Engineering of formate dehydrogenase for the acceptance of a biomimetic nicotinamide-based cofactor in the reduction of CO₂

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January 2020

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1. Introduction

1.1. Formate Dehydrogenase

- **EC 1.17.1.9**
- **Types**
  - Metal-independent
  - Metal-dependent
- **Applications**
  - Cofactor regeneration
  - $\text{CO}_2$ conversion (under research)

(Amao, 2018)
1. Introduction

1.2. CO₂ Conversion by FDH

\[ \text{HCOO}^- \rightleftharpoons \text{CO}_2 + \text{H}^+ + 2e^- \]

\[ \text{CO}_2 + \text{H}^+ + 2e^- \rightleftharpoons \text{HCOO}^- \]

(Castillo et al., 2008)

(Marpani et al., 2017)
1. Introduction

1.3. Biomimetic Cofactors

(Guarneri et al., 2019)
1. Introduction

1.4. Enzyme Evolution

Directed Evolution

vs

Rationally Design
2. Objective

• Determine at least one beneficial mutation for doing protein engineering to the enzyme formate dehydrogenase to allow the use of a nicotinamide biomimetic cofactor instead of the natural cofactor NADH
3. Research Method

3.1. Work Pipeline

Select the enzyme and residues for mutations
- FDH enzyme that requires a cofactor and thermostable
- Residues that interact with the cofactor

Select the biomimetic cofactor
- Binding position similar to the natural cofactor NADH
- Method: Molecular docking using SwissDock

Create mutants and determine the 3D structure
- Method: Homology modelling using SwissModel

Select the best mutant
- Interactions of each mutant with the biomimetic cofactor
- Method: Molecular docking using Autodock Vina

Evaluation of the viability of the mutant
- Stability of the protein (Method: CUPSAT)
- Ligand transport (Method: Caver Web)
3. Research Method

3.2. Project Planning
4. Results

4.1. Selection of the FDH Enzyme

PsFDH (formate dehydrogenase from *Pseudomonas sp. 101*)

- Natural cofactor: NADH
- Tm: 63°C

(Filippova et al., 2005)

Apoenzyme
PDB: 2NAC

Holoenzyme
PDB: 2NAD
4. Results

4.2. Target Residues for Mutations

- Catalytic domain
- Reaction mechanism
- Interaction between subunits
4. Results

4.3. Selection of the Biomimetic Cofactor

(Nowak, Pick, Csepei, et al., 2017)

E (V) vs NHE

-0.6  -0.55  -0.5  -0.45  -0.4  -0.35  0.3  -0.35  -0.2

NADH  NADP⁺  P3NAH  MNA⁺  P2NA⁺  BNA⁺

Increasing oxidation ability

Increasing reducing ability

P3NAH docking in PsFDH

NADH  P3NAH  P2NAH  MNAH

Biomimetic cofactors

Natural cofactor
4. Results

4.4. Enzymes with Mutations

**π–π Stacking Interactions**

- Ala 283
- Phe
- Tyr
- Gly 123
- Phe
- Tyr

**Wider Cofactor Binding Groove**

- Thr 376 → Gly
- Ser 380 → Gly
- Tyr 381 → Gly
- Arg 222 → Gly

- 2NADa_A283F
- 2NADa_A283Y
- 2NADa_G123F
- 2NADa_G123Y
- 2NADa_T376G
- 2NADa_S380G
- 2NADa_Y381G
- 2NADa_R222G
4. Results

4.5. Mutants Evaluation

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<th>WT</th>
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<tr>
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<td>Distance (Å)</td>
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4. Results

4.5. Mutants Evaluation

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<thead>
<tr>
<th></th>
<th>2NADa_A283F</th>
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4. Results

4.5. Mutants Evaluation

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<th>2NADa_T376G</th>
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<th>2NADa_Y381G</th>
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4. Results

4.6. Mutant Viability Evaluation

Protein Stability

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<tr>
<th>Amino acid</th>
<th>Overall Stability</th>
<th>Torsion</th>
<th>Predicted ΔΔG (kcal/mol)</th>
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<tbody>
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Ligand Transport

<table>
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<th>2NADa_S380G</th>
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<tbody>
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<td>Bottleneck radius (Å)</td>
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<td>Length (Å)</td>
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<td>Curvature</td>
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<tr>
<td>Number of residues</td>
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<td>38</td>
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5. Conclusions

• Viable mutant: 2NADa_S380G
  • Access tunnel Ser -> Gly
  • Acceptable protein stability and ligand transport

• Residues added with aromatic groups: steric hindrance

• Potential of free software for planning protein engineering
6. Limitations and Improvements

• Limitations
  • Steps are not automatically connected
  • Dependence of tools based on web servers

• With more computational power
  • Molecular dynamics
  • More mutations
Thank you for your attention!

Please, send me your questions!