DEVELOPMENT OF P450-BM3 USING MOLECULAR DYNAMICS SIMULATIONS - A TRIBUTE TO THE LATE PROFESSOR HIDEAKI YAMADA-

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Cytochrome P450 enzymes constitute a large family of enzymes that are remarkably diverse oxygenation catalysts found in archaea, bacteria, fungi, plants, and animals. Because of the catalytic diversity and broad substrate range of P450s, they are the potential biocatalyst candidates. Cytochrome P450 (P450-BM3; CYP102A1) from *Bacillus megaterium* is a self-sufficient cytochrome P450 and catalyzes NADPH-dependent oxidation of medium and long chain saturated fatty acids.

P450-BM3 is an attractive biocatalyst candidate for the production of fatty acids, fine chemicals, and biomolecules. Also, it could be largely prepared by recombinant expression in *Escherichia coli* (*E coli*) or other bacteria. Protein engineering of P450-BM3 to obtain activities towards various substrates was widely reported (1). The catalytic efficiency, substrate specificity and regioselectivity are the key factors for industrial applications. P450-BM3 converted eicosapentaenoic acid (EPA) into 17,18-epoxyeicosatetraenoic acid (17,18-EpETE) in a stereoselective manner. 17,18-EpETE is a new class of anti-allergy and anti-inflammatory lipid mediator that inhibits the development of food allergy and contact hypersensitivity (2).

To design enzymes for these functions improvement, the molecular dynamics (MD) simulation has become a valuable tool to effectively explore the amino acid substitutions on enzymes. To enhance the regioselectivity of 17,18-EpETE, we carried out MD and docking simulation. Here, mutation site prediction for enhancing the regioselectivity (MSPER) is an analytical method for identifying candidate substitution residues based on conformational differences from docking poses between a target product and byproducts (3). We generated docking poses of P450-BM3 with the substrates (EPA) or the target product (17,18-EpETE) using MD simulations and applied MSPER on them to predict mutation sites. To evaluate the predictions, amino acid substitution experiments were carried out. P450-BM3 was expressed in E coli and the saturation mutagenesis of selected amino acids was performed. We found several mutants showed improved the regioselectivity of 17,18-EpETE. Finally, the combination of mutation sites, predicted for the target products, 17,18-EpETE, succeeded in enhancing the regioselectivity up to 96%.

Our results suggest that protein engineered variants of P450-BM3 would be one of key enzymes for industrial applications. In addition, it demonstrated that MSPER is a powerful tool to obtain improved enzymes in regioselectivity.

References
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