Characterization of CgX Tautomerase and Mutants with Acetylenecarboxylic Acid

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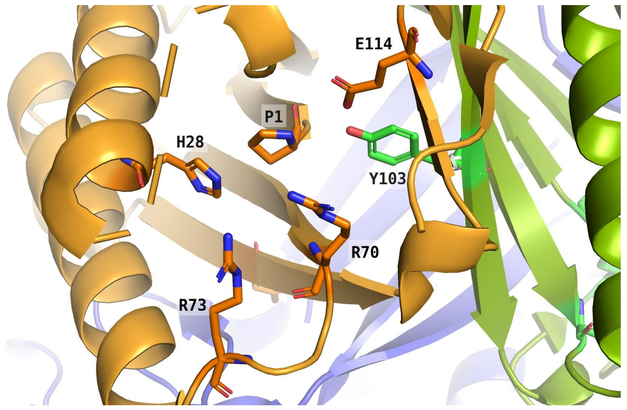
Background

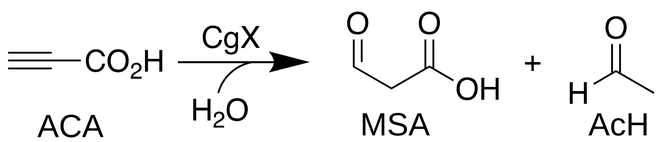
- CgX, a tautomerase native to *Corynebacterium glutamicum*, catalyzes the hydration and subsequent decarboxylation of acetylenecarboxylic acid (ACA).
- ACA can be formed from CH_4 and CO_2



Active Site Residues of CgX Protein

Objectives

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



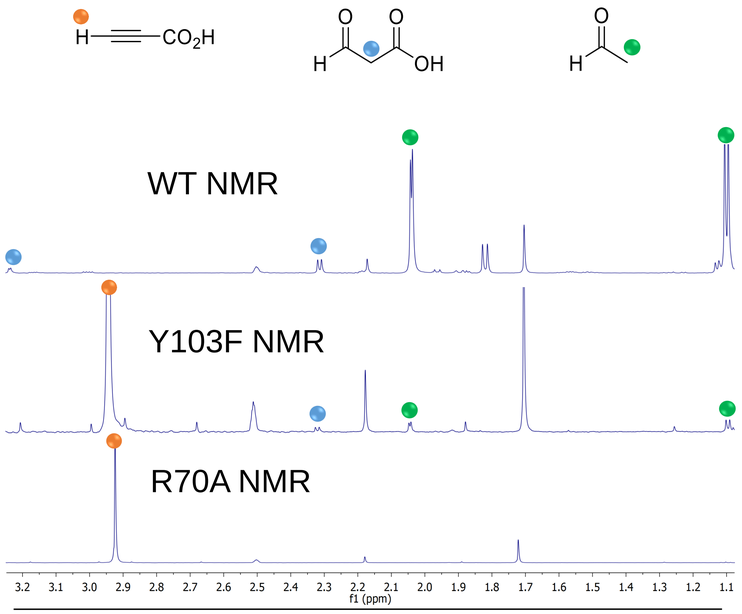
Methods

- CgX mutations performed using Q5 site-directed mutagenesis.
- BL21(DE3) with a T7 promoter system for protein expression.
- Protein purification using ÄKTA 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: ^{1}H NMR of WT, Y103F, and R70A



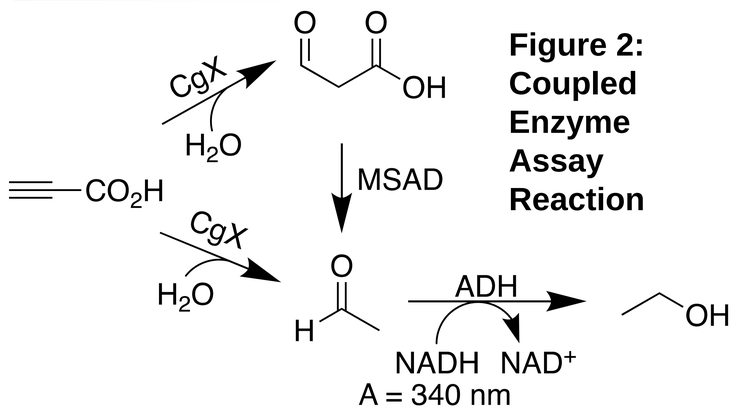


Figure 2: Coupled Enzyme Assay Reaction

Table 1: Specific Activities

Enzyme	+ MSAD	– MSAD
	μmol/min·mg	μmol/min·mg
CgX	29	24
Y103A	0.72	0.41
Y103F	2.2	1.4
H28A	0.23	0.091
E114A	0.064	0.035

*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

Enzyme	Product Ratio	
	MSA	AcH
CgX	17%	83%
Y103A	43%	57%
Y103F	36%	64%
H28A	60%	40%
E114A	45%	55%

*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.

Acknowledgments

This material is based upon work supported by the National Science Foundation under Grant No. (CHE-1851776). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Many thanks to Professor Karen Draths and Amaya Sirinimal for their critical role and support and to Michigan State University Department of Chemistry for this opportunity.