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Mutations at the putative active cavity of styrene monooxygenase from *Pseudomonas* sp. LQ26 creates mutants with altered substrate preference

Abeer Ahmed Qaed Ahmed[1], Lin Hui[2], Tang Da-Fang[2] and Wu Zhong-Liu[2]
[1]Faculty of Medical Sciences, Al-Saeed University, Yemen
[2]Center of Applied and Environmental Microbiology, Chengdu Institute of Biology, Graduate University of Chinese Academy of Sciences, China

Enzymes are able to perform highly chemo-, regio-, and enantioselective reactions. Many enzymes have been used in industrial, food, pharmaceutical, and environmental applications. Styrene monooxygenase (SMO) is a promising enzyme, serves as a friendly environmental cleaning tool and as excellent biocatalysts in producing chemical building blocks. Improvement of enzymes is highly important. It is critical to find biocatalysts with high robustness and wide range of substrate. Thus it is important to design new mutants with improved activity for non-natural substrates which normally cannot be easily accomplished by the wild types.

Materials & Methods: With the aim of enhancing the robustness and adaptation of SMO to broaden the range of substrate and based on the X-ray crystal structure of the dimeric FAD-specific oxygenase subunit of the SMO from *P. putida S12 (PDB ID:3IHM)*, point of mutations were constructed using SMO from *Pseudomonas* sp. LQ26 as parental enzyme on amino acid residues that could possibly interact with the vinyl group of styrene which were identified using automatic docking. Mutants and wild type were examined in the enzymatic epoxidation of styrene, α-methylstyrene, and α-ethylstyrene. Then, their enzymatic activities were analyzed.

Results: Docking results identified four amino acid residues to be adjacent to the vinyl group of styrene. Wild type displayed decrease in activity when substrate increased in size. All four mutants altered their substrate's preference compared to the wild type. Three of these mutants exhibited high preference toward α-methylstyrene. In contrary to the wild type, one mutant increased in activity with the increase of the substrate's size with clear preference toward the bulkiest substrates, α-ethylestyrene.

Conclusions: This study revealed mutants with altered substrate's preference. Our data showed that rational design approach based on crystal structure of SMO provides an efficient way to identify critical residues, and to design mutants with enhanced biocatalytic properties.

ahmed01abeer01@yahoo.com