IN SILICO METHODS IN ENZYME SCREENING AND GENE EXPRESSION

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INTMSAlign is a software to assign consensus residues of target protein utilizing large amount of their family sequences. We generated three protein sequences with S-selective hydroxynitrile lyase (S-HNL) activity, which we call designed S-HNLs; these proteins folded as efficiently as the native S-HNL (1). a-Amino-e-caprolactam (ACL) racemase from Achromobacter obae has been shown to be an effective catalyst for the dynamic kinetic resolution of amino acid amide and a-aminonitriles to form chiral amino acids. We searched for ACL racemase in silico with INTMSAlign software. By fixing Lys 241 as one of the key residues, we discovered thirteen ACL racemase genes from 413 fold type-I PLP genes (2).

Insolubility of proteins expressed in Escherichia coli expression hinders the progress of both basic and applied research. Insoluble proteins contain residues that decrease their solubility (aggregation hotspots). We discovered a phenomenon of soluble expression of HNL from Manihot esculenta, in E. coli. By random mutagenesis, we found that a single point mutation H103L, and mutation with alterations at three positions (Lys-Pro mutations at positions 176, 199 and 224) cause total solubility in E. coli even when grown at 37°C (3). If a relationship between soluble expression and mutation points could be established, it will become very easy to generate a mutant for correctly folded expression in E. coli. Using a combination of approaches involving directed evolution and primary sequence analysis, we found two rules of thumb to help identify hotspots: one focuses on the hydrophobicity of amino acids in the a-helix structure, and another one focuses the difference in hydrophobicity relative to the corresponding amino acid in the consensus protein. Using these two relationships together, we succeeded in developing methods to improve the solubility of expressed proteins in E. coli (4).

References:

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