Biomolecular Chemical Simulations toward Elucidation of the Enantioselectivity and Reactivity of Lipases in Organic Synthesis

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Abstract

We are presently continuing to perform biomolecular chemical simulations for *Burkholderia cepacia* lipase (BCL) and *Candida antarctica* lipase typeB (CALB) to predict their enantioselectivity and reactivity toward various organic compounds. Here, we describe molecular dynamics (MD) and fragment molecular orbital (FMO) calculations on the complexes of CALB with primary and secondary alcohol esters. For esters with high enantioselectivity, the fast-reacting enantiomer of esters is located near the active site of CALB, whereas the slow-reacting enantiomer of esters moves away from the active site of CALB. On the other hand, for the esters with low enantioselectivity, we found that both (R)- and (S)-enantiomers of esters remain the active site of CALB. The FMO computations indicate that for the esters with high enantioselectivity, each fast-reacting enantiomer shows strong interactions with some particular amino acid residues, including Thr40, whereas for the esters with low enantioselectivity, both (R)- and (S)-enantiomers interact with identical amino acid residues including Thr40. It is predictable that Thr40 in CALB plays an important role in the chiral recognition of enantiomers through lipase-catalyzed biotransformations.

Key Words: MD calculation, FMO calculation, lipase, enantioselectivity, reactivity, organic synthesis, alcohol esters, interaction energy

Area of Interest: Molecular recognition and molecular modeling
1. Introduction

Lipase enzymes owing to their ease of use are now widely utilized as environmentally friendly and efficient biocatalysts capable of achieving chemo-, regio-, and enantioselective reactions under extremely mild conditions. Our group has investigated the enantioselectivity of such enzyme lipases as *Burkholderia cepacia* lipase (BCL) and *Candida antarctica* lipase typeB (CALB) through their biocatalytic synthetic reactions with lipases [1-5]. The degree of enantioselectivity is given by an experimentally obtained $E$ value that is an enantiomeric ratio for each lipase-catalyzed reaction [6]. As the $E$ value increases, enantioselectivity becomes higher. Although a large amount of experimental data, involving our results, is available for both BCL and CALB lipases, the mechanism responsible for their enantioselectivity is still not completely elucidated.

Recent advances in computational technology and molecular simulation software make it possible for organic chemists and biochemists to efficiently perform *ab initio* quantum chemical computations for biopolymer compounds as well as for small organic compounds. Over the last several years, we have been focusing our attention on biomolecular chemical simulations of the complexes of lipases with organic compounds to elucidate the enantioselectivity and reactivity of these enzymes in organic synthesis. We have recently shown that both molecular dynamics (MD) and fragment molecular orbital (FMO) calculations are useful means for the prediction of the enantioselectivity of BCL toward a variety of primary and secondary alcohol esters [7]. In this paper, we performed MD and FMO calculations toward CALB complexes with eight different primary alcohol esters (Figure 1) and thirteen different secondary alcohol esters (Figure 2).

![Figure 1. $E$ values of CALB toward eight different substrate primary alcohol esters 1-8 [8]](image)

![Figure 2. $E$ values of CALB toward thirteen different substrate secondary alcohol esters 9-21](image)
Experimentally obtained $E$ values of CALB toward substrate primary and secondary alcohol esters are shown in Figures 1 and 2, respectively. As shown in Figures 1 and 2, CALB shows high enantioselectivity toward secondary alcohol esters 9-21, whereas for primary alcohol esters 1-8, CALB gives low enantioselectivity. For the secondary alcohol esters with high enantioselectivity, it is observed that the fast reacting enantiomer of esters 9-20 is $(R)$-enantiomer and that of ester 21 is $(S)$-enantiomer. The purpose of this study is to clearly predict lipase enantioselectivity and the extent of the enantioselectivity on the basis of biomolecular computations. This work also aims to elucidate the mechanism for enantiomeric recognition of enzyme lipases.

2. Computational Methods

The structures of $(R)$- and $(S)$-enantiomers of primary and secondary alcohol esters were built by using GaussView. The structures of all molecules were optimized by two steps quantum chemical calculations at both the HF/6-31G* level and the B3LYP/6-31G* level with Gaussian 03 [9]. The X-ray crystallographic structure of CALB was obtained from the Protein Data Bank (PDB ID: 1LBS [10]). CALB is made from a single polypeptide chain bearing 317 amino acid residues. The active-site amino acid residue of CALB is Ser105 and the catalytic triad comprises Ser105, Asp187, and His224. We used AMBER11 [11] to construct CALB complexes with the optimized esters and to carry out the energy minimizations. Each ester is manually placed near the active site Ser105 of CALB (Figure 3). The structures of CALB-ester complexes were solvated with TIP3P model water molecules within 8.0Å of the respective complexes. Counterions were placed to neutralize the systems. Energy minimizations in the presence of the TIP3P model water molecules were performed.

**Figure 3.** Complex of CALB and $(R)$-enantiomer of secondary alcohol ester 9

Color code: green C, red O, blue N, white H, yellow Cys.
2.1 Molecular Dynamics Calculations

After energy minimization, CALB-ester complexes with TIP3P model water molecules were subsequently subjected to an MD calculation over a period of 2000ps at 300K by using AMBER11 with a GPGPU calculation system. A periodic boundary condition was applied by controlling the pressure. The temperature was kept constant by using the weak-coupling algorithm [12] with a coupling time of 1.0ps. Only bond lengths involving hydrogen atoms are constrained by the SHAKE method [13]. The non-bonded interactions were calculated by a cutoff method. The distance of the cutoff was 10.0Å. The time step for the MD calculation was 1fs. The whole system temperature was gradually increased by heating to 300 K for 50ps and kept at 300 K for the next 2000ps.

We estimated the C-O interatomic distance, $R_{\text{C-O}}$, between the carbonyl carbon of esters and the oxygen of the active site Ser105 side chain OH in each CALB-ester complex (Figure 4) by using VMD (Visual Molecular Dynamics) Version 1.8.6 [14], and examined the time dependence of the C-O interatomic distance during the MD trajectory.

![Diagram showing C-O interatomic distance between the carbonyl carbon of esters and the oxygen of the active site Ser105 side chain OH in CALB-ester complex.]

**Figure 4.** C-O interatomic distance between the carbonyl carbon of $(R)$-enantiomer of the secondary alcohol ester 9 and the oxygen of the active site Ser105 side chain OH in the CALB-ester complex.

Color code: green C, red O, blue N, white H.

2.2 Fragment Molecular Orbital Calculations

After MD calculations, the surrounding TIP3P model water molecules and counterions were removed and the resulting complexes were subsequently subjected to an FMO calculation at the FMO2-MP2/6-31G level with the ABINIT-MP/BioStation program which was developed by Nakano et al. [15-17]. The ABINIT-MP/BioStation program can be obtained from a Website [18]. We computed the IFIE (InterFragment Interaction Energy) in order to examine the interactions between esters and amino acid residues in CALB. In our FMO calculations, each amino acid residue of CALB was treated as a single fragment. On the other hand, the esters were not divided. The computations were performed under vacuum conditions.
3. Results and Discussion

3.1 Molecular Dynamics Calculations

For the secondary alcohol esters that have a large $E$ value ($E > 150$), MD calculations show that the C-O interatomic distance, $R_{C-O}$, for the fast reacting enantiomers, such as the (R)-enantiomer of esters 9-20 and (S)-enantiomer of ester 21, remains roughly unchanged, while $R_{C-O}$ for the slow reacting enantiomers, such as the (S)-enantiomer of esters 9-20 and (R)-enantiomer of ester 21, increases with the elapsed time. This result indicates that for esters with high enantioselectivity, the fast reacting enantiomer of esters is located near the active site of CALB, whereas the slow reacting enantiomer of esters moves away from the active site of CALB. On the other hand, for esters with a small $E$ value ($E < 50$), such as primary alcohol esters 1-8, we found that both (R)- and (S)-enantiomers of esters remain the active site of CALB. Similar MD calculations were performed for some initial structures, and we obtained the same results mentioned above. Figure 5 shows the time dependence of $R_{C-O}$ in the complexes of CALB with the (R)- and (S)-enantiomer of primary alcohol ester 1, 5 and secondary alcohol ester 9, 14 as an example.

![Figure 5](image.png)

**Figure 5.** Time dependence of $R_{C-O}$ in the complexes of CALB with the (R)- and (S)-enantiomers of primary alcohol ester 1, 5 and secondary alcohol ester 9, 14

In addition, we estimated the difference in $R_{C-O}$ between the (R)- and (S)-enantiomer complexes, $\Delta R_{C-O}$, at 2000ps of the MD trajectory. For the esters with a large $E$ value, $\Delta R_{C-O}$ was more than
5.0Å, whereas for the esters with a small $E$ value, $\Delta R_{C-O}$ was less than or equal to 3.5Å. All esters showed the above behavior. Figure 6 shows the relationship between the experimentally obtained $E$ value and $\Delta R_{C-O}$ for primary alcohol esters 1-8. As shown in Figure 6, the values of $\Delta R_{C-O}$ for each ester are strongly correlated to the $E$ values for the corresponding esters.

**Figure 6.** Relationship between the $E$ value and $\Delta R_{C-O}$ for primary alcohol esters 1-8

### 3.2 Fragment Molecular Orbital Calculations

For a detailed discussion on the interactions between esters and CALB, the IFIE values between esters and the selected amino acid residues in CALB were calculated at the FMO2-MP2/6-31G level. As shown in our previous works [7, 19], the IFIE analysis is a useful method for gaining detailed information on protein-ligand binding. For the esters with high enantioselectivity, each fast
reacting enantiomer showed a strong interaction with Thr40 in CALB. In addition to the interaction with Thr40, it was found that each fast reacting enantiomer interacted with some particular amino acid residues in CALB such as Gly41, Gln106, Asp134, Gly157, Asp187, and Ile189. These amino acid residues are located near the active site of CALB. In contrast, the interactions of each slow reacting enantiomer with these amino acid residues were very weak. The reason for this result is considered to be that the slow reacting enantiomer of esters departs from the active site of CALB. In this paper, we omitted showing the IFIEs for the slow reacting enantiomer. Figure 7 shows the IFIE values between the fast reacting enantiomer and the selected amino acid residues in CALB. As an example, the structures of the fast reacting enantiomers of ester 10, 14 and the interacting amino acid residues are illustrated in Figure 8. The IFIE between (R)-10 and Thr40 is larger than that between (R)-14 and Thr40. The methyl group of (R)-10 orients to Thr40, whereas that of (R)-14 does not face Thr40.

On the other hand, for esters with low enantioselectivity, both (R)- and (S)-enantiomers interact...
with the identical amino acid residues, including Thr40, and the interactions of both enantiomers with amino acid residues in CALB were similar to each other in their intensities and patterns except for primary alcohol ester 7. The IFIE values between the (R)- and (S)-enantiomers and the selected amino acid residues in CALB are shown in Figures 9 and 10, respectively. The value of IFIE between the (R)-enantiomer of alcohol ester 7 and Thr40 is rather large compared with that between the (S)-enantiomer and Thr40. The selectivity of CALB toward the primary alcohol ester 7 might be moderate rather than low. Figure 11 shows the structures of the (R)- and (S)-enantiomers of ester 2 and the interacting amino acid residues, as an example. IFIE between (S)-2 and Thr40 is slightly larger than that between (R)-2 and Thr40. The acetyl group of (S)-2 orients to Thr40, whereas that of (R)-2 does not face Thr40.

Interestingly, regardless of the extent of enantioselectivity, we hardly observed interactions between each enantiomer and the active site amino acid residue Ser105. These FMO computational results suggest that the IFIEs revealed in the present calculations can also be related to the enantioselectivity of CALB toward the primary and secondary alcohol esters observed by synthetic investigations.
4. Conclusion

To predict the lipase enantioselectivity and the extent of enantioselectivity toward non-natural organic compounds, for the complexes of CALB and eight different primary and thirteen different secondary alcohol esters, we performed the MD calculation over a period of 2000 ps and estimated the C-O interatomic distance between the carbonyl carbon of esters and the oxygen of the active site Ser105 side chain OH for each CALB-ester complex. The FMO simulations carried out on the CALB-ester complex structures obtained by MD calculations were subsequently carried out and the IFIE between esters and amino acid residues in CALB were calculated.

From our MD calculations, we found that the difference in the C-O interatomic distance between (R)- and (S)-enantiomer complexes, $\Delta R_{C-O}$, for the esters with high enantioselectivity (large $E$ value) was larger than that for the esters with low enantioselectivity (small $E$ value). Additionally, for the esters with low enantioselectivity, $\Delta R_{C-O}$ can be correlated to the experimentally observed $E$ value for each ester. Our computational results indicate that it is certainly possible to predict the CALB enantioselectivity and the extent of enantioselectivity.

Another aim of our study is to elucidate the mechanism of enantiomeric recognition of enzyme lipases. For the esters showing high enantioselectivity, it is also found that each fast-reacting enantiomer strongly interacts with particular amino acid residues including Thr40 in CALB. On the contrary, each slow reacting enantiomer showed very few interactions with these amino acid residues. The reason for this result is considered to be that the slow reacting enantiomer of esters with high enantioselectivity moves away from the active site of CALB. In contrast, for the esters bearing low enantioselectivity, we observed that both (R)- and (S)-enantiomers interact with the same amino acid residues, including Thr40. The IFIE for the amino acid residue Thr40 is particularly noteworthy. It is predictable that Thr40 can play an important role in the chiral recognition of enantiomers by CALB. Although the reasons why there are few interactions between esters and the active site amino acid residue Ser105 in CALB are not clear, the additional FMO calculations with higher accuracy, such as the FMO3 or FMO4 calculations for CALB-ester complexes, might give detailed information on the interactions between esters and amino acid residues in CALB. Otherwise, we may need to examine the structure changes of complexes and the time change of IFIE between esters and Ser105. Moreover, we made CALB-ester complexes manually by using AMBER in the present work, but we are planning to carry out a docking simulation for constructing CALB-ester complexes. Our biomolecular simulations including the MD calculation and FMO calculations may enable us to elucidate the mechanism responsible for enantiomeric recognition of enzyme lipases. We have reached a conclusion that biomolecular chemical simulations are useful tools for predicting and understanding the reactivity and the enantioselectivity of lipase-catalyzed biotransformations.

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