IMPROVING THERMOSTABILITY OF TRYPTOPHAN 2-MONOOXYGENASE BY SEMI-RATIONAL ENGINEERING

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Tryptophan 2-monooxygenase (TMO) is an FAD-bound flavoenzyme in the L-amino acid oxidase family. The enzyme catalyzes the oxidative decarboxylation of L-tryptophan to form indole-3-acetamide (IAM), carbon dioxide, and water. TMO originates from *Pseudomonas savastanoi* and is involved in the tryptophan conversion pathway in bacteria to generate secondary metabolites as precursors for biosynthesis. In order to utilize the enzyme for industrial applications, thermostable enzymes are essential to withstand high temperatures. In this work, TMO was engineered to increase the enzyme thermostability using a semi-rational design. Computational tools were used together with structural analysis to select candidate residues which were further subjected to site-saturation mutagenesis. After obtaining an enzyme library, we subsequently screened for thermostable enzymes using a high-throughput product analysis system. The hit variants from the screening were identified by DNA sequencing and further characterized their enzymatic and biophysical properties. The improvement on the variant thermostability was determined by thermal shift assays and residual activities after incubating at elevated temperatures to identify candidates with more thermostability than the wild-type enzyme.