초임계 이산화탄소에서 Lipase에 의한 Racemic Naproxen의 Esterification 반응에 관한 모델링 및 실험적 확인

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Modeling and Experimental Verification of Lipase-catalyzed Enantioselective Esterification of Racemic Naproxen in Supercritical Carbon Dioxide

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INTRODUCTION

Supercritical carbon dioxide ($SCCO_2$) is a new reaction media and offers several advantages as an alternative to organic solvents [1]. Especially, enzymatic reactions in $SCCO_2$ [2] can be achieved with high efficiency, resolution and selectivity compared with conventional organic solvents. Some attempts have been made [3, 4] to calculate enantioselectivity by experiments, with fair prediction of fast-reacting enantiomer. Recently, Kwon [5] proposed a method to analyze catalytic reaction by *Candida antartica* lipase (CALB) using a simplified model for quantum mechanical and molecular dynamics studies. In this study, simplified quantum mechanical calculations have been performed to identify energies and configurations of whole reaction pathways for CALB and racemic naproxen in $SCCO_2$. Also molecular dynamic simulations were performed to identify selectivity of (S)-form and (R)-form eantiomer. The calculation results were compared with experimental data in qualitative manner using racemic naproxen as a substrate.

MATERIALS AND METHODS

1) Preparation of the enzyme and the substrates

The active site of CALB is illustrated in Figure 1(a). Positions of all non-hydrogen atoms in active sites were held constant and positions of hydrogen atoms were optimized during the calculations.

Naproxen was used as a substrate to investigate the reaction pathway and the selectivity of the lipase-catalyzed reaction. (R)-, (S)-naproxen used in this study are described in Figure 1 (b-1, b-2).

(a) **(b-1)** **(b-2)**

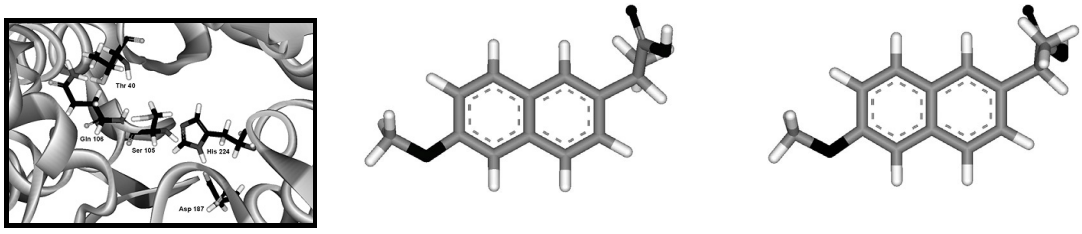

Figure 1. The structures of the enzyme and the substrates **(a)** Close-up of active sites of *Candida Antarctica* Lipase B structure, **(b-1)** Substrate of (R)-naproxen, **(b-2)** Substrate of (S)-naproxen.

2) Quantum mechanical and molecular dynamics simulation

The energy values and molecular conformations were also calculated in this study by optimizing the molecular structure of each system. In order to perform this simulation, the CHARMM force fields were used with Accerlys DS Modeling 1.7 software for MD simulation.

RESULTS AND DISCUSSION

1) Simulation of enzymatic reaction in $SCCO_2$

Several studies have reported the behaviors of in compressed supercritical fluids including carbon dioxide according to the exceptionally low values of the dielectric constant, typically 1.5. In this work, the dielectric constant (1.5) is used for carbon dioxide compared with water (78.4). This mechanism was consisted of an esterification reaction by CALB in $SCCO_2$ to make (R, S) naproxen ethyl ester. Then, the minimization energy value and the reaction pathway in $SCCO_2$ are compared with those in vacuum and water in Figure 2 (a), respectively. As shown in this figure, the minimization energy value in $SCCO_2$ is lower than in vacuum and in water, in other words, enzyme reaction velocity in $SCCO_2$ is still faster than other solvent's conditions due to the high diffusivity.

To identify the conformational preference of (R, S)-forms, the hydrogen bond lengths were examined on seven parts (from ① to ⑦) of the tetrahedral intermediate (7th reaction step) on the basis of function-based subset in Figure 2 (b-1, b-2). The significant hydrogen bonding in the active site was studied by analysis of MD results, and we found that the (S)-form ester was more stable than the (R)-form ester in Figure 3.

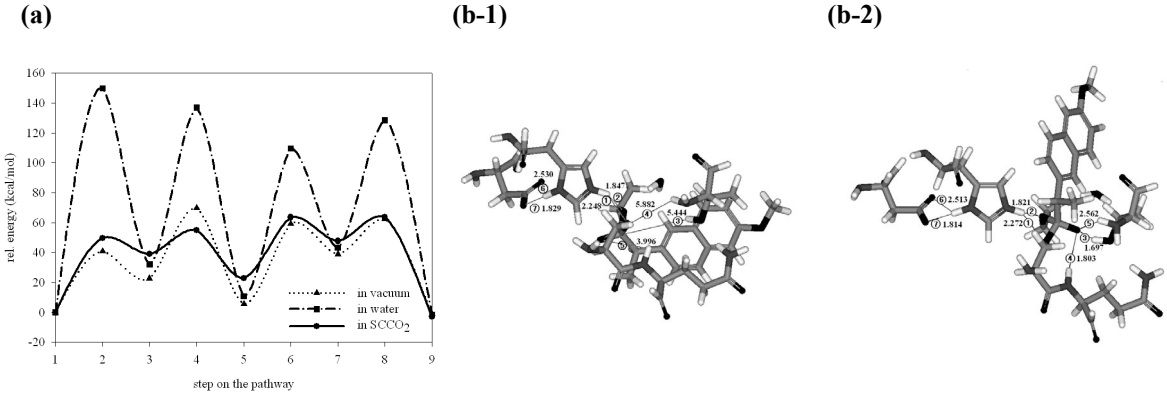

Figure 2. (a) Complete reaction pathway by the proposed reaction mechanism. **(b-1)** Structures of the sites of significant hydrogen bonding in the CALB-(R)-naproxen ethyl ester; **(b-2)** in the CALB-(S) naproxen ethyl ester.

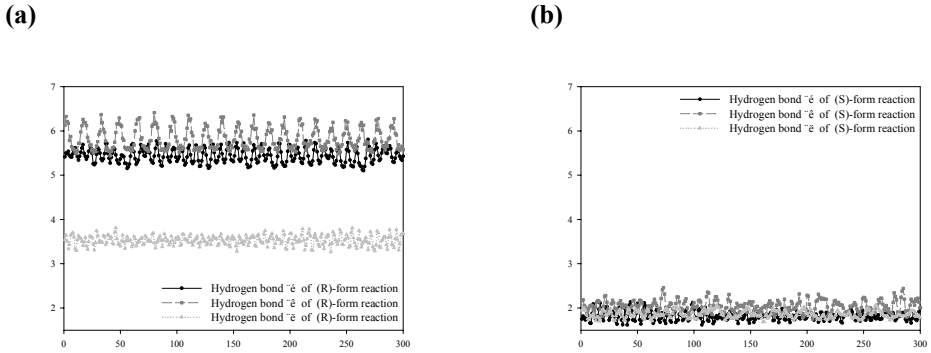

Figure 3. Trajectories and histograms of the hydrogen bonds (③, ④ and ⑤) of CALB-(R, S)-ethyl ester **(a)** (R)-naproxen ethyl ester, **(b)** (S)-naproxen ethyl ester.

2) Experimental verification of molecular dynamics study

In the study of molecular modeling simulation, CALB-(S) form naproxen ethyl ester was more stable than CALB-(R) form. For experimental verification of molecular modeling calculation, esterification reaction was carried out with racemic naproxen by CALB in ambient condition and supercritical condition. Experiments were performed in a high-pressure cell at 325.15 K and 130 bar for 5 hrs in supercritical carbon dioxide, and 48 hrs in the ambient condition with a stirring rate of 150 rpm, 7 g of enzyme amount and 2 % water content. The esterification reaction products were analyzed by HPLC using a chiralcel OD column.

Conversion yields of (S)-naproxen ethyl ester of the catalyzed reaction supercritical carbon dioxide were compared with those at ambient condition. In figure 4(a), (b), the conversion yields of (S) and (R) form in $SCCO_2$ were represented 86.6 % and 17.3 % (a), respectively. In the ambient condition, conversion yield of the esterification reaction were shown 43.6 % and 6.4 % (b), respectively. This results shows higher production of (S) form than (R) form and it also prove that hydrogen bonds of CALB-(S) form enantiomer complex of were more stable than CALB-(R) form enantiomer complex.

The conversion yield of (S)-form ester in $SCCO_2$ were compared with those in ambient condition as 86.6 %, 43.6 %, respectively as shown in figure 4(c). According to these results, we could also verify that the productivity in $SCCO_2$ condition is much higher than those in the ambient condition.

(a) **(b)** **(c)**

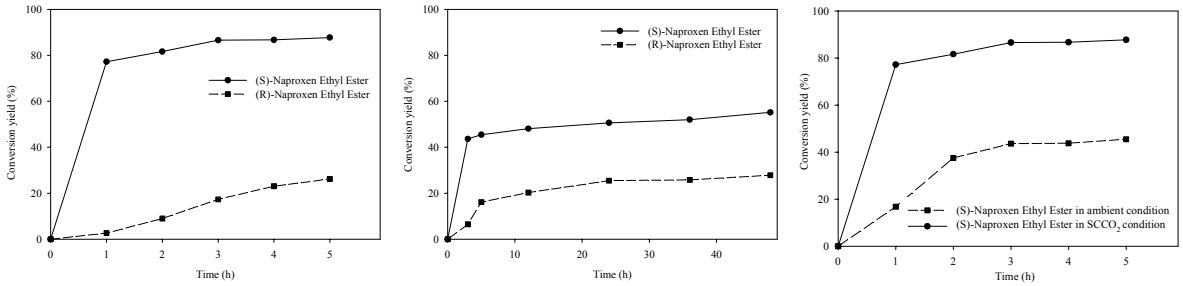

Figure 4. Comparison of the Productivity by the Experimental Conditions **(a)** Esterification of racemic naproxen in $SCCO_2$ at 323.15 K and 130 bar. **(b)** Esterification of racemic naproxen in ambient condition. **(c)** Comparison of (S)-naproxen ethyl ester productivity: esterification of racemic naproxen in $SCCO_2$ and atmospheric pressure.

Conclusion

Quantum mechanical and molecular dynamics simulation analysis has been performed on the CALB- naproxen complex in $SCCO_2$. From the analysis of the trajectories and histograms of the molecular dynamics simulation, the enantioselectivity of lipase could be explained by calculating the binding energy and structural characteristics. These results shows higher production of (S) form than (R) form and it also prove that hydrogen bonds of CALB-(S) form complex were more stable than CALB-(R) form enantiomer complex.

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