Monte Carlo Simulations on the Cyclosophoraose as a Host for the Complexation of Indomethacin

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Cyclosophoraoses are a class of unbranched cyclic β-(1→2)-D-glucans produced by Gram-negative bacteria, *Agrobacterium* and *Rhizobium* species, varying in size from 17 to 40 glycosyl units.[1,2] These molecules are known to be involved in the osmolarity regulation in response to external osmotic shock,[3,4] and the initial stage of root-nodule formation of *Rhizobium* species during the nitrogen fixation.[2] Throughout this process, cyclosophoraoses are suspected to be involved in complexation with various plant flavonoids. Much attention has been focused on their potential abilities to form inclusion complexes with other molecules.[5,6] Cyclosophoraoses have some advantages as host molecules on the conventional host molecule, β-cyclodextrin (β-CD) including lower toxicity and higher aqueous solubility.[5] The inclusion complex forming ability can be exploited commercially and is of interest to the pharmaceutical industry, where inclusion phenomena can be advantageously employed to improve the properties of drug molecules.

Computer-aided molecular modeling has become a powerful tool in analyzing the functions of biomolecules. In order to rationalize the application of cyclosophoraoses as host molecules, it would be very useful to be able to predict the structures and properties of the resulting complexes. In this study, the inclusion complex formation of cyclosophoraose and β-CD with indomethacin, an anti-inflammatory drug, was discussed by Monte Carlo (MC) docking simulations to search a possible mechanism of recognition and specificity of the host-guest complex formation. The purpose of this study was to determine energetically the most favorable mode(s) of binding between the host and guest molecule. The findings from this study will be employed in predicting the three-dimensional structures and complex formation abilities of various host-guest complexes.

Experimental Section

Molecular mechanics calculations were performed with InsightII/Discover program (version 97.0, Molecular Simulations Inc. U.S.A.) using consistent valence force field (CVFF)[7] on a SGI R4600 platform (Silicon Graphics Inc. U.S.A.).

As the three dimensional structures of cyclosophoraoses are currently not available, we constructed a molecular model of cyclosophoraose which consisted of 21 glycosyl units (Cys-21mer) referring to Palleschi and Crescenzi.[3] This Cys-21mer molecule was chosen because of its medium size among the family of cyclosophoraoses.[8] The molecular model

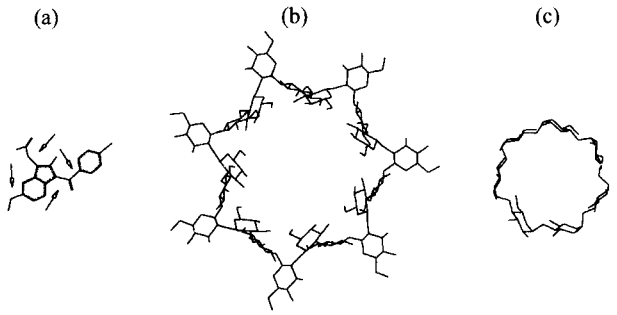

Figure 1. (a) Molecular model of indomethacin. The arrows indicate the four rotatable single bonds in MC docking simulations. (b) Molecular model of Cys-21mer proposed by Palleschi and Crescenzi. (c) Molecular model of β-CD.

of β-CD was obtained from the crystallographic geometry.[9] The Conformational search of indomethacin was performed by simulated annealing molecular dynamicsfull minimization strategy[10] and the lowest energy conformation was selected for the MC docking simulations. These molecular models were fully energy minimized before MC simulations. The conformations of these molecules are depicted in Figure 1.

The host and guest molecules were positioned in the neighborhood with a distance of ~15 Å. After 100 iteration conjugate gradient energy minimization, this configuration was used as the starting point for the following MC docking simulations. Several initial configurations were tried. In the course of trial to a new configuration, the guest molecule indomethacin could take transnational movement of maximum 7 Å to x, y, and z axis and rotation of maximum 180° around x, y, and z axis. For docking with guest molecule flexibly, the torsional angles of four single bonds could rotate upto 180° (see Figure 1). Total 10 degrees of freedom was present for this system (3 translational, 3 rotational, and 4 dihedral). Each cycle began with a random change of up to 5 degrees of freedom among them.[11] If the energy of the resulting host-guest system was within 1000 kcal/mol from the last accepted structure, the system was subjected to the 100 iterations of conjugate gradient energy minimization. The energy tolerance of 1000 kcal/mol was imposed to avoid significant overlap of van der Waals radii in the random move. The resulting structure was accepted based on two criteria: (a) energy check was the Metropolis criteria at the temperature of 300 K,[12] and (b) root mean squared displacement (RMSD) check was to compare the RMSD of a new structure against structures accepted so far. RMSD check was

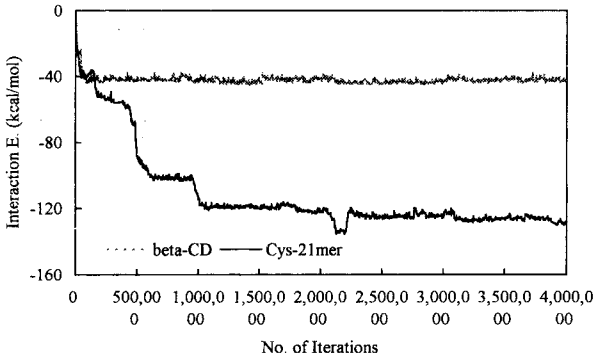

Figure 2. Energy profile of Metropolis Monte Carlo docking simulations. The interaction energy was defined as the difference between the sum of independently calculated energy of each host-guest molecule and the energy of each configuration in the process of MC docking simulation.

performed to eliminate the possibility of accepting a very similar configuration, and the configuration that was within 0.1 Å RMSD of pre-existing one was not accepted. The Monte Carlo simulation was performed until the complete energy convergence was reached. The non-bonded interactions were calculated by cell multipole method[13] and the dielectric constant was set to 1.

Results and Discussion

We chose a Monte Carlo technique because of its proven effectiveness in a wide range of molecular recognition problem. The pathways of Monte Carlo docking simulations showed a general tendency of inclusion complex formation. But it indicated that Cys-21mer could form energetically more stable inclusion complexes with indomethacin than β-CD did. The interaction energy was defined as the difference between the sum of independently calculated energy of each host-guest molecule and the energy of each configuration in the process of MC docking simulation.[10] Figure 2 compares the interaction energies in Monte Carlo runs for Cys-21mer-indomethacin and β-CD-indomethacin complexes. The Low energy conformations of Cys-21mer-indomethacin complex were found at -123.6±3.7 kcal/mol and those of β-CD-indomethacin complex were found at -42.1±1.3 kcal/mol, indicating that the inclusion complex formation of Cys-21 with indomethacin was energetically more favorable than that of β-CD. Figure 3 shows representative snapshots during the MC docking simulations. Indomethacin was fully embedded in the central cavity of Cys-21mer, whereas substantial part of it was exposed out of the cavity of β-CD. In the case of β-CD, two types of configurations were found according to the orientation of indomethacin. The indole ring of indomethacin was trapped in the cavity of β-CD in Type I and the chlorophenyl ring was trapped in the cavity in Type II. Both types appeared alternately in the course of MC simulation. To guarantee a global solution, several docking simulations with different initial positions of the guest molecule were done. But similar results were found.

The lower interaction energies of Cys-21mer-indomethacin complexes could be possibly caused by the bigger and flexible inner cavity of Cys-21mer compared with that of β-CD. For β-CD-indomethacin complex, electrostatic interaction energy occupied -13.7 kcal/mol and van der Waals interaction energy occupied -31.9 kcal/mol out of the total interaction energy (-45.1 kcal/mol) of the lowest energy configuration (Figure 3B (c)). For Cys-21mer-indomethacin complex, electrostatic interaction energy occupied -59.8 kcal/mol and van der Waals interaction energy occupied -77.6 kcal/mol out of the total interaction energy (-135.6 kcal/mol) of the lowest energy configuration (Figure 3A (d)). The inner cavity of Cys-21mer provided stronger intermolecular energy for both the electrostatic and the van der Waals interactions compared with that of β-CD. Internal energies were raised in the course of inclusion complex formation but the values were very small compared with nonbonded interaction energies (0.5 kcal/mol for β-CD and 1.8 kcal/mol Cys-21mer complex cited above).

The RMSD of host molecules from the initial conformations during the MC runs showed that the molecular conformation of Cys-21mer changed much whereas that of β-CD kept almost unchanged. These results indicated that Cys-21mer itself arranged its molecular conformation for the preferential energetic stability during the MC runs, whereas β-CD showed no critical conformational variations. Thus, it

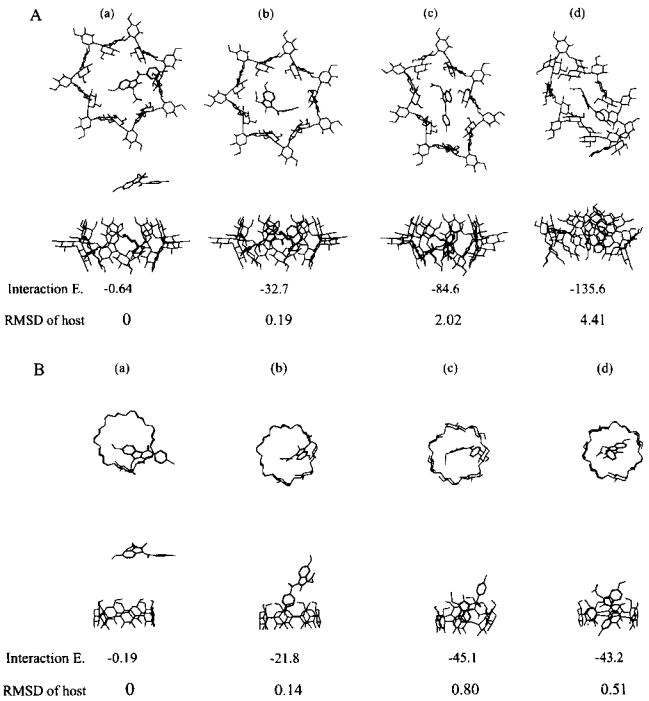

Figure 3. (A) Snapshots of Cys-21mer-indomethacin complex during the MC docking simulation. The interaction energies (kcal/mol) and RMSDs (Å) of the hosts from the initial conformation were displayed below each configuration. (a) The initial configuration. (b) At iteration 30,100. (c) At iteration 496,900. (d) The lowest energy configuration. (B) Snapshots of β-CD-indomethacin complex during the MC docking simulation. (a) The initial configuration. (b) At iteration 14,800. (c) Type I, at iteration 1,521,400. The lowest energy configuration. (d) Type II at iteration 2,796,700.

could be pointed out that Cys-21mer formed the inclusion complex much more dynamically than β-CD did. Flexibility of the dihedral angles of glucose units in the complexed Cys-21mer allowed conformational changes to fit indomethacin closely in the cavity for further energetic preferential stability. Therefore, the recognition process of Cys-21mer could be explained by the "induced fit" model and the flexibility of Cys-21mer would be essential for effective inclusion complex formation. The conformational flexibility of Cys-21mer may provide larger number of accessible states. This means that the entropic term (-TΔS) of Cys-21mer-indomethacin complex formation is also favorable than that of β-CD to give larger free energy change (ΔG), as well as the energetic term.

The result from the MC docking approach suggests a practical computational approach for the assessment of the preferences of host-guest complex formation, and a systematic prediction method of the inclusion complex forming abilities of cyclosophoraoses with many other guest molecules compared with that of β-cyclodextrin. This information will be invaluable both for the theoretical determination of the usefulness of cyclosophoraoses as host molecules and for the computational selection of guest molecules for cyclosophoraoses.

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