Chapter 4: Enzyme Kinetics

Purpose:
1) Investigate the kinetics of LDH purified from bovine heart and muscle
2) Learn how to determine kinetic information
3) Understand the effects of inhibitors on enzyme activity
Enzyme Kinetics

- Rate of enzyme catalyzed reaction depends on substrate concentration
- Want to measure initial rate, \( V_o \) – [E] low, [S] high
- As [S] increases, \( V_o \) increases to certain point and then levels off – \( V_{max} \)
Michaelis-Menton Mechanism for Enzyme Action

- **1st Step**: Fast reversible binding of Enzyme to Substrate (Enzyme-Substrate complex)
- **2nd Step**: Slower breakdown of the ES complex to Enzyme + Product

\[
E + S \overset{k_1}{\rightleftharpoons} ES \overset{k_2}{\rightarrow} E + P
\]

- At any time during reaction the enzyme is present as both E and ES
- Maximal rate \((V_{max})\) observed when \([ES]\) is highest, and \([E]\) is lowest
  - Enzyme is saturated with substrate
• **Pre-Steady State**
  Initial mixing of $E + S$, while $[ES]$ builds up

• **Steady-State**
  $[ES]$ remains approximately constant

• **Steady-State Kinetics**
  Measurements of $V_o$ while $[ES]$ is relatively stable
Michaelis-Menton Kinetics

\[ K_M - \text{Michaelis constant} = \frac{(k_2 + k_{-1})}{k_1} \]

\[ V_o = \frac{V_{max} [S]}{K_M + [S]} \]

\[ \frac{V_{max}}{2} = \frac{V_{max} [S]}{K_M + [S]} \]

\[ K_M = [S], \text{when } V_o = \frac{1}{2} V_{max} \]
Lineweaver-Burk Manipulation

Double-Reciprocal Plot

\[ V_o = \frac{V_{\text{max}}[S]}{K_M + [S]} \]

\[ \frac{1}{V_o} = \frac{K_M + [S]}{V_{\text{max}}[S]} \]

\[ \frac{1}{V_o} = \frac{1}{[S]} \left( \frac{K_M}{V_{\text{max}}} \right) + \frac{1}{V_{\text{max}}} \]

\[ y = x \ (m) + b \]

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

\[ 1/\left[ S \right] \]

Slope = \( K_M / V_{\text{max}} \)

[S] = 0.5 \( K_M \)

[S] = 5 \( K_M \)
Eadie-Hofstee Manipulation

Eadie-Hofstee Plot

\[ y = -5.5398x + 0.8114 \]

\[ R^2 = 0.9834 \]

Vo (mM/min)

Vo/[S] (1/min)

Slope = - \( K_M \)

\[ Vo = \frac{V_{max} [S]}{K_M + [S]} \]

\[ Vo = -K_M \left( \frac{V_o}{[S]} \right) + V_{max} \]

\[ y = m \times (x) + b \]
Introduction of an Inhibitor

- **Competitive Inhibition** – Competes with substrate for active site

- **Uncompetitive Inhibition** – Binds to distinct site from substrate active site and binds only to ES complex

- **Non-Competitive Inhibition (Mixed)** – Binds to both substrate active site and distinct site

- **Pure Non-Competitive Inhibition** – Binds to a distinct site on the enzyme complex that decreases overall activity
Competitive Inhibition

\[ V_o = \frac{V_{max} [S]}{K_M \left(1 + \frac{[I]}{K_I}\right) + [S]} \]

\[ \frac{1}{V_o} = \frac{1}{[S]} \left( \frac{K_M \left(1 + \frac{[I]}{K_I}\right)}{V_{max}} \right) + \frac{1}{V_{max}} \]

\[ y = x \ (m) + b \]

See pp. 99-100 for equations
Uncompetitive Inhibition

\[ V_o = \frac{V_{\text{max}} [S]}{K_M + [S] \left( 1 + \frac{[I]}{K_I} \right)} \]

\[ \frac{1}{V_o} = \frac{1}{[S]} \left( \frac{K_M}{V_{\text{max}}} \right) + \frac{1}{V_{\text{max}}} \left( 1 + \frac{[I]}{K_I} \right) \]

\[ y = x \ (m) + b \]

See pp. 99-100 for equations
Non-Competitive Inhibition (Mixed)

\[
V_o = \frac{V_{max} [S]}{(K_M + [S]) \left(1 + \frac{[I]}{K_I}\right)}
\]

\[
\frac{1}{V_o} = \frac{1}{[S]} \left(\frac{K_M \left(1 + \frac{[I]}{K_I}\right)}{V_{max}}\right) + \frac{1}{V_{max}} \left(1 + \frac{[I]}{K_I}\right)
\]

\[
y = x \ (m) + b
\]

See pp. 99-100 for equations
Pure Non-Competitive Inhibition

\[
\frac{1}{V_0} = \frac{1}{V_{\text{max}}} \left( 1 + \frac{[I]}{K_I} \right)
\]

Y-Intercept = \( \frac{1}{V_{\text{max}}} \left( 1 + \frac{[I]}{K_I} \right) \)

Slope = \( \frac{K_M}{V_{\text{max}}} \left( 1 + \frac{[I]}{K_I} \right) \)

(noncompetitive inhibitor)

X-Intercept = \( \frac{1}{V} \)

Slope = \( \frac{K_M}{V_{\text{max}}} \)

(no inhibitor present)

Intercept = \( \frac{-1}{K_M} \)
Chapter 4: Procedure

- Make **new** cocktail with Tris-Buffer pH 8.2 – **Cocktail A**
  - This cocktail gives a higher $K_M$ value for LDH
- Perform activity assays where you vary [pyruvate] without inhibitor
  - Starting $\Delta A_{340}/\text{min} = 0.02-0.04$
  - Dilute appropriately to get in range
Chapter 4: Procedure

- Make new cocktail with Tris-Buffer pH 8.2 and inhibitor (your choice) – **Cocktail B**
  - Make sure to write down letter and concentration of inhibitor
- Perform activity assays where you vary [pyruvate] in presence of the inhibitor
  - Rates with inhibitor < Rates of uninhibited reactions

Make sure to prepare data tables p. 106-7 **BEFORE LAB**!
Include all cocktail recipes in your notebook!
Lab Notebook: Chapter 4

- Raw Data for uninhibited and inhibited LDH

- Calculation of rates in mM:
  \[
  \left( \frac{\Delta A_{340}}{\text{min}} \right) \left( \frac{\left(3 \text{ mL total volume}\right) \left(\text{Dilution Factor}\right)}{\left(0.1 \text{ mL enzyme used}\right)} \right) = \text{Rates in mM/min}
  \]

- Michaelis-Menton and Lineweaver-Burk Plots for uninhibited and inhibited LDH

- Calculation of \( K_M \) and \( V_{max} \) – Show unit calculations!

- Calculation of \( K_I \) for your type of inhibition

What type of inhibition is pictured here?