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Methylformamide Conversion by Formate Dehydrogenase to Fuel Reductive Aminase with NADPH and Amine in a Cascade Setup

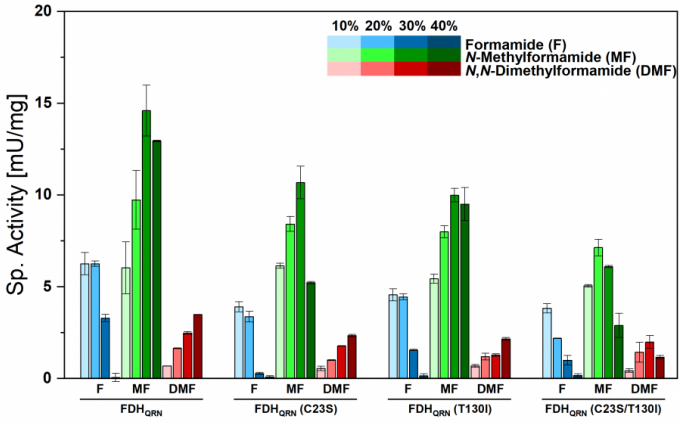
AUTHORS

Artur MAIER / RUHR UNIVERSITY BOCHUM, UNIVERSITAETSSTRASSE 150, BOCHUM
Tanja KNAUS / UNIVERSITY OF AMSTERDAM, POSTBUS 94157, GD AMSTERDAM
Francesco MUTTI / UNIVERSITY OF AMSTERDAM, POSTBUS 94157, GD AMSTERDAM
Dirk TISCHLER / RUHR UNIVERSITY BOCHUM, UNIVERSITAETSSTRASSE 150, BOCHUM

PURPOSE OF THE ABSTRACT

Formate dehydrogenase (FDH) from Candida biodinii is well studied and applied in various setups as a NADH-regeneration system. Attempts were made to increase stability and due to its clear preference for NAD+, to shift the substrate specificity towards NADP+ through mutagenesis. Here, the impact of various known stability mutations [1,2] on the NADP+ accepting FDH (D195Q/Y196R/Q197N) [3], in the following FDH-QRN, and the bioinformatically predicted T130I mutation on wild type and mutants are elucidated. FDH (C23S/T130I) showed a 6-fold lower Km value (8.5 ± 0.32 µM vs 51.0 ± 1.0 µM) and an approx. 3.5-fold increase in catalytic efficiency (235.7 s-1¬ mM-1¬) compared to the wild type (66.4 s-1 mM-1). FDH-QRN (C23S/T130I) showed a 2-fold lower Km and increase of the catalytic efficiency to 18.37 ± 1.4 s-1 mM-1 compared to the FDH-QRN (16.74 ± 1.55 s-1 mM-1). FDHs are known to accept formate derivatives such as methyl-, ethyl- and phenylformate, among others [4]. In silico docking and molecular dynamics simulations suggested that formamides might be suitable substrates for FDHs as well. All variants were screened for activity towards formamide (F), N-methylformamide (MF) and N,N-dimethylformamide (DMF), showing that all variants have the ability to accept formamides, with highest activities, with NAD+ as cofactor, achieved by FDH (T130I) for F with 83.1 mU/mg and for MF and DMF by FDH (C23S) with 68.3 mU/mg and 7.4 mU/mg, respectively. With NADP+ as cofactor, the FDH-QRN reached activities with F, MF and DMF of 6.2, 14.6 and 3.5 mU/mg. The NADP+-accepting variants were employed for NADPH regeneration in a cascade reaction for the reductive amination of cyclohexanone by reductive aminase from Aspergillus oryzae [5] with MF as the sole electron and amine donor, reaching conversion rates up to 63% in a whole cell approach, broadening the applicability of FDHs in biocatalysis.

FIGURES



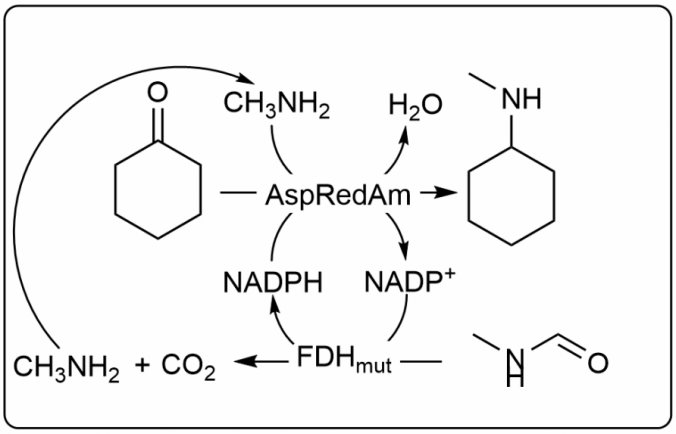


FIGURE 1

FDH Activity with formamide derivatives

Specific activity of the respective NADP+ accepting FDH variants with formamide (F), N-methylformamide (MF) and N,N-dimethylformamide (DMF) as the sole electrone donor at concentrations ranging from 10-40% (v/v).

FIGURE 2

Reductive amination cascade

Scheme of the reductive amination of cyclohexanone catalyzed by AspRedAm coupled with a FDH mutant utilizing N-methylformamide for NADPH regeneration. N-methylformamide serves as the sole electrone and methylamine donor.

KEYWORDS

NADPH regeneration | cascade | FDH | reductive amination

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