Analysis of the Conformational Stability and Activity of Candida antarctica Lipase B in Organic Solvents

INSIGHT FROM MOLECULAR DYNAMICS AND QUANTUM MECHANICS/SIMULATIONS

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The conformational stability and activity of Candida antarctica lipase B (CALB) in the polar and nonpolar organic solvents were investigated by molecular dynamics and quantum mechanics/molecular mechanics simulations. The conformation change of CALB in the polar and nonpolar solvents was examined in two aspects: the overall conformation change of CALB and the conformation change of the active site. The simulation results show that the overall conformation of CALB is stable in the organic solvents. In the nonpolar solvents, the conformation of the active site keeps stable, whereas in the polar solvents, the solvent molecules reach into the active site and interact intensively with the active site. This interaction destroys the hydrogen bonding between Ser¹⁰⁵ and His²²⁴. In the solvents, the activation energy of CALB and that of the active site region were further simulated by quantum mechanics/molecular mechanics simulation. The results indicate that the conformation change in the region of active sites is the main factor that influences the activity of CALB.

Because of its high enantioselectivity and catalytic activity, wide range of substrates, and thermal stability, Candida antarctica lipase B (CALB)³ is widely used in many industrial applications and scientific researches (1). CALB is composed of 317 amino acid residues and has a molecular mass of 33 K (2). Similar to other serine hydrolyzes, a serine-histidine-aspartate catalytic triad is responsible for the catalytic activity of CALB. The mechanism is outlined in Fig. 1. It is a two-step mechanism with an acylation step and a deacylation step separated by a covalent acyl-enzyme intermediate (3).

The activity of the enzymes is strongly affected by the choice of solvent (4–7). As a matter of fact, even reversal of substrate specificity (8, 9) and enantipreference (10, 11) has been reported. A higher thermostability and altered stereoselectivity for CALB in organic solvents have also been observed (12, 13). Many researchers have put effort into elucidating the underlying mechanisms responsible for the observed solvent effects. The most widely accepted model was described by Laane (14), who summarized the influence of organic solvents on the enzymatic reactions and concluded that the enzyme activity is higher in the environment surrounded by nonpolar and mid-polar solvents, whereas the lowest activity is expressed in polar solvents. The Laane model has been widely used in solvent selection in enzymatic reactions. However, the Laane model does not describe the mechanisms of solvent effect on a molecular level.

Molecular dynamics simulations have been proven to be a useful tool in understanding protein structure and have been used to get insights into the structure and behavior of the enzymes (15–18). The effect of solvents on the activity of CALB might be the results of the conformational change around the activity site or some particular area. In this work, the overall conformational change of CALB and the local conformational change around the active site in polar and nonpolar organic solvents were investigated through molecular dynamics simulation. Through constructing the tetrahedral intermediate model, the solvent effect of the conformation change on the activity of CALB was investigated by quantum mechanics (QM)/molecular mechanics (MM) simulation.

MATERIALS AND METHODS

General Simulation Approach—The crystal structure of CALB was solved in six solvent boxes: methanol, acetone, tetrahydrofuran, chloroform, cyclopetane, and hexane. Molecular dynamics simulations of the protein-solvent system were performed using the AMBER 10 program package (19) and the all-atom amber force field ff99 (20). The structural models of tetrahedral intermediates were constructed using the last structures of the equilibration systems with its natural substrate 3-methyl-2-butyl acetate. The QM/MM simulations of the tetrahedral intermediate systems were performed using the AMBER 10 program package (19) with the active site of the enzyme and the substrate described by a QM Hamiltonian.

Protein Structure Used—The structure from Candida antarctica (Protein Data Bank code 1LBS) (21) taken from the Protein Data Bank was used as the initial structure of CALB. The two sugar units, which are far away from the active site, were deleted, and the 92 water molecules were reserved. Protonation states of titratable groups Arg, Lys, Asp, His, and Glu were calculated at pH 7. For His²²⁴, the proton on ND1 was selected in the free enzyme simulations, whereas the protons on both

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³ The abbreviations used are: CALB, C. antarctica lipase B; QM, quantum mechanics; MM, molecular mechanics; RMSD, root mean square deviation.
ND1 and NE2 were selected in the simulation of tetrahedral intermediate.

Parameterization of Solvent Models—The organic solvent molecules of methanol, acetone, tetrahydrofuran, chloroform, cyclopentane, and hexane were parameterized. The geometric parameters were derived by \textit{ab initio} geometry optimization on the HF/6–31G* level using Gaussian 98 (22) in the gas phase. The partial charges were derived by fitting partial charges using the RESP program (23) of AMBER 10 to the electrostatic potential. For each solvent, a periodic solvent box was created and equilibrated by molecular dynamics simulations. The calculated densities of the boxes in equilibrium were in good agreement with experimental data as shown in Table 1 (24, 25, 32).

Molecular Dynamics Simulations—The initial structures were energy-minimized (500 steepest descent and 500 conjugate gradient) and then heated gradually from 0 to 300 K in 20 ps with the positions of all of the calcium atoms restrained using a harmonic potential. Molecular dynamics simulations of the protein-solvent systems were performed at 300 K and 1 bar using a time step of 1 fs. The temperature and pressure of the system were controlled using a weak coupling to an external heat bath (26) with a temperature coupling constant of 1.0 ps and a pressure coupling constant of 1.2 ps. The simulations were carried out in a truncated octahedral box under periodic boundary conditions. Nonbonded interactions were calculated at a cut-off distance of 10 Å. The SHAKE algorithm (27) was applied to all bonds.

QM/MM Simulation—To evaluate the effect of conformation change induced by solvents on the activity of CALB, the tetrahedral intermediate model, which is a good approximation of transition state (28), was constructed by docking the substrate 3-methyl-2-butyl acetate into the active site of the enzyme manually. The tetrahedral intermediate systems were optimized (1000 steepest descent and 1000 conjugate gradient) and equilibrated with 20 ps of molecular dynamics simulation at 300 K and 1.0 bar, during which the QM/MM models were selected to handle the system. The QM part consisted of 25 atoms, including the substrate and the side chains of Ser105, Asp187, and His224. The rest formed the MM part. The QM part was described by the semiempirical model of AM1, and the MM part was described by the amber force field ff99.

Method for Evaluating Solvent Effect on the Activation Energy—To quantitatively evaluate the effect of solvents on CALB activity, the activation energy was calculated using the method described below. The process of enzyme reaction in the solvents is simplified as presented in Fig. 2. The ground state is defined as the state at which there is no interaction between the enzyme and the substrate.

The ground state energy \( E_{(G)}^A \) in solvent A was calculated according to Equation 1,

\[
E_{(G)}^A = E_{\text{sub}(G)}^A + E_{\text{enz}(G)}^A + E_{\text{sol}(G)}^A + E_{\text{sub-sol}(G)}^A + E_{\text{enz-sol}(G)}^A
\]

(Eq. 1)

TABLE 1

| Solvent          | Experimental density g/cm\(^3\) | Simulated density g/cm\(^3\) | Log \(P^*\) |
|------------------|--------------------------------|------------------------------|-------------|
| Methanol         | 0.79                           | 0.83                         | -0.74       |
| Acetone          | 0.79                           | 0.81                         | -0.24       |
| Tetrahydrofuran  | 0.89                           | 0.9                          | 0.46        |
| Chloroform       | 1.47                           | 1.48                         | 1.97        |
| Cyclopentane     | 0.75                           | 0.76                         | 3.00        |
| Hexane           | 0.65                           | 0.65                         | 4.00        |

\(P^*\), partition coefficient. \(\log P^* > 4\): nonpolar solvent; \(2 < \log P^* < 4\): midpolar solvents; \(\log P^* < 2\): polar solvents.
where $E_{\text{sub}(G)}$, $E_{\text{enz}(G)}$, and $E_{\text{sol}(G)}$ are the energies of substrate, enzyme, and solvent, respectively. $E_{\text{sub-sol}(G)}$ is the interaction energy between substrate and solvent, and $E_{\text{enz-sol}(G)}$ is the interaction energy between enzyme and solvent at ground state.

The transition state energy $E^{A}_{(T)}$ in solvent A was evaluated according to Equation 2,

$$E^{A}_{(T)} = E^{A}_{\text{sub}(T)} + E^{A}_{\text{enz}(T)} + E^{A}_{\text{sol}(T)} + E^{A}_{\text{sub-enz}(T)} + E^{A}_{\text{sub-sol}(T)} + E^{A}_{\text{enz-sol}(T)}$$

(Eq. 2)

where $E^{A}_{\text{sub}(T)}$, $E^{A}_{\text{enz}(T)}$, and $E^{A}_{\text{sol}(T)}$ are the energies of substrate, enzyme, and solvent at transition state, respectively. $E^{A}_{\text{sub-enz}(T)}$ is the interaction energy between substrate and enzyme, $E^{A}_{\text{sub-sol}(T)}$ is the interaction energy between substrate and solvent, and $E^{A}_{\text{enz-sol}(T)}$ is the interaction energy between enzyme and solvent at transition state.

The activation energy $\Delta E^A$ in solvent A was calculated according to Equation 3,

$$\Delta E^A = E^A_{(T)} - E^A_{(G)}$$

(Eq. 3)

Because the energy of solvent, the interaction energy between substrate and solvent, and the interaction energy between enzyme and solvent do not have much difference in the same solvent, Equation 3 is approximated by Equation 4,

$$\Delta E^A = (E^A_{\text{sub}(T)} + E^A_{\text{enz}(T)} + E^A_{\text{sol}(T)}) - (E^A_{\text{sub}(G)} + E^A_{\text{enz}(G)}) = E^{A}_{\text{tetra}} - (E^A_{\text{sub}(G)} + E^A_{\text{enz}(G)})$$

(Eq. 4)

where $E^{A}_{\text{tetra}}$ is the energy of tetrahedral intermediate, including the energy of substrate, the energy of enzyme, and the interaction energy between substrate and enzyme at transition state.

Similarly, the activation energy $\Delta E^B$ in solvent B was calculated according to Equation 5,

$$\Delta E^B = E^{B}_{\text{tetra}} - (E^B_{\text{sub}} + E^B_{\text{enz}})$$

(Eq. 5)

and the activation energy difference caused by changing from solvent A to solvent B can be evaluated by Equation 6.

$$\Delta E^A - \Delta E^B = \Delta E^A - \Delta E^B$$

(Eq. 6)
methanol, acetone, and tetrahydrofuran, the RMSD of backbone atoms became stable after 1 ns of simulation. In the nonpolar solvents chloroform, cyclopentane, and hexane, the RMSD of backbone atoms changed little after 0.5 ns of simulation. The average RMSD of backbone atoms increases as the polarity of the solvents decreases, as presented in Fig. 4.

The effect of solvents on the secondary structural change of CALB is shown in Fig. 5. The CALB in the solvents preserves ~90% of the $\beta$-sheets of the crystal structure. The preservation ratio of the $\alpha$-helix contents of CALB increases with the solvent polarity. In methanol, acetone, and tetrahydrofuran, the CALB preserves 88.8, 86.9, and 82.9% of the $\alpha$-helix of the crystal structure, respectively. In chloroform, cyclopentane, and hexane, 80.5, 79.7, and 78.7% of the $\alpha$-helix of the crystal structure are preserved, respectively. The $\alpha$-helix structure, which resides at the surface, is easier to destroy and more sensitive to the solvents. Although the $\beta$-sheet structure tends to reside in the inner region of a protein, the preserved $\beta$-sheet structure is higher than the preserved $\alpha$-helix structure.

Fig. 6a shows the surface area of CALB in the solvents. Fig. 6b shows the change of the surface area of CALB in the solvents in comparison with the surface area of the crystal structure (Protein Data Bank code 1LBS). The hydrophobic surface areas of CALB in the solvents are larger than that of the crystal structure, although the hydrophilic surface area of CALB decreases with the polarity of the solvents. In the nonpolar solvents, some
of the polar side chains, which are originally accessible to the solvents in the crystal structure, are buried into the interior of CALB, and the nonpolar side chains become exposed to the solvents. This results in the increase of the hydrophobic surface area and the decrease of hydrophilic surface area. Unlike the effects of the solvent on the surface area of the CALB, the radius of gyration of CALB changes slightly in the solvents, as shown in Fig. 7.

**The Local Conformation Change of CALB**—The active site of CALB is located at the bottom of a deep funnel as shown in Fig. 8, which is based on the crystal structure (Protein Data Bank code 1LBS). The conformation of the funnel plays an important role in the activity of CALB (29). To examine the effect of solvents on the conformation change of the funnel, the RMSD of the residues forming the funnel was calculated, and the results are shown in Fig. 9. The RMSD of the funnel in the nonpolar solvents is slightly higher than that in the polar solvents. The RMSD of the funnel in the organic solvents is below 1.0 Å, which means that the conformation of the funnel keeps stable in the organic solvents.

The average RMSD in the last 1 ns for each residue is shown in Fig. 10. In the polar solvents, most residues of CALB keep stable, and only small changes can be observed for the region 243–268. In the nonpolar solvents, the region 243–268 undergoes significant change with the value of the RMSD larger than 1.0 Å. The change of the region 243–268 in the nonpolar solvents is dramatic, but these residues are far from the active site as illustrated by Fig. 11, so it should not be the main reason for the activity change in the solvents.

The Effect of Solvents on the Active Site—The active site of CALB consists of Ser\(^{105}\), His\(^{224}\), and Asp\(^{187}\), as shown in Fig. 12. In the key step of the enzymatic reaction, Ser\(^{105}\) (nucleophile) attacks carbonyl carbon of the ester to form the transition state, and this attack is promoted by His\(^{224}\), which acts as the general base and accepts a proton from Ser\(^{105}\). The hydrogen bonding between OG (Ser\(^{105}\)) and NE2 (His\(^{224}\)) and the hydrogen bonding between OD2 (Asp\(^{187}\)) and ND1 (His\(^{224}\)) are very important for the stabilization of the transition state (30). The average distance between OG (Ser\(^{105}\)) and NE2 (His\(^{224}\)) is presented in Fig. 13. The hydrogen bonding between OD2 (Asp\(^{187}\)) and ND1 (His\(^{224}\)) is preserved well in the organic solvents. The hydrogen bonding between OG (Ser\(^{105}\)) and NE2 (His\(^{224}\)) is preserved well in the nonpolar solvents, but in polar solvents, the hydrogen bonding cannot be formed, as indicated by the distance between OG (Ser\(^{105}\)) and NE2 (His\(^{224}\)), which is larger than 3.5
Å. The Mulliken charge (31) of atom OG (Ser^{105}), which attacks carbonyl carbon of the ester to form the transition state in the step of the enzymatic reaction, was obtained by the QM/MM simulation, and the results are presented in Fig. 14. The OG atom was more negative in the nonpolar solvents than that in the polar solvents, which means that CALB attacks the ester more easily.

In the crystal structure (Protein Data Bank code 1LBS), there are two water molecules around the active site: Wat^{318} and Wat^{325}, as shown in Fig. 15. Wat^{318} interacts with Asp^{187} through the hydrogen bonding between OD2 (Asp^{187}) and O (Wat^{325}), and Wat^{318} interacts with Ser^{105} through the hydrogen bonding between O (Ser^{105}) and O (Wat^{318}). In the solvents, the two hydrogen bonds were monitored, and the results are presented in Fig. 16. The results indicate that the two hydrogen bonds are preserved well, so the crystal water molecules around the active site should not be responsible for the breakdown of the hydrogen bonding between OG (Ser^{105}) and NE2 (His^{224}) in polar solvents.

The organic solvent molecules around the active site were investigated, and the results are presented in Fig. 17. In the nonpolar solvents, the solvent molecules are 5 Å away from the active site, although in the polar solvents, some solvent molecules interact intensively with the active site. Hydrogen bonds between methanol (or acetone) molecules and Ser^{105} are found. The intensive interaction between the active site and the polar

![FIGURE 16. Distance between OD2 (Asp^{187}) and O (Wat^{325}) and the distance between O (Ser^{105}) and O (Wat^{318}) in the solvents calculated from the last 1-ns simulation. The abbreviations are as defined in the legend to Fig. 3.](image)

![FIGURE 17. Last snapshots of the active site of CALB and organic solvent molecules around the active site in the solvents. The abbreviations are as defined in the legend to Fig. 3.](image)
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The active site of CALB consists of Ser\textsuperscript{105}, His\textsuperscript{224}, and Asp\textsuperscript{187}. The hydrogen bond between OG (Ser\textsuperscript{105}) and NE2 (His\textsuperscript{224}) and the hydrogen bond between OD2 (Asp\textsuperscript{187}) and ND1 (His\textsuperscript{224}) are very important for the stabilization of the transition state of the enzymatic reaction. The results of the conformation change of the active site indicate that the breakdown of the hydrogen bond between OG (Ser\textsuperscript{105}) and NE2 (His\textsuperscript{224}) leads to the low activity of CALB in the polar solvents, and the breakdown of the hydrogen bond is due to the intensive interaction between the active site and the polar solvent molecules.

To evaluate the effect of conformation change induced by solvents on the CALB activity, the tetrahedral intermediate model was constructed and was evaluated by the QM/MM simulation. The results indicate that the activation energy of CALB and the activation energy of the active site region are lower in the nonpolar solvents than that in the polar solvents, and the effects of solvent on the active site region are the main cause of the difference in the activity of CALB.

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![FIGURE 18. Activation energy (a) and the activation energy of the active site region (b) in the solvents. The ratio of the activation energy of the active site region to the activation energy is shown (c). The abbreviations are as defined in the legend to Fig. 3.](image-url)
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