PROTEIN ENGINEERING OF *CANDIDA RUGOSA* LIPASE

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Lipases (EC 3.1.1.3) catalyze the hydrolysis of emulsified long-chain triacylglycerol at the lipid–water interface. *Candida rugosa* lipase (CRL) is one of important industrial enzymes that are widely used in biotechnological applications such as the production of fatty acids and the synthesis of various esters. The catalytic efficiency and substrate specificity are seemed to be the key factors for industrial applications. Several approaches have been used to increase the stability of different lipases. For example, the immobilized CRL on carriers led to significant improvement in the catalyst's activity and stability (Ref 1). In this study, we have focused on protein engineering to improve the properties of CRL.

We found the mutations of amino acids at locations in the substrate binding region resulted in a different substrate preference (Figure 1). Two of mutants (Ser365x, Gly414x) had a preference for short chain lengths by hydrolysis assay using triacylglycerol and pNP-esters as substrates (Ref 2). The enantioselectivity of WT and mutants (S365x, G414x) was compared in the resolution of by esterification reaction with 1-propanol. The resolution of (*R, S*)-ibuprofen of WT and S365x mutants showed a good selectivity while G414x mutants showed lower esterification activity. These residues are responsible for the differences of substrate specificity and important factors in the variation of the catalytic activity. For improving thermostability, we used four different strategies; (1) introduction of disulfide bridges, (2) stabilization of loops, (3) increasing hydrophobic bond, and (4) making higher number of hydrogen bonds. We found several mutants showed higher remaining activities after incubation at 50°C for 30 min and higher relative activities at 60°C compare at 30°C.It suggested that the thermostability of CRL was improved by various ways. Our results provide new insights on the protein structure and function relationship and potentially useful catalysts for applications.

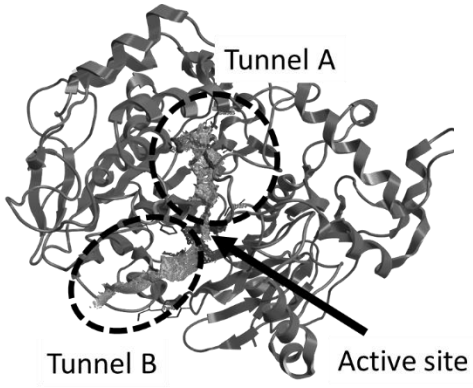

Figure 1. Structure of CRL. (PDB code: 1crl) The active site (Ser209, Glu341, and His449) and two tunnel regions are indicated. Ser365 and Gly414 are located in tunnel A.

References

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