Molecular Dynamics Studies on T1 Lipase: Insight into a Double-Flap Mechanism

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T1 lipase is isolated from the palm *Geobacillus zalihae* strain T1 in Malaysia, functioning as a secreted protein responsible for the catalyzing hydrolysis of long-chain triglycerides into fatty acids and glycerol at high temperatures. In the current study, using 30 ns molecular dynamics simulations at different temperatures, an aqueous activation was detected for T1 lipase. This aqueous activation in T1 lipase was mainly caused by a double-flap movement mechanism. The double flaps were constituted by the hydrophobic helices 6 and 9. Helix 6 employed two major components with the hydrophilic part at the surface and the hydrophobic part inside. In the aqueous solution, the hydrophobic part could provide enough power for helix 6 to move away, driving the protein into an open configuration and exposing the catalytic triad. Our findings could provide structural evidence to support the double-flap movement, revealing the catalytic mechanism for T1 lipase.

I. INTRODUCTION

Due to their ability to catalyze the hydrolysis of long-chain triglycerides with the formation of diacylglyceride, monoglyceride, glycerol, and free fatty acids, lipases are of wide interest for biotechnological applications, such as detergents and food production as well as pharmaceuticals and industrial synthesis of fine chemicals.[1−7] In recent years, many good attempts have been made to determine the three-dimensional structures of lipases. By now, 133 crystal structures of bacterial, fungal, and mammalian lipases have been released in the RCSB Protein Data Bank.[8] All lipase structures share a common fold known as the α/β hydrolase fold.[9] The standard architecture of the α/β hydrolase fold has eight typically parallel β strands with the second one antiparallel, which are connected by α helices packing on either side of the β strands. The active site of these lipases contains the classical catalytic triad constituted of Ser, Asp/Glu, and His. Besides, these lipases share an interfacial activation mechanism. It is indicated by both experimental and computational studies that the interfacial activation occurs through flap or lid movements; it moves toward closing the hydrophobic binding pocket in aqueous surroundings and opening in an oil−water interface.[10,11]

The lipase isolated from palm *Geobacillus zalihae* strain T1 in Malaysia, the so-called T1 lipase, is a thermoalkalophilic extracellular lipase.[12] T1 lipase is a secreted protein with responsibility for catalyzing the hydrolysis of long-chain triglycerides into fatty acids and glycerol at high temperatures. It is reported that this enzyme is still stable with considerable enzyme activity at 60 °C and with maximal activity at 70 °C.[12] Like other lipases, T1 lipase conserves a catalytic triad composed of Ser−His−Asp to perform its catalytic function. However, after its crystal structure was determined,[13] it was found that the catalytic triad of T1 lipase was not detected in the protein surface. So, we supposed that T1 lipase might expose its catalytic triad or active site by significant structural transitions. Thus, molecular dynamics (MD) simulations were employed with aqueous surroundings at five different temperatures to provide structural evidence. Such simulation techniques have been successfully applied to solve problems of structural biology, providing detailed information at the atomic level.[13−15]

II. MATERIALS AND METHODS

The X-ray crystal structure of T1 lipase (PDB code 2dsn.pdb)[16] was used to construct the initial models for our MD simulations. All the MD simulations were performed with the GROMACS 3.3.3 package, with GROMACS force field parameters and periodic boundary conditions.[17,18] The initial models were solvated in 8.5 × 8.5 × 8.5 nm boxes with SPC waters and a space of 10 Å around the solute. To neutralize the redundant charges, six sodiums were added to the system. Then, the system was subjected to a 1000 step energy minimization with the steepest descents method. Finally, 30 ns MD simulations were performed at 30, 60, and 70 °C with NPT ensembles and at 100 °C with the NVP ensemble (at 100 °C the NPT ensemble is unstable). During our MD simulations, all bonds in the systems were constrained by the LINear Constraint Solver (LINCS) algorithm, and atom velocities for start-up runs were obtained according to a Maxwell distribution at 310 K. To maintain the simulated systems at a constant temperature and pressure, the Berendsen thermostat was applied with a coupling time of 0.1 and 1.0 ps. At least 3 MD trajectories with different starting vectors were generated. The particle mesh Ewald algorithm was employed to treat electrostatic interactions with inter-

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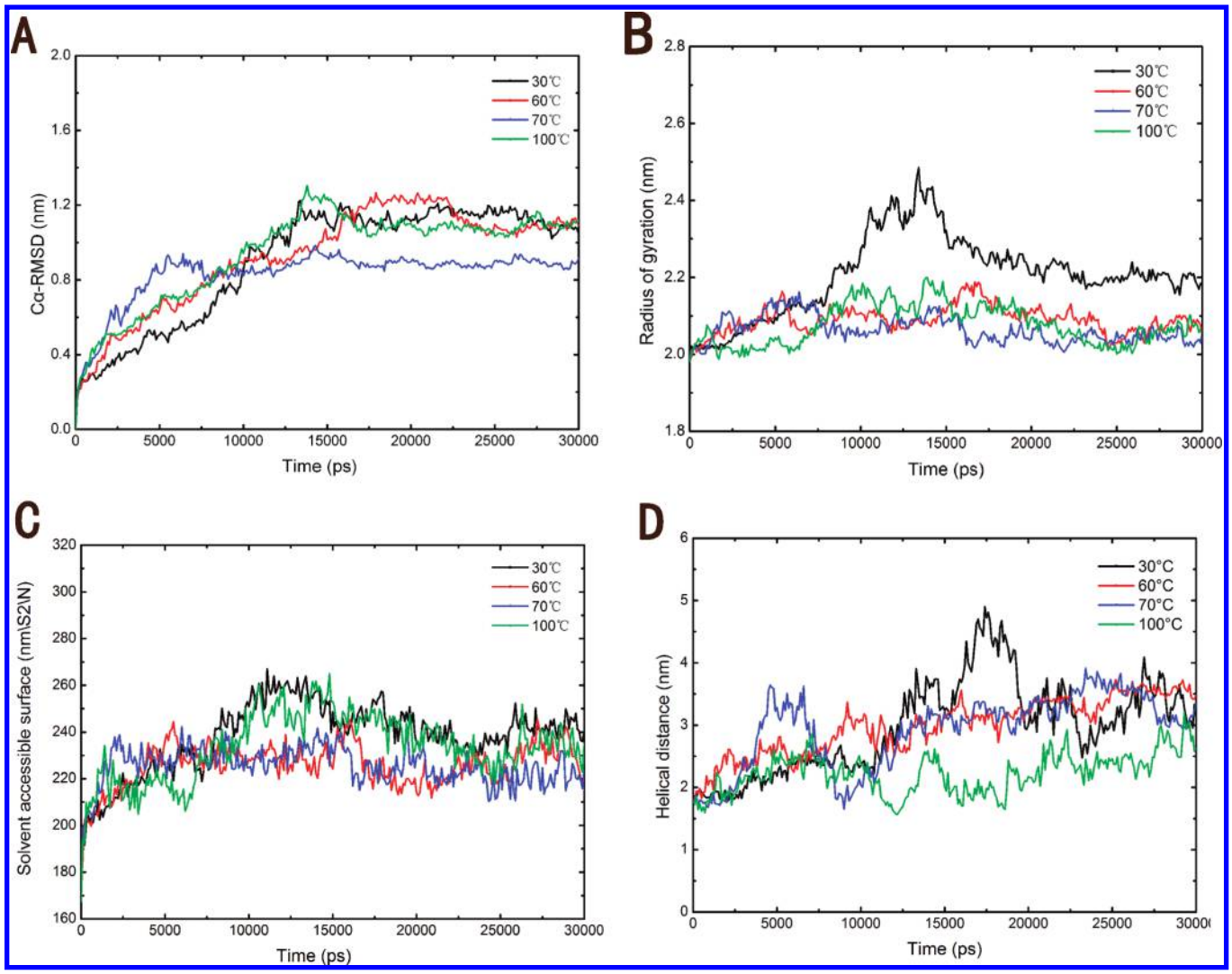

Figure 1. The global properties of T1 lipase at different temperature conditions. (A) The rms deviations for all the Cα atoms from the initial structure; (B) the radius of gyrations for Cα atoms; (C) the time-dependent solvent accessible surface areas; and (D) the time-dependent helical distances between the hydrophobic helices 6 and 9, where the black, red, blue, and green curves stand for the systems at 30, 60, 70, and 100 °C, respectively.

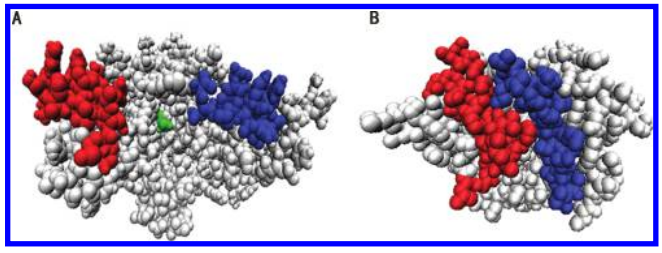

Figure 2. The open and closed configurations of T1 lipase generated from MD simulations. (A) The space-filling representations of the open configuration; and (B) the space-filling representations of the closed configurations, where helices 6 and 9 and catalytic traid were colored in blue, red, and green, respectively.

polation order of 4 and a grid spacing of 0.12. The van der Waals interactions were treated by using a cutoff of 12 Å. The integration step is 2 fs, and the coordinates were saved every 1 ps.

III. RESULTS AND DISCUSSION

According to the MD trajectories, T1 lipase had quite different dynamic behaviors at different temperatures. First, we calculated the root-mean-square (rms) deviations for all the Cα atoms from the initial structure, which are considered as a central criterion to measure the convergence of the protein system concerned (Figure 1A). The rms deviation curve for T1 lipase at 70 °C was notable due to its exceptional convergence value, giving an indication that the protein adopted a comparatively stable configuration at this temperature. It was thus helpful for T1 lipase to reach its maximal activity. Second, we computed the radius of gyration for the Cα atoms, as shown in Figure 1B. The curves of the radius of gyrations for T1 lipase at 60, 70, and 100 °C were similar, however, that at 30 °C showed an exceptional value. The large fluctuation in the radius of gyrations at 30 °C indicated that the protein might be undergoing a significant structural transition. This was also supported by the fluctuations in the solvent accessible surface areas (Figure 1C). In contrast, judging from the stable curves for the radius of gyration and solvent accessible surface areas, T1 lipase at 60 and 70 °C was in a suitable configuration that was favorable for its catalytic activity.

After carefully observing the MD trajectories, it was found that the structural transitions at 30 °C were caused by the hydrophobic helices 6 (Asp175−Ala190) and 9 (Val294−

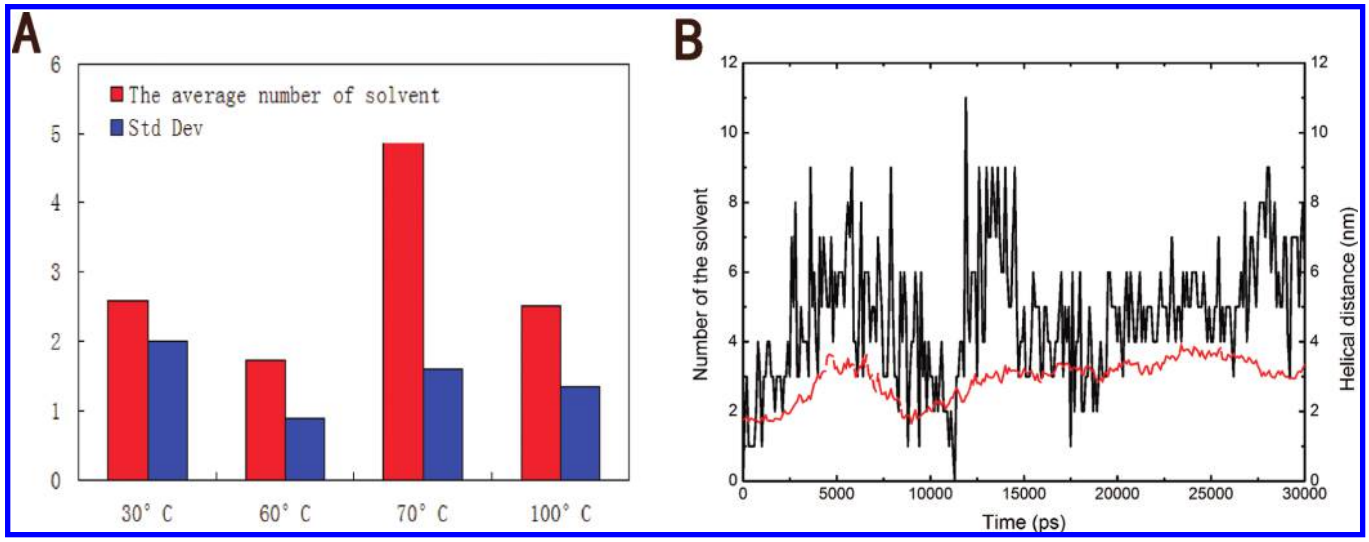

Figure 3. The statistical information for the solvents in the active site at different temperature conditions. (A) The average number of solvent molecules in the active site at 30, 60, 70, and 100 °C, respectively; and (B) the correlation between the number of solvent molecules in the active site and the helical distances at 70 °C.

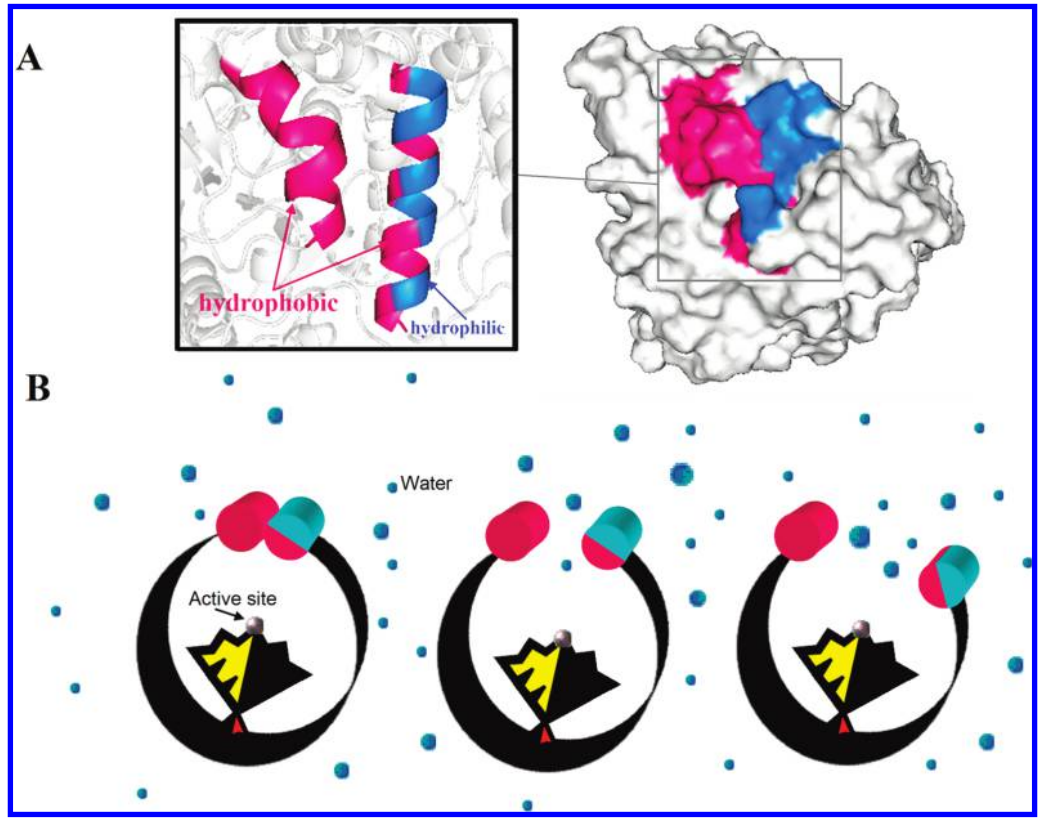

Figure 4. Schematic illustration of the allosteric mechanism of the structural transitions. Only helices 6 and 9 are marked in this figure, and their hydrophobic and hydrophilic surfaces are in red and blue, respectively.

Ser301). So, we calculated the distances between the two helices during 30 ns MD simulations (Figure 1D). According to the distances between helices 6 and 9, two different configurations for T1 lipase can be identified: the open form (Figure 2A) with interhelical distances of 3.5−5 nm and the closed form (Figure 2B) with interhelical distances of ∼2 nm. Huge fluctuations were detected in the curves for the protein at 30 and 70 °C. At 70 °C (the blue curve in Figure 1D), T1 lipase was in the closed form for the first 5 ns simulations, subsequently transforming into the open form. After a short closed process at 10 ns, the protein adopted the open form again and finally kept this configuration to the end. Different from the trajectory at 70 °C at the beginning of MD simulations, T1 lipase at 30 °C (the black curve in Figure 1D) kept its closed form for a long time (0−10 ns), and its open form showed larger interhelical distances (the maximum 4.9 appeared at ∼17 ns).

Additionally, to assess how the structural transition influenced the catalytic activity, we have calculated the number of solvent molecules that could enter into the active site of T1 lipase within a 5 Å distance from the catalytic Ser. As displayed in Figure 3A, at 70 °C the lipase had the most solvents in the active site, which was propitious for the catalytic reactions, thus it could reach the maximal

activity. Also, after mapping the numbers of solvents in the active site onto the interhelical distance at 70 °C (Figure 3B), it was found that the numbers of solvents in the active site seemed to have a positive correlation with the helical distance. So, it was believed that the structural transition exposed the catalytic triad and let solvent molecules into the active site. Furthermore, due to the smallest deviations, the solvents in the active site at 60 °C had little changed, revealing that T1 lipase adopted a comparatively stable configuration at this temperature.

It was known that the lipases employed an interfacial activation; the flap moved toward closing the hydrophobic binding pocket in an aqueous environment and opening in an oil−water interface. However, both closed-to-open and open-to-closed processes were detected in our simulations with explicit water surroundings. The structural transitions from either closed-to-open or open-to-closed might provide molecular insights into the catalytic mechanism for T1 lipase. As distinct from other lipases, the catalytic triad of T1 lipase with the closed configuration was in the protein core, thus the enzyme could not perform catalysis. So, for the catalytic duties, T1 lipase had to adopt an open configuration with the catalytic triad or active site exposed to the surrounding solution. So, a structural transition occurred, which was mainly caused by the hydrophobic helices 6 and 9. By studying the structural features for the aforementioned two helices, it was believed that the transitions were derived from the hydrophobic interactions between the two helices. In the starting structures of our simulations, helix 6 could be divided into two major components with the hydrophilic part at the surface and the hydrophobic part inside (Figure 4). In aqueous solution, the hydrophobic part could provide enough power for helix 6 to move away, driving the protein into the open configuration and exposing the catalytic triad.

IV. CONCLUSION

As a special member of the lipase family, T1 lipase is a secreted protein with responsibility for catalyzing the hydrolysis of long-chain triglycerides into fatty acids and glycerol at high temperatures. It is reported that this enzyme is still stable with considerable enzymatic activity at 60 °C and with maximal activity at 70 °C. In the present study, we employed molecular dynamics (MD) simulations on the T1 lipase with GROMACS force field parameters, periodic boundary conditions, NPT (or NVT) ensembles in the explicit water surroundings at 30, 60, 70, and 100 °C, respectively. It was found from the MD trajectories that, in contrast to other lipases with interfacial activations, T1 lipase employed an aqueous activation by the double-flap movement. The motions of the flaps consisted of helices 6 and 9 leading to the open-to-closed and closed-to-open transitions, which were driven by the hydrophobic part of helix 6. These findings are consistent with the experimental result, revealing the catalytic mechanism for T1 lipase. Of most importance, our findings may provide structural information for the rational design of lipases.

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