Effect of an arginine-to-isoleucine active site mutation on *Escherichia coli* malate dehydrogenase enzymatic activity

Jonathan M. Zatorski
Bruce J. Heyen, Ph.D.
Biochemistry Research at Olivet:

I. This Talk
   a. Protein research (general)
   b. Independent Research Experience

II. Next Talk
   a. Research in Advanced Biochemistry Course
   b. Future Research in the Chemistry Department
Protein Review

• are large **biomolecules**, or **macromolecules**, consisting of one or more long chains of **amino acid residues**.
• Proteins perform a vast array of functions within **organisms**, including
  • catalysing metabolic reactions – called **enzymes**,
  • DNA replication,
  • responding to stimuli, and
  • transporting **molecules** from one location to another.
• Proteins differ from one another primarily in their sequence of **amino acids**
• Proteins are produced from **gene expression** – central **dogma**
Protein Research – Structure and Function

Crystal → Diffraction pattern → Electron density map → Protein model
Protein Research – Structure and Function

- Substrate Specificity
- Enzyme Promiscuity
- Inhibitors
- Optimal Conditions
Protein Research – Structure and Function

- General function
- Active site location
- Allosteric site
- Hypothesize a catalytic mechanism
Protein Research – Structure and Function

- Uncertain mechanism of action
- Substrate specificity?
- Overall conformational changes
Protein Research – Mutations

Analyze Mutant Protein

Diffraction pattern → Electron density map → Protein model

Mutation

Known Protein
Protein Research – Mutations

Analyze Mutant Protein

Mutation

Known Protein

Diffraction pattern → Electron density map → Protein model

Reaction rate

\[ \frac{1}{V} \]

Substrate concentration

\[ \frac{1}{V_{\text{max}}} \]

\[ \frac{1}{K_m} \]

\[ \frac{1}{V_{\text{max}}} \]

\[ [S] \]
Purpose of Protein Research:

a. Understand catalytic mechanisms
b. Design inhibitors – drug discovery
c. Gain knowledge of structural motifs within protein families
d. Evolution of protein families
E. Coli MDH & LDH: A Peculiar Pair of Proteins
• Model for identification of functional paralog shift mutations in dehydrogenases
Malate $\rightleftharpoons$ Oxaloacetate

NAD$^+$ $\rightarrow$ NADH + H$^+$

MDH

Lactate $\rightleftharpoons$ Pyruvate

NAD$^+$ $\rightarrow$ NADH + H$^+$

LDH
Malate dehydrogenase

PDB: 1IB6


Lactate dehydrogenase

PDB: 3WX0

Furukawa, N., Togawa, M., Miyanaga, A., Nakajima, M., Taguchi, H.
PDB: 1IB6

NAD$^+$

R81
Malate $\leftrightarrow$ Oxaloacetate

NAD$^+$ $\leftrightarrow$ NADH + H$^+$

Lactate $\leftrightarrow$ Pyruvate

NAD$^+$ $\leftrightarrow$ NADH + H$^+$

R81
\[
\text{Malate} \rightleftharpoons \text{Oxaloacetate} \quad \text{NAD}^+ \; \text{NADH} + \text{H}^+
\]

\[
\text{Lactate} \rightleftharpoons \text{Pyruvate} \quad \text{NAD}^+ \; \text{NADH} + \text{H}^+
\]
• Hypothesis
• The arginine-to-isoleucine (R81I) mutation in MDH would alter enzyme activity in a way that favors the lactate-pyruvate reaction because of the methyl group on lactate and pyruvate.
Method – Expression, Isolation & Purification

WT

R81I

IPTG

I.
Method – Expression, Isolation & Purification

I. IPTG

II. PEI

-80°C 5x
Method – Expression, Isolation & Purification

I. Expression
   - WT
   - R81I
   - IPTG

II. Isolation
   - -80°C
   - 5x
   - PEI

III. Purification
   - Ni-NTA
Results – Expression, Isolation & Purification

Cell lysate PEI Ni-NTA

1 2 3 4 5 6

Protein conc. (mg/mL)

Column Flow (mL)

WT eMDH Equil. Load Wash Elution

0 1 2 3

Protein conc. (mg/mL)

Column Flow (mL)

R81I eMDH

0 1 2 3
Method – Characterization

Wild Type R81I

- Bradford assay
- Enzyme assay with malate
- Enzyme assay with lactate
- Enzyme assay with pyruvate

Specific activity per substrate
$\text{Malate} + \text{NAD}^+ \rightarrow \text{Oxaloacetate} + \text{NADH} + \text{H}^+$

$\text{Lactate} + \text{NAD}^+ \rightarrow \text{Pyruvate} + \text{NADH} + \text{H}^+$
Malate $\rightleftharpoons$ Oxaloacetate

Lactate $\rightleftharpoons$ Pyruvate

$\text{NAD}^+ \rightleftharpoons \text{NADH} + \text{H}^+$
Questions to Answer:

1. Dehydration of the active site / hydration potential of active site enzymes
2. Steric or electrostatic forces guide the transition?
3. Contribution from neighboring positively charged amino acids?
Thank You

• Pence-Boyce Research Experience
• Dr. Heyen
• Department of Chemistry & Geosciences