Engineering the substrate specificity of oxidoreductases by redesign of enzyme-substrate intermolecular interactions

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Engineering the substrate-specificity of enzyme is a promising approach that can expand the applicability of enzymes in the area of biotechnology. In this study, two oxidoreductases, 3-hydroxybutyrate dehydrogenase (3HBDH) and succinic semialdehyde reductase (AKR7A5) were selected as model enzymes. The enzyme-substrate interatomic contact analysis was applied for the redesign of enzymes. In engineering of the substrate-specificity of 3HBDH toward levulinic acid, 16 variants of the 3HBDH were generated and a double mutant, His144Leu/Trp187Phe, showed the most enhanced catalytic activity (33-fold) toward the target substrate. In addition, the substrate-specificity of AKR7A5 toward levulinic acid was engineered, and four out of six tested mutants showed improved catalytic properties. Among the improved variants, Met13Trp exhibited the most enhanced activity (7.0-fold) toward the target substrate. On the other hand, the structural effect of the positive mutations on the substrate-specificity of the enzymes was analyzed by employing the interatomic contact analysis to understand the structural basis for the substrate specificity of the enzyme.