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Protein Engineering of a Spectroscopic Probe into Malate Dehydrogenase (MDH)

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Introduction

Malate dehydrogenase (MDH) is an enzyme that plays a key role in many metabolic processes, such as the Krebs cycle. Specifically, it reversibly catalyzes the interconversion of (S)-malate and NAD^+ to oxaloacetate and NADH (Figure 1). Once oxaloacetate is synthesized, MDH dispatches it to citrate synthase (CS) in the Krebs cycle, but it is unclear how this happens. One theory is that MDH channels oxaloacetate to CS by forming a metabolon, a mechanism for direct channeling that prevents diffusion of reaction intermediates into a bulk matrix. However, the lack of a spectroscopic probe in MDH makes it difficult to visualize any conformational changes that might happen upon interaction with CS, since MDH does not have tryptophan (Trp) residues in its primary sequence. Thus, this research focuses on incorporating Trp as a fluorescent marker into MDH's structure to reveal interactions between MDH and CS, as fluorescence emissions and λ_{max} shift due to protein-protein binding. Therefore, if a fluorescent and active Trp-MDH mutant can be engineered, then monitoring its fluorescence spectrum in the presence of CS could indicate binding, suggesting metabolon formation.

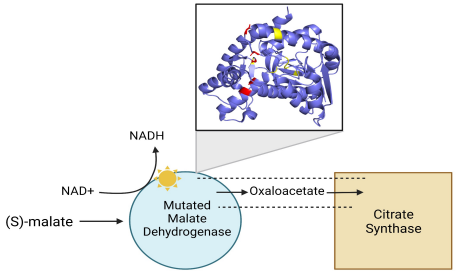
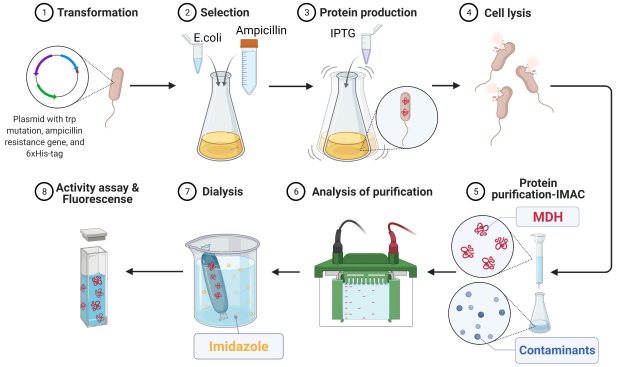


Figure 1. Malate dehydrogenase with Trp mutation passing on oxaloacetate to citrate synthase via an unknown mechanism. PyMOL image[5] shows the amino acids that were mutated to act as a spectroscopic probe (yellow), as well as the active site residues (H220, D193, R124, R130, R196) (red).

Materials and Methods[6]



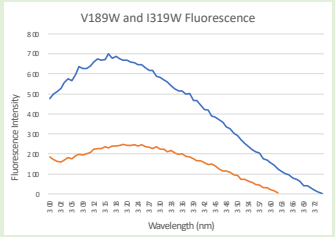
Results

Six Trp MDH mutants were produced and evaluated via fluorescence spectroscopy and specific activity. Since MDH does not contain Trp in its primary sequence, it did not fluoresce, which is supported by the data. (Table 1). At similar concentrations, mutants I319W and V189W successfully fluoresced. I319W exhibited an intensity of 243 and λ_{max} of 323 nm, while V189 had an intensity of 701 and λ_{max} of 316 nm (Table 1). However, mutant I136W showed no fluorescence. Mutants I319W, V189W, and I136W showed minimal specific activity compared to wild type (WT), but I319W had significantly higher activity than V189W and I136W (Table 1). Mutants P119W and G218W could not be purified or overexpressed due to instability.

MDH Protein	Specific Activity (min^{-1})	Fluorescence Intensity	λ_{max} (nm)
WT	108,087 ± 13,938	0	0
I319W	1554 ± 75	243	323
V189W	19 ± 2	701	316
A120W			
P119W	Not determined	Not determined	Not determined
I136W	4 ± 2	0	0
G218W	Not determined	Not determined	Not determined

Table 1. Specific activity, fluorescence intensity, and λ_{max}, for WT, I319W, V189W, A120W, P119W, and I136W MDH.

Figure 2. Fluorescent emission of V189W and I319W MDH excited at 290 nm both at concentration of 1.713 mM.



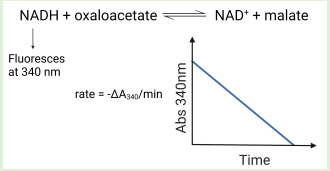


Figure 3.[7] Protein concentration (mg/mL) and enzymatic activity (absorbance/minute) are used to determine specific activity, specifically ((absorbance/minute)/(mg/mL)). Since NADH fluoresces at 340 nm, fluorescence decreases with time: rate = ΔA_{340}/min.
Specific activity = rate/[MDH]

Conclusions

Compared to wild type, I319W was 1.4% as active, V189W was 0.018% as active, and I136W was 0.0037% as active (Table 1). I136W failed to fluoresce in contrast to I319W and V189W. A120W is still in the process of being characterized. The inability to purify or overexpress both P119W and G128W leads to the conclusion that these mutations caused instability. Therefore, none of the tested mutants can be used to determine the presence of metabolon formation between MDH and CS because none of them met the two requirements of fluorescing and retaining native activity close to the level of wild-type MDH.

Future Work

- Research and design new MDH mutants to be tested for fluorescence and specific activity.
- Metabolon formation in the Krebs cycle; study how MDH interacts with citrate synthase in the Krebs cycle.

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5. Figure 1 Image source: The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC
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7. Figure 3 Image source: Created with BioRender.com (2022).

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