ENGINEER FLEXIBLE LOOPS FOR IMPROVED ENZYME THERMOSTABILITY

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Enzyme thermostability is a critical factor for its wide applications in industrial fields. Flexible sites are potential targets for engineering the stability of enzymes. Nevertheless, the success rate of the rigidifying flexible sites (RFS) strategy is still low due to a limited understanding of how to determine the best mutation candidates. The stereospecifically controlled carbon-carbon bond forming ability of *Escherichia coli* transketolase (TK) makes it very promising as a biocatalyst in industry. However, as a mesophilic enzyme, it suffers the limitation of low stability to elevated temperatures and extremes of pH, limiting its current use in industrial processes. In order to improve thermostability of TK, we have applied two parallel strategies to identify mutation candidates within the flexible loops. The first was a “back to consensus mutations” approach, and the second was computational design based on ΔΔG calculations in Rosetta. Forty-nine single variants were generated and characterized experimentally. From these, three single-variants I189H, A282P, D143K were found to be more thermostable than wild-type TK. The combination of A282P with H192P, a variant constructed previously, resulted in the best all-round variant with a 3-fold improved half-life at 60 °C, 5-fold increased specific activity at 65 °C, 1.3-fold improved k_{cat} and a T_m increased by 5 °C above that of wild type. Based on a statistical analysis of the stability changes for all variants, the qualitative prediction accuracy of the Rosetta program reached 65.3%. Furthermore, molecular dynamics (MD) simulations of variants confirmed a good inverse correlation between protein stability and local flexibility which was determined by the magnitude of fluctuations with respect to the average conformations. Both of the two strategies investigated were useful in guiding mutation candidates to flexible loops, and had the potential to be used for other enzymes.