Enzymes II: kinetics

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Specific aims: Enzymes II: kinetics

You must be able to:
1. Know what is kinetics;
2. Understand the concepts of Michaelis-Menten Kinetics and its conditions
3. Define Velocity (initial velocity $V_0$) and how it is determined
4. Understand how the experiment of initial velocity $V_0$ versus substrate concentration $[S]$ is achieved
5. Define steady state kinetics, (M-M kinetics: $E$ and $ES$ changes very small)
6. Define Pre-steady State Kinetics and its importance:
7. Understand the concept of Vmax, and Km
8. Understand Michaelis-Menten Equation
9. Compare Michaelis-Menten kinetics enzymes with enzymes that do not follow M-M kinetics (allosteric enzymes)
10. Describe Perfect enzymes,
11. Understand the concept of $K_{cat}/K_m$
12. Know how Lineweaver Burk blot is established
13. Interpret data from Lineweaver Burk blot
E+S <=> ES <=> ES* <=> EP <=> E+P
Michaelis-Menten Kinetics

\[ E + S \leftrightarrow ES \leftrightarrow ES^* \leftrightarrow EP \leftrightarrow E + P \]
Michaelis-Menten Kinetics

- Substrate
- Product
- Enzyme
- Enzyme-Substrate Complex

Graph showing the concentration of substrate, product, enzyme, and enzyme-substrate complex over time.
Michaelis-Menten Kinetics

Substrate high

Enzyme high

ES low

Product low
Kinetic Considerations

Pre-steady state: 
- [E] and [ES] vary widely

Steady state: 
- [E] and [ES] relatively constant
Pre-steady State Kinetics

Can give info on reaction mechanism, rate of ES formation
Enzymes
• Kinetic Considerations

Substrate | Enzyme | Low substrate | High substrate
Enzymes
• Kinetic Considerations

Initial Velocity ($V_0$) - Measured as [Product]/Time

Low [S], Low $V_0$ (Enzymes Often Idle)

High [S], High $V_0$ (Enzymes Almost Always Busy)

Substrate Concentration (Molarity)
Kinetic Considerations

![Graph showing enzyme kinetics](image)

- $V_{\text{max}}$
- $\frac{1}{2}V_{\text{max}}$
- $K_m$

**Graph Explanation:**
- **Reaction rate** vs. **Substrate concentration**
- The graph illustrates the relationship between the reaction rate and substrate concentration, highlighting the concept of $V_{\text{max}}$ (maximum reaction rate), $\frac{1}{2}V_{\text{max}}$, and $K_m$ (Michaelis constant).
Michaelis-Menten Kinetics

- Considerations

\[ V_0 = \frac{V_{\text{max}}[S]}{K_m + [S]} \]

Enzymes That Don’t Follow Michaelis-Menten Kinetics Include Those That Bind Substrate Cooperatively - Binding of One Substrate Affects Binding of Others
Control of Enzyme Activity

- Allosteric Control

**Allosteric Enzyme Kinetics**

Substrate Does Not Change Enzyme Binding of Substrate

Substrate Does Change Enzyme Binding of Substrate
Michaelis-Menten Equation

\[ v = \frac{V_{\text{max}} [S]}{K_m + [S]} \]

\( V_{\text{max}} \) occurs when an enzyme is saturated by substrate
\( V_{\text{max}} \) varies with amount of enzyme used
\( K_m \) is a measure of an enzyme’s affinity for its substrate
\( K_m \) value inversely related to affinity

High \( K_m \) = Low Affinity
Low \( K_m \) = High Affinity
Michaelis-Menten Kinetics

- $V_{\text{max}}$ and $K_{\text{cat}}$

$V_{\text{max}}$ is Proportional to the Amount of Enzyme Used in an Experiment - Not Useful for Comparing Enzymes

Since $V_{\text{max}}$ is a Velocity, and Velocity = [Product]/Time,

\[
\frac{V_{\text{max}}}{[\text{Enzyme Used}]} = \frac{[\text{Product}]}{[\text{Enzyme Used}] \times \text{Time}}
\]

The Two Concentrations Cancel Out.

The Result is a Number Per time (say 1000/second). (It is the Number of Product Molecules Made by Each Enzyme Molecule Per Time.

It is Also Known as the Turnover Number or $K_{\text{cat}}$ and Does Not Vary with the Amount of Enzyme)
Perfect Enzymes

Maximum $K_{cat}/K_M$
Mutation leads to reduced $K_{cat}/K_M$
Diffusion of substrate limiting
Triose Phosphate Isomerase
# Enzyme Co-factors

<table>
<thead>
<tr>
<th>Cofactor</th>
<th>Enzyme</th>
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<tbody>
<tr>
<td><strong>Coenzyme</strong></td>
<td></td>
</tr>
<tr>
<td>5'-Deoxyadenosyl cobalamin</td>
<td>Methylmalonyl mutase</td>
</tr>
<tr>
<td>Biotin</td>
<td>Pyruvate carboxylase</td>
</tr>
<tr>
<td>Coenzyme A (CoA)</td>
<td>Acetyl CoA carboxylase</td>
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<tr>
<td>Flavin adenine nucleotide</td>
<td>Monoamine oxidase</td>
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<tr>
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<td>Pyruvate dehydrogenase</td>
</tr>
<tr>
<td><strong>Metal</strong></td>
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<tr>
<td>K⁺</td>
<td>Propionyl CoA carboxylase</td>
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<tr>
<td>Mg²⁺</td>
<td>Restriction Endonucleases; Hexokinase</td>
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<tr>
<td>Mo</td>
<td>Nitrate reductase</td>
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<tr>
<td>Ni²⁺</td>
<td>Urease</td>
</tr>
<tr>
<td>Se</td>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>Carbonic anhydrase; Carboxypeptidase</td>
</tr>
</tbody>
</table>
Michaelis-Menten Kinetics

• Lineweaver Burk Plot

Also Called Double Reciprocal Plot

Uses Same Data as V vs. [S] Plot, but Inverts All Data for the Plotting

Linear for Enzymes Following Michaelis Menten Kinetics

Direct Reading of $-1/K_m$ and $1/V_{max}$

Y-Intercept $= \frac{1}{V_{max}}$

X-Intercept $= -\frac{1}{K_m}$

$\frac{1}{V_0}$