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Cry3Aa-formate dehydrogenase as a platform for regeneration of NADH for coupling reactions

AUTHORS
Reza YEKTA SAADABAD / THE CHINESE UNIVERSITY OF HONG KONG, CENTRAL AVE, HONG KONG
Xu XIONG / THE CHINESE UNIVERSITY OF HONG KONG, CENTRAL AVE, HONG KONG
Marianne M LEE / THE CHINESE UNIVERSITY OF HONG KONG, CENTRAL AVE, HONG KONG
Corresponding author : Michael KENNETH CHAN / michaelkchan88@yahoo.com

PURPOSE OF THE ABSTRACT
NADH-dependent enzymes play an important role in a wide range of biological oxidation-reduction reactions. Their use in commercial biocatalysis is limited, however, due to the high cost of the required NADH cofactor. One attractive solution is the development of NADH regenerations systems which reduce the NADH required. Formate dehydrogenase (FDH) is a commonly used enzyme for this purpose since it can efficiently regenerate NADH by oxidizing formic acid into CO2 gas. However, it has low stability, cannot be recycled for multiple reaction cycles, and requires time-consuming and costly purification.

Here, we present a strategy to stabilize FDH by fusion to Cry3Aa, a protein that naturally produces micrometer sized crystals within the bacterium Bacillus thuringiensis (Bt). The resulting Cry3Aa-FDH fusion crystals can be directly isolated from Bt by density centrifugation, greatly simplying its production costs. Moreover it can be co-immobilized with a second NADH-dependent enzyme allowing for direct coupling of the NADH produced for catalysis within same crystal.

Two different enzymes, leucine dehydrogenase and alcohol dehydrogenase, were independently co-immobilized in Cry3Aa-FDH crystals as a coupling reaction to produce chiral intermediates to validate this system. According to the findings of this study, Cry3A-FDH/Cry3A-LDH and Cry3A-FDH/Cry3A-ADH can efficiently synthesize L-tert-leucine and ethyl (S)-3-hydroxybutyrate products without the minimal addition of exogenous NADH. This biosystem is reusable and can be used for multiple reactions, which is essential for industrial applications. We hope to investigate the platform's ability to simplify reactions and reduce associated costs for other industrial enzymes.

FIGURES

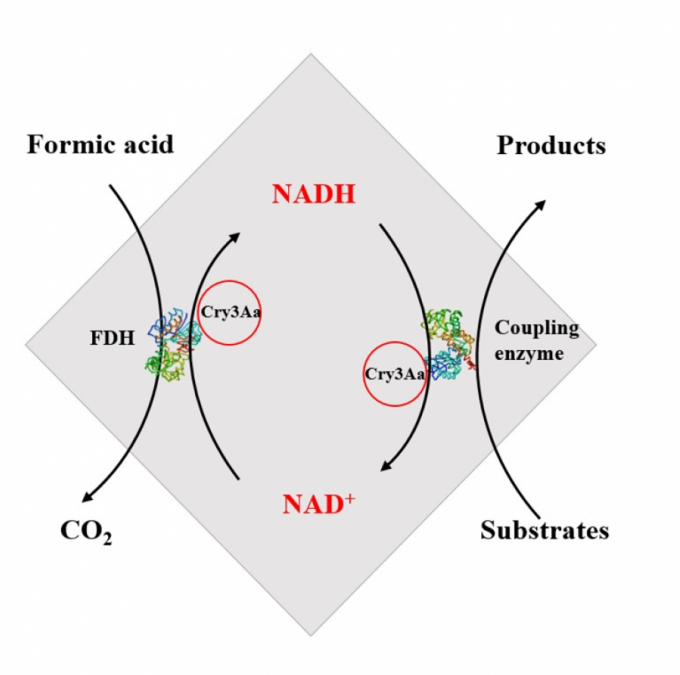

FIGURE 1

Graphical Abstract

Cry3Aa -formate dehydrogenase (FDH) that is coupled with another enzyme (leucine dehydrogenase or alcohol dehydrogenase) to continuously regenerate NADH cofactors by oxidation of formic acid into CO2 gas, which is then utilized by the second enzyme.

FIGURE 2

KEYWORDS

Cry3Aa | FDH | NADH regeneration | Genetically immobilization

BIBLIOGRAPHY

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