ENZYME ENGINEERING OF GLUTAMATE DEHYDROGENASE FOR PRODUCTION OF L-AMINO ACIDS

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L-Amino acids are high-value chemicals widely used in pharmaceuticals, pesticides, cosmetics, food and feed additives industries. Amino acid dehydrogenase such as glutamate dehydrogenase (GluDH) has been used to catalyze asymmetric reductive amination of α-keto acid to produce L-amino acids. However, GluDHs commonly have strict substrate specificities and display limited activities towards non-natural substrates, hindering their wide applications. Hence, investigation of the potential hot spots and development of high-efficiency and general molecular engineering strategies to improve activities of GluDHs toward novel substrates would promote the applications of GluDHs in synthesis of L-amino acids. Based on the homology modeling and substrate docking, we hypothesized that enlarging the “cave” region determining substrate specificity in the GluDH binding pocket would contribute to the binding of substrates with large size and improve the catalytic efficiencies. With the glutamate dehydrogenase from *Pseudomonas putida* (PpGluDH) as the target, the “cave” region was enlarged by site-directed mutagenesis and two variants including A167G and V378A were obtained showing 123-fold and 116-fold improved catalytic efficiencies, respectively, towards 2-oxo-4-[(hydroxy)(methyl) phosphinyl]butyric acid (PPO). This molecular engineering method was then applied to ten other GluDHs from different sources and with different properties. All engineered GluDHs acquired substantial improvements in PPO-oriented catalytic activity, and the most efficient mutant showed up to 1820-fold increased activity. This indicated that this method could be generally used to engineer the GluDHs from different sources. GluDHs are typical “hinge-bending” enzymes. During catalysis, the cofactor-binding domain rotates with respect to the substrate-binding domain around an α-helix structure, which is defined as the hinge, causing the subunits of the GluDHs to transform between open and closed conformations. Several mutants located in this hinge region were acquired using error-prone PCR-based directed evolution that significantly affected the catalytic efficiency. Inspired by this, we selected the hinge region as the hot spots for semi-rational engineering of GluDHs for improving activity. Five mutation libraries were constructed and mutants exhibiting significantly improved catalytic activities towards PPO were obtained from four of them, and the highest activity increase reached 104-fold. These variants also showed significantly improved activities toward several other α-keto acids substrates, indicating that the hinge region engineering was an effective strategy to improve reductive amination activity of GluDHs. We have systematically engineered the activities of GluDHs toward non-natural substrates by modifying the substrate binding and modulating the conformations transformation, with the mutation target on different functional regions. A set of GluDHs variants were obtained, promising to be used in industrial synthesis of L-amino acids.