442b Development of a Phosphite Dehydrogenase-Based Nicotinamide Cofactor Regeneration System

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NAD(P)H-dependent oxidoreductases are valuable tools for synthesis of chiral compounds. The expensive cost of the cofactors, however, requires *in situ* cofactor regeneration for preparative applications. We have attempted to develop an enzymatic system based on phosphite dehydrogenase (PTDH) from *Pseudomonas stutzeri* to regenerate the reduced nicotinamide cofactors NADH and NADPH. We used directed evolution to address one of the main limitations with the wild-type PTDH enzyme, its low stability. After three rounds of random mutagenesis and high throughput screening, twelve thermostable amino acid substitutions were identified. These twelve mutations were combined by site-directed mutagenesis, resulting in a mutant whose T_{50} is 20°C higher and half-life of thermal inactivation at 45°C is >7000-fold greater than that of the parent PTDH. The engineered PTDH has a half-life at 50°C that is 2.4-fold greater than the *Candida boidinii* formate dehydrogenase (FDH), an enzyme widely used for NADH regeneration. The improved stability and effectiveness of the thermostable PTDH mutant was shown using the industrially important bioconversion of trimethylpyruvate to L-*tert*-leucine. Site-directed mutagenesis was also used to incorporate a cofactor specificity mutation (A176R) identified in previous work into the thermostable PTDH construct to create a powerful new NADPH regenerating enzyme. Several regeneration reactions were selected and conducted in small-scale batch reactions and in an enzyme membrane reactor to evaluate the capabilities of the PTDH mutants. The engineered PTDH will be useful in NAD(P)H regeneration for industrial biocatalysis.