Site-Directed Mutagenesis of Malate Dehydrogenase: A Class Project

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Challenge:

Make your own mutant protein:
1. Design the mutation
2. You have 8 weeks to create the protein
Order supplies,
Express the protein in a host,
Purify,
Characterize.
Continuing the Malate dehydrogenase project

Goals:

1. To engineer an dehydrogenase enzyme with new substrate specificity towards lactate
2. To propose a catalytic mechanism for *E. coli* malate dehydrogenase
Hypothesis:

A R81I/K82I dimutant will create a hydrophobic pocket in the active site that will lead to a change in substrate specificity from malate to lactate.
Methods
Site Directed Mutagenesis

- Mutating the plasmids
Site Directed Mutagenesis

Promoter to drive target gene expression

WT mdh

Plasmid

ampR
Site Directed Mutagenesis

Plasmid → Annealed mutated Primer → DNA Synthesis
PCR

Polymerase chain reaction (PCR)
Purifying the Mutagenic Plasmids

- Spin column
Spin Column

Purifying DNA via a Spin Column

PCR product

Site Directed Mutagenesis

Transformation

- Inserting the plasmids into the cells
Transformation

Cell Growth

- Ampicillin Broth
- Selects for transformed cells
Expression

- Expressing MDH
Protein Expression

- When lactose is present, the gene is expressed.
- IPTG is a lactose analog that binds with higher affinity.
- This allows for high gene expression.
Purification

- Isolating protein
Cell Lysis

- Freeze
- Thaw
- Sonicate

-80°C

3x
Precipitation

- Added protease inhibitor
- PEI precipitates DNA
Purification

- The protein was engineered to have a histidine tag
- Histidine has an imidazole R-group, which is attracted to our Ni-NTA purification column.
Purification

- Combined all fractions from the elution step
- Centrifugal filtration
Results
SDS-PAGE

- Protein analysis
Enzyme Activity

- Bradford assay
- Comparing malate vs lactate specificity
Conclusion
Conclusion

Hypothesis not supported. Results provide a new insight into the catalytic mechanism of E. coli

- Arginine-81 may have greater influence on malate-oxaloacetate catalysis
- Lysine-82 may have greater influence on specificity
- Arg-81 was previously listed as a functional paralog shift mutation, but Lys-82 was not.
Near Future - Other methods, other proteins

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Methods

- pH probe enzyme assays
- Bioinformatics
- Structure techniques
  - X-ray
  - Protein NMR

Proteins

- Any protein
- Any organism
  - CHO cells
Reflections

- Opportunity for independent research
- Importance of independent research
- Requirement for getting into grad school/jobs
- Class Bonding
- Critical thinking/ problem solving
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Questions?