Poster

Structure-based design and optimization of fungal lipases to improve substrate binding and enzyme activity

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Lipase is an enzyme that catalyses the hydrolysis of fats, converting triacylglycerols into the glycerol and fatty acids. Lipases are widely used in processing of dietary lipids, production of biodiesel, and synthesis of bioactive esters. Substrate specificity of lipases often limits the application, so that enhanced selectivity and engineered catalytic sites are desired among food and medicinal industry. We investigated fungal lipases from *Beauveria bassiana* (BBL) to understand the structural basis of its substrate specificity. BBL belongs to the canonical αβ hydrolase family, and exhibits lipase activity against various lipid substrates. The three-dimensional structure of BBL adopts the conserved hydrolase fold of fungal sterol esterase with a distinct hydrophobic channel at the substrate binding site (Fig. 1). BBL shares ~30% sequence identity with the sterol esterase, such that template-based (Phyre2) or template-free (AlphaFold2) modelling provided very similar backbone folds. We employed mutations at the substrate binding interfaces and also at the lid domain that covers the binding site to examine their impact on the biochemical activity.

Deletion of the lid domain did not perturb the overall scaffold of BBL based on the AlphaFold2 prediction. We prepared the recombinant mutant proteins using the Sf9 insect cell expression system, and purified the mutants using the column chromatography. Circular dichroism of the mutants indicated that secondary structures were well maintained. The enzymatic activity was largely maintained regardless of the lid domain, but modulated by the mutations at the binding interface. We identified mutations that slightly enhanced the lipase activity, demonstrating that the binding interface can be engineered for enhanced activity.

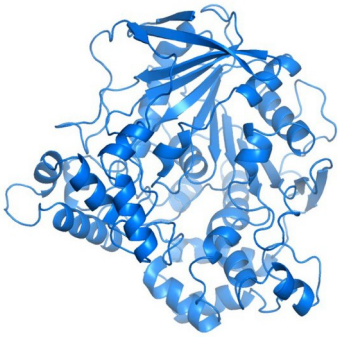

Figure 1. Three-dimensional structure of fungal lipase in a cartoon diagram.