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 over-expressed pathway enzymes and improve pathway efficiencies.