OFFICIAL ABSTRACT and CERTIFICATION

Enhancement of Oxidoreductase Cofactor Systems Through the Site-Saturation Mutagenesis of CboFDH for Enzymatic Activity with 3'NADP Serving as a Novel Model for NAD-capped RNA

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Oxidoreductases working with their cofactors are the sole enzymes that catalyze the transfer of electrons between molecules or to electrodes. Formate dehydrogenases are enzymes with considerable biotechnological potential as they can be used to produce optically active compounds from non-chiral ones. Oxidoreductase cofactor systems may also serve as an effective means for the enzymatic capture of CO2. However, the separation of cofactors from enzymes is expensive and can cause harmful over purification, stunting the numerous biocatalysis reactions that could ensue. Recently discovered naturally produced NAD-capped RNA in Escherichia coli can solve this issue of resupplying cofactors in oxidoreductase applications in a time-efficient, cost effective manner. In order for these capped molecules to be manipulated for in vitro and in vivo reactions, readily available 3' NADP served as a novel model for NAD-capped RNA because there is a parallel in their structures: a phosphate is attached in the same location in 3'-NADP as the RNA attaches in the NAD-capped RNA. In this study, formate dehydrogenase from Candida boidinii (CboFDH) was mutated through site-saturation mutagenesis, changing its cofactor specificity to demonstrate activity with 3'NADP, providing sufficient evidence for activity with NAD-capped RNA. The results from spectrometer enzyme assays indicated that two CboFDH mutants demonstrated increased activity with 3'NADP. Thus, the innovative use of 3'NADP in place of NAD-capped RNA demonstrated enzymatic activity with mutated CboFDH samples. This newfound information is the stepping-stone to more efficient use of oxidoreductases as biocatalysts and a maintainable process to limit excess CO2 in the atmosphere.

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