SITE-DIRECTED MUTAGENESIS OF STRUCTURAL HOT SPOTS FOR ENHANCED SOLUBILITY OF DEOXYXYLULOSE PHOSPHATE PATHWAY ENZYMES

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Increasing the metabolic flux through a biochemical pathway is highly desirable for metabolic engineering. One strategy is to enhance the solubility of overexpressed pace-making enzymes. Accurate theoretical prediction of target mutation sites is instrumental to reduce the experimental efforts and speed up the optimization process. In this study, the rate-limiting steps along the non-mevalonate (DXP) pathway, namely *E. coli* Dxs and IspG, were used as the model enzymes to learn and develop a set of bioinformatics tools that would enable rational optimization of enzyme solubility. TANGO prediction was first used to identify the aggregation-prone regions (APRs), and then SIFT analysis was carried out to eliminate the non-tolerable amino acids in the APRs. Preliminary results have shown that 5 out of 8 tested mutations have resulted in an increase in Dxs solubility. Similarly, 7 out of 12 IspG mutants have displayed enhanced solubility. Importantly, the *in vivo* activities of the more soluble mutants were improved. Taken together, the solubility of both Dxs and IspG were enhanced by ~2-fold, by targeted single amino acid mutation. The study demonstrated rapid improvement of enzyme solubility by combinations of computational tools. The information gained would be useful for rational engineering of overexpressed pathway enzymes and improve pathway efficiencies.