluate the dispersion forces. An average structure of each conformation obtained using MM/PBSA was optimized at the RHF/6-31G level using the polarizable continuum model (PCM) with the integral equation formalism variant (IEFPCM). These structures were used for FMO calculations. PIEDA were decomposed into the electrostatic (ES), exchange-repulsion (EX), charge transfer (CT), and dispersion (DI) components, as shown in the following equation:

$$\Delta E_{int} = \Delta E_{ES} + \Delta E_{EX} + \Delta E_{CT} + \Delta E_{DI} \quad (8)$$

3. RESULTS AND DISCUSSION

3.1. β-CD + Ampicillin

The binding free energies using MM/PBSA analysis are listed in Table **1**. Lact_BCDAmp is more stabilized (-47.1 kJ mol^{-1}) than Phen_BCDAmp. The structure in which the phenyl ring of ampicillin exists outside the β-CD cavity is stabilized compared with that in which the lactam ring exists outside the cavity. The average structure of Lact_BCDAmp determined using PMEMD indicates that Lact_BCDAmp forms an intermolecular hydrogen bond on the primary hydroxyl side of β-CD, and the O-H distance is 0.09739 nm, the H···O distance is 0.18749 nm, and the O-H···O angle is 168.739°, as shown in Figure **3a**. The lactam ring, which has large molecular volume, makes the closest contact with the atoms in the β-CD cavity. On the other hand, as shown in Figure **3b**, Phen_BCDAmp forms an intermolecular hydrogen bond on the secondary hydroxyl side of β-CD, and the N-H distance is 0.10186 nm, the H···O distance is 0.20447 nm, and the N-H···O angle is 173.985°. The molecular volume of the phenyl ring is not large and it cannot make close contact with the atoms in the β-CD cavity. It seems that the difference in binding free energies was caused by forces between the ampicillin molecule and the inner walls of the β-CD cavity for different conformations. The van deer Waals interactions of Lact_BCDAmp are greater than those of Phen_BCDAmp and the entropy decrease of Lact_BCDAmp is larger than that of Phen_BCDAmp. Lact_BCDAmp is stabilized significantly upon inclusion of ampicillin into the β-CD cavity, which is accompanied by a large entropy decrease, and the stabilization is due to van deer Waals interactions resulting from the favorable fit. As

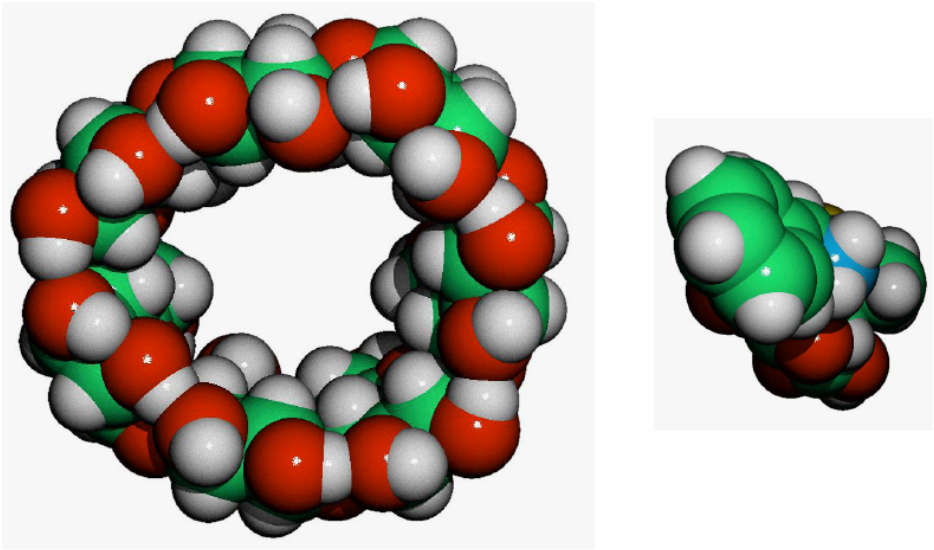
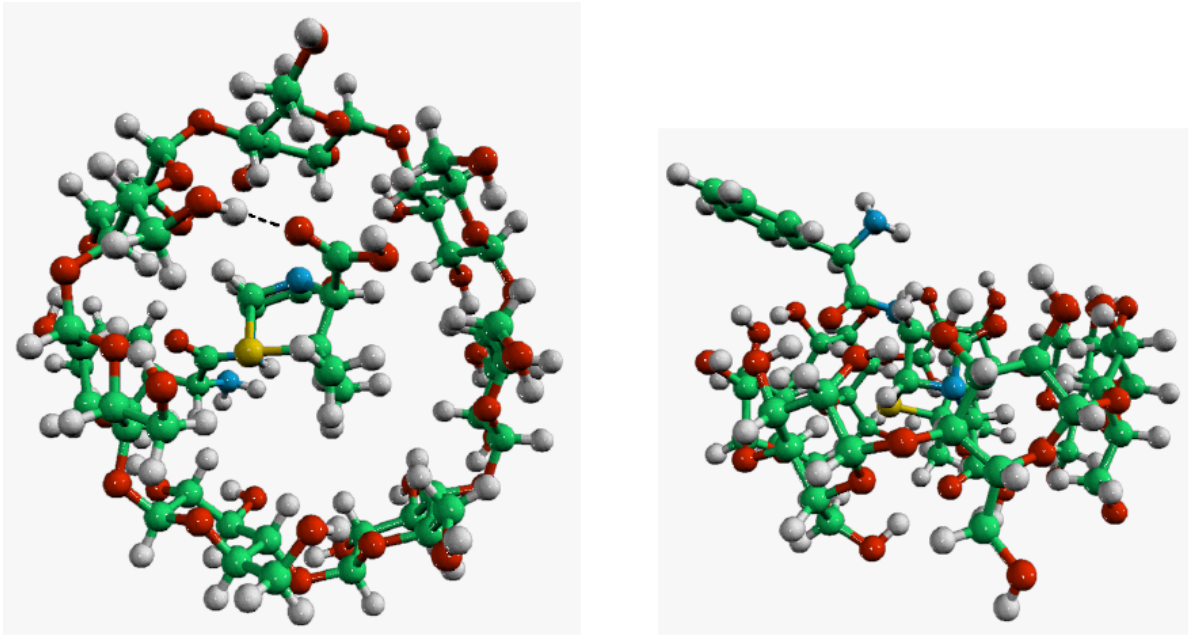


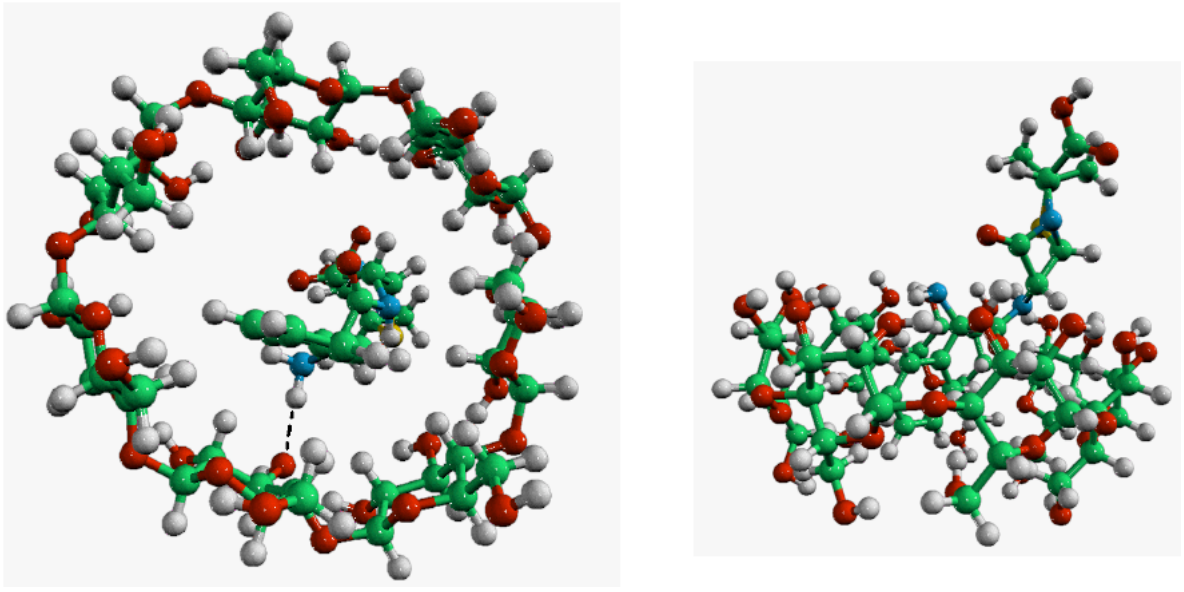
Figure 2: The solvated structures ampicillin and β-CD used in solvation free energy calculations.

Table 1: Binding Free Energies Predicted on the Basis of MM/PBSA Analysis for the Systems of β-CD + Drug. All Data are Given in kJ mol^{-1}

System	ΔG_{vdw}	ΔE_{MM}	$-T\Delta S$	ΔG_{gas}	ΔG_{solv}	ΔG_{bind}
Lact_BCDAmp	-134.8	-273.3	74.1	-199.2	56.6	-142.6
Phen_BCDAmp	-113.3	-225.4	69.6	-155.8	60.3	-95.5
Isob_BCDIbup	-96.3	-168.3	61.2	-107.2	-7.9	-115.1
Prop_BCDIbup	-93.0	-208.9	65.6	-143.3	45.5	-97.8



(**a**)



(**b**)

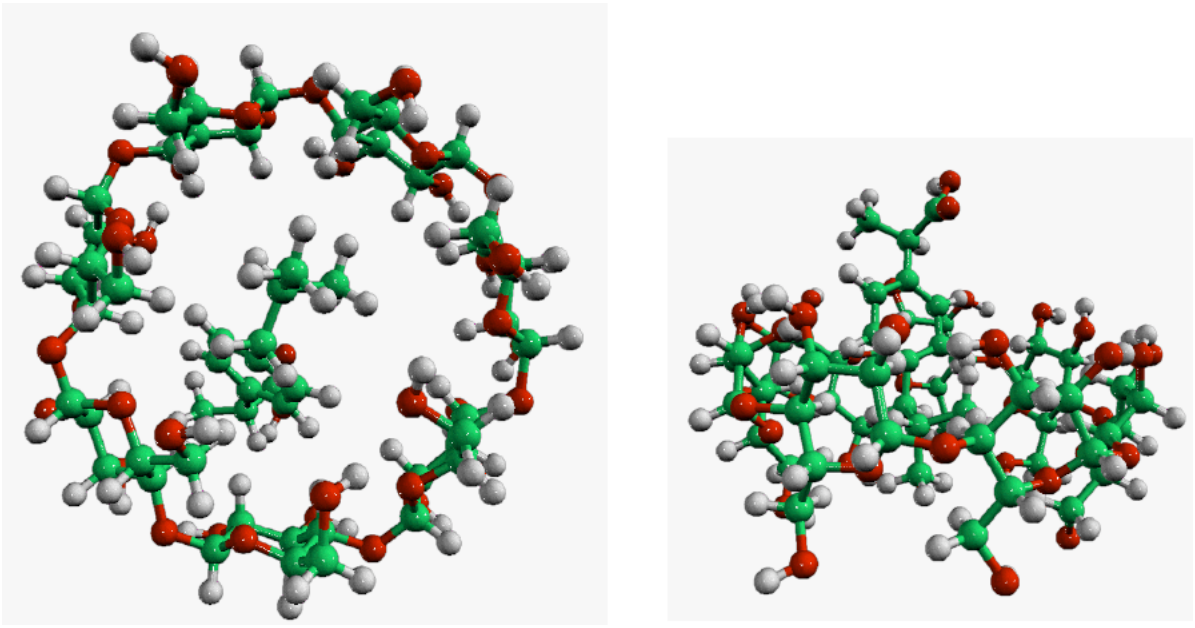
Figure 3: a. The average structures Lact_BCDAmp obtained using MM/PBSA analysis (without water molecules). Vertical view (left, dotted line represents the intermolecular hydrogen bond) and side view (right).

b. The average structures Phen_BCDAmp obtained using MM/PBSA analysis (without water molecules). Vertical view (left, dotted line represents the intermolecular hydrogen bond) and side view (right).

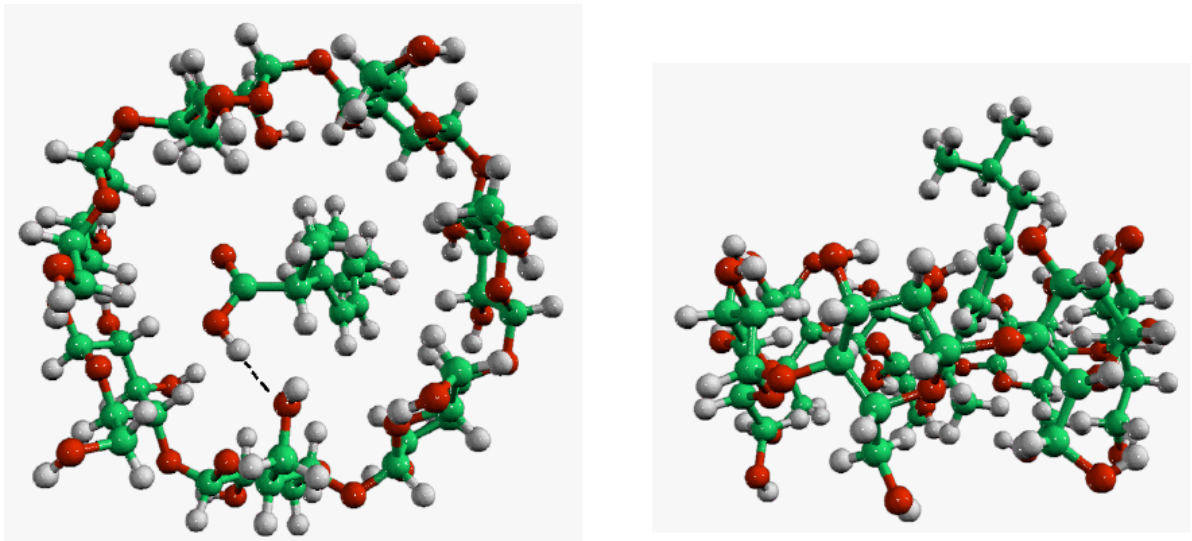
shown in Table **2**, the dispersion energy and interaction energy of Lact_BCDAmp are larger than those of Phen_BCDAmp; these results further clarify the large stabilization of Lact_BCDAmp.

3.2. β-CD + Ibuprofen

Consideration of these inclusion phenomena and the MM/PBSA results shows that Isob_BCDIbup is more stabilized (-17.3 kJ mol^{-1}) than Prop_BCDIbup and the van der Waals stabilization of Isob_BCDIbup is larger than that of Prop_BCDIbup. As shown in Figures **4a** and **4b**, ibuprofen in Isob_BCDIbup does not form intermolecular hydrogen bonds with the β-CD cavity. Prop_BCDIbup appears to form an intermolecular hydrogen bond on the primary hydroxyl side of β-CD, with the O-H distance of 0.09737 nm, the H···O distance of 0.20505 nm, and the O-H···O angle of 168.651°. The interaction energy of Prop_BCDIbup is larger than that Isob_BCDIbup, as shown in Table **2**. Conversely, the dispersion energy of Isob_BCDIbup is larger than that of Prop_BCDIbup. It seems that the affinity of the isobutyl group for the β-CD cavity is



(**a**)



(**b**)

Figure 4: a. The average structures Isob_BCDIbup obtained using MM/PBSA analysis (without water molecules). Vertical view (left) and side view (right).

b. The average structures Prop_BCDIbup obtained using MM/PBSA analysis (without water molecules). Vertical view (left, dotted line represents the intermolecular hydrogen bond) and side view (right).

Table 2: PIEDA Results for the Systems of β-CD + Drug. All Data are Given in kJ mol^{-1}

System	ΔG_{vdw}	ΔE_{MM}	$-T\Delta S$	$\Delta G_{gas}\,\Delta G_{solv}$	ΔG_{bind}
Lact_BCDAmp	-237.3	430.6	-93.7	-206.3	-106.7
Phen_BCDAmp	-109.4	290.0	-92.7	-156.3	-68.4
Isob_BCDIbup	-42.0	61.8	-33.9	-137.4	-151.5
Prop_BCDIbup	-97.2	67.7	-43.6	-132.7	-205.8

favorable since the inner walls of β-CD are considerably hydrophobic.

4. CONCLUSION

By considering these inclusion phenomena along with the MM/PBSA analysis results, we predict that a phenyl ring of ampicillin exists outside of the secondary hydroxyl side of β-CD and that the lactam ring exists inside the β-CD cavity. Further, we predict that the isobutyl group of ibuprofen exists inside the β-CD cavity and that the propanoic acid exists outside of the secondary hydroxyl side of β-CD. It seems that the inclusion occurs to give the form in which the hydrophobic group of the drug is not exposed to water. These observations were borne out by the dispersion forces determined using PIEDA. Thus, the lactam ring of ampicillin was inside the β-CD cavity in Lact_BCDAmp, which protected it from aggregation. This indicates that the inclusion complexes of ampicillin and β-CD would be useful as drug delivery systems. The structures of the complexes could be predicted using two different methods that contain no empirical parameters. The experimental Gibbs energies of β-CD + ampicillin[13] and β-CD + ibuprofen[21] are -17.3 kJ mol^{-1} and -23.4 kJ mol^{-1}, respectively. Thus, the theoretical values do not agree with experimental absolute values. In the future, we plan to perform thermodynamic integration calculations to quantitatively reveal the mechanism of interaction between CD and drugs in aqueous solutions.

APPENDIX

ΔG_{bind} = Binding free energy between CD and a drug to form a complex

ΔE_{MM} = Change in the gas phase MM energy

ΔG_{solv} = Solvation free energy

$-T\Delta S$ = Entropy term upon binding

$\Delta E_{internal}$ = Summation of bond, angle, and dihedral energies

ΔE_{els} = Electrostatic energy

ΔE_{vdw} = van der Waals energy

ΔG_{PB} = ΔG_{solv} calculated with the Poisson–Boltzmann (PB) method

ΔG_{SA} = ΔG_{solv} calculated from the solvent-accessible surface area

ΔG_{gas} = Interaction energy between the complex, β-CD, and the drug in the gas phase

ΔG_{solv}(drug) = Solvation free energy of the drug

ΔG_{solv}(β-CD) = Solvation free energy of β-CD

ΔG_{solv}(complex) = Solvation free energy of the complex

ΔE_{ES} = Electrostatic component of ΔE_{int}

ΔE_{EX} = Exchange-repulsion component of ΔE_{int}

ΔE_{CT} = Charge transfer component of ΔE_{int}

ΔE_{DI} = Dispersion component of ΔE_{int}

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