Characterization of CgX Tautomerase and Mutants with Acetylenecarboxylic Acid

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Background
- CgX, a tautomerase native to Corynebacterium glutamicum, catalyzes the hydration and subsequent decarboxylation of acetylenecarboxylic acid (ACA).
- ACA can be formed from CH₄ and CO₂

Objectives
- The main objective is to characterize CgX mutants to determine enzyme activities and product ratios.
- CgX catalyzes the following reaction.

\[ \text{CO}_2 + \text{H}_2 \xrightarrow{\text{CgX}} \text{HCO}_2 \text{H} \rightarrow \text{MCA} + \text{AcH} \]

Methods
- CgX mutations performed using Q5 site-directed mutagenesis.
- BL21(DE3) with a T7 promoter system for protein expression.
- Protein purification using AKTA Start FPLC.
- Coupled enzyme assay carried out on SpectraMax iD3 plate reader.

Results
- CgX activity on ACA revealed malonic semialdehyde, acetaldehyde (and hydrates).

**Figure 1:** ^1H NMR of WT, Y103F, and R70A

**Figure 2:** Coupled Enzyme Assay Reaction

**Table 1:** Specific Activities

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>+ MSAD (μmol/min·mg)</th>
<th>− MSAD (μmol/min·mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CgX</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>Y103A</td>
<td>0.72</td>
<td>0.41</td>
</tr>
<tr>
<td>Y103F</td>
<td>2.2</td>
<td>1.4</td>
</tr>
<tr>
<td>H28A</td>
<td>0.23</td>
<td>0.091</td>
</tr>
<tr>
<td>E114A</td>
<td>0.064</td>
<td>0.035</td>
</tr>
</tbody>
</table>

*R70K, R73K, R70A, and R73A were determined to be inactive.

Analysis
- Enzyme activities as determined by NMR and UV assays:
  - Active: WT, Y103A, Y103F, H28A*, E114A
  - Inactive: R70K, R73K, R70A, R73A
  - H28A NMR is not available, but it is predicted to be similar to the other active enzymes due to the coupled enzyme assay.
- Table 2 shows the product ratio of MSA to AcH from the enzyme assay.

**Table 2:** Product Ratios

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Product Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CgX</td>
<td>MSA: AcH 17%:83%</td>
</tr>
<tr>
<td>Y103A</td>
<td>43%:57%</td>
</tr>
<tr>
<td>Y103F</td>
<td>36%:64%</td>
</tr>
<tr>
<td>H28A</td>
<td>60%:40%</td>
</tr>
<tr>
<td>E114A</td>
<td>45%:55%</td>
</tr>
</tbody>
</table>

*Calculated from Table 1.

Conclusion
- WT remains the most active.
- Mutations on active site residues result in decreased activity.
- H28A has a higher product ratio of MSA: AcH compared to other mutants.
- All R70 and R73 mutants are inactive, confirming that these residues are critical for activity.

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