LECTURE 3: ENZYME KINETICS

To succumb is healthier than to conquer, as we may calm down and develop affection and love, as well as avoid emotion, anger or madness, and hate.

LECTURE OUTCOMES

After the completion of the lecture and mastering the lecture materials, students will be able to

1. to explain enzyme substrate interaction in the conversion of substrate to product
2. to explain the order of chemical reactions
3. to explain enzyme kinetics particularly the rate of reactions as a function of substrate concentration
4. to calculate $K_M$ and $V_{max}$ of reactions catalyzed by enzymes
5. to explain characteristics of enzymes based kinetic analysis of enzymatic reactions
LECTURE OUTLINE

I. INTRODUCTION
   1. Definition
   2. Basic Enzyme Reactions
   3. The rate of reaction

II. ENZYME-SUBSTRATE INTERACTION
   1. Lock and Key" Hypothesis
   2. The "Induced Fit" Hypothesis

III. MICHAELIS-MENTEN MODEL
   1. The formation of ES Complex
   2. Steady-State Assumption
   3. Initial Velocity Assumption
   4. Total Enzyme

IV. PENETUAN K_M DAN V_MAX

I. INTRODUCTION

1. Basic Enzyme Reactions
   1. Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzymes or the study of the rates of enzyme-catalyzed reactions.

The branch of chemistry which deals with the rates of chemical processes is known as chemical kinetics.
2. Enzymes, functioning as catalysts, increase the speed of a chemical reactions, and are not used up in the reactions.
   The basic enzymatic reaction can be represented as follows
   \[ S + E \rightarrow P + E \] (1)
   where \( E \) = enzyme, \( S \) = substrate, and \( P \) = product of the reaction.

3. A theory to explain the catalytic action of enzymes was developed by Savante Arrhenius in 1888, a Swedish chemist. He proposed that the substrate (S) and enzyme (E) formed some intermediate substance known as the enzyme substrate complex as shown below.
   \[ S + E \rightarrow ES \] (2)

4. If this reaction is combined with the original reaction equation (1), the following results:
   \[ S + E \rightarrow ES \rightarrow P + E \] (3)

5. The existence of an intermediate enzyme-substrate complex has been demonstrated in the laboratory, for example, using catalase and a hydrogen peroxide derivative.

6. At Yale University, Kurt G. Stern in 1937 observed spectral shifts in catalase as the reaction it catalyzed proceeded.
   - This experimental evidence indicates that the enzyme first unites in some way with the substrate and then returns to its original form after the reaction is concluded.
2. The rate of reaction

1. The rate of reaction \( V \) is the quantity of substrate \( \partial S \) converted to product, or the amount product \( \partial P \) produced per unit time.

2. Reactions follow zero order kinetics when substrate concentrations are high.
   - Zero order means there is no increase in the rate of the reaction when more substrate is added.

\[
S \rightarrow P
\]

Where \( V \) is the rate (velocity) of reaction

\[
V = -\frac{\partial S}{\partial t} = \frac{\partial P}{\partial t} = k
\]

\[
\int_{[S]}^{[S]_b} \partial S = -k \int_{0}^{t} \partial t
\]

<table>
<thead>
<tr>
<th>Order</th>
<th>Rate</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>zero</td>
<td>( k )</td>
<td>rate is independent of substrate concentration</td>
</tr>
<tr>
<td>first</td>
<td>( k[S] )</td>
<td>rate is proportional to the first power of substrate concentration</td>
</tr>
<tr>
<td>second</td>
<td>( k[S][S] = k[S]^2 )</td>
<td>rate is proportional to the square of the substrate concentration</td>
</tr>
<tr>
<td></td>
<td>( k[S_1][S_2] )</td>
<td>rate is proportional to the first power of each of two reactants</td>
</tr>
</tbody>
</table>
2. ENZYME-SUBSTRATE INTERACTION

- The rate of enzymatic reaction is influenced by the interaction of enzyme and substrate.
- The two types of interaction between enzyme and substrate
  1. "Lock and Key" Hypothesis
  2. The "Induced Fit" Hypothesis

1. "Lock and Key" Hypothesis
   - Emil Fischer in 1890 proposed "Lock and Key" Hypothesis.

   - The shape, or configuration (form in space), of the active site is especially designed for the specific substrate involved.
   - As the active site is made up of protein, the form of active site in space (configuration) is determined by the amino acid sequence of the enzyme in the active site.
- It is also important that the **native configuration** of the entire enzyme molecule must be intact for the active site to have the correct configuration.

- In such a case, the substrate then **fits into the active site** of the enzyme in much the same way as a key fits into a lock.

- Enzyme examples follow the lock and key hypothesis are **RNase A, cyclophilin A, and dihydrofolate reductase** (Sullivan & Holyoak, 2008)

2. The "Induced Fit" Hypothesis

- Enzymes are **highly flexible, conformationally dynamic molecules**.

- Many of their remarkable properties, including substrate binding and catalysis, are due to their **structural pliancy**.

- Realization of the conformational flexibility of proteins led Daniel Koshland to hypothesize that the binding of a substrate (S) by an enzyme is an interactive process.

- The shape of the enzyme’s **active site** is actually modified upon binding **S**, in a process of dynamic recognition between enzyme and substrate aptly called induced fit.
In essence, substrate binding alters the conformation of the protein, so that the protein and the substrate "fit" each other more precisely. The process is truly interactive in that the conformation of the substrate also changes as it adapts to the conformation of the enzyme. An example of enzyme follow “Induced-fit hypothesis” is **Rubisco**.

The two dimension of Rubisco active site as a fuction of amio acid sequence
**Hexokinase** has a large induced fit motion that closes over the substrates *adenosine triphosphate* (ATP) and *xylose*.

Hexokinase catalyzes the following reaction.

\[
\text{Gucose} + \text{ATP} \rightarrow \text{Glucose-6P} + \text{ADP}
\]

- Binding sites in blue, substrates in black and Mg\(^{2+}\) cofactor in yellow. (PDB: 2E2N, 2E2Q)

**Induced Fit Model**

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**III. MICHAELIS-MENTEN MODEL**

Leonor Michaelis and Maud L. Menten proposed a general theory of enzyme action in 1913 while studying the hydrolysis of sucrose catalyzed by the enzyme *invertase*.

1. **The formation of ES Complex**

   - Their theory was based on the assumption that the enzyme (E) and its substrate (S) associate reversibly to form an enzyme-substrate complex, ES

   \[
   \text{E} + \text{S} \underset{k_2}{\overset{k_1}{\longrightarrow}} \text{ES} \underset{k_4}{\overset{k_3}{\longrightarrow}} \text{E} + \text{P}
   \]

   E = Enzyme, S = Substrate, P = Product, ES = Enzyme-Substrate complex, \(k_1, k_2, k_3, k_4\) = rate constants. \(k_4\) is very small and ignored.
For instance, the breakdown of sucrose to glucose and fructose through a hydrolysis reaction catalyzed by *sucrase (invertase)*

\[ \text{Sucrose} + H_2O \rightarrow \text{Glucose} + \text{Fructose} \]

- If environmental factors are constant, the rate of product formation (reaction rate of velocity, \( V \)) is dependent upon the concentration of enzyme and substrate

- \([E] = \) enzyme concentration
- \([S] = \) substrate concentration

**Effect of \([E]\)**

- to study the effect of increasing the \([E]\) upon the reaction rate, the substrate must be present in an excess amount so that the reaction is independent of the \([S]\).

\([S] \gg [E]\)

- The formation of product proceeds at a rate which is linear with time.
- Any change in the amount of product formed over a specified period of time is dependent upon the level of enzyme present.
The reactions with the rates independent of substrate concentration but equal to some constant $k$ are said to be "zero order".

**The influence of [S]**

- The concentration of substrate [S] greatly influences the rate of product formation (the velocity of a reaction, $V$)
  - Studying the effects of [S] on the velocity of a reaction is complicated by the reversibility of enzyme reactions, e.g. conversion of product back to substrate
  - To overcome this problem, initial velocity ($V_0$) measurements are used. At the start of a reaction,
    - [S] is in large excess of $[P] \rightarrow [S] \gg [P]$
  - thus the initial velocity of the reaction ($V_0$) will be dependent on substrate concentration [S]

- When initial velocity is plotted against [S], a hyperbolic curve is found and characterized by an approximately constant maximum of reaction rate ($V_{\text{max}}$)
- \( V_{\text{max}} \) represents the maximum reaction velocity
- This occurs at \([S] >> E\), and all available enzyme is "saturated" with bound substrate, meaning only the ES complex is present

- The association/dissociation of ES complex is assumed to be a rapid equilibrium, and the enzyme:substrate dissociation constant is \( K_s \).
  At equilibrium,
  \[ k_2[ES] = k_1[E][S] \]
  and
  \[ K_s = \frac{[E][S]}{[ES]} = \frac{k_2}{k_1} \]

**2. Steady-State Assumption**
- The interpretations of Michaelis and Menten were refined and extended in 1925 by Briggs and Haldane, by assuming \([ES]\) quickly reaches a constant value in such a dynamic system.
- That is, ES is formed rapidly from \( E + S \), and disappears by its two possible fates
  - dissociation to regenerate \( E + S \), and
  - reaction to form \( E + P \)
- This assumption is termed the steady-state assumption and is expressed as
  \[ \frac{\partial[ES]}{\partial t} = 0 \]
3. **Initial Velocity Assumption**

- Enzymes accelerate the rate of the reverse reaction as well as the forward reaction, then the conversion of E+P to ES or $k_4[E][P] = k_3[ES]$
- If we observe only the initial velocity for the reaction immediately after E and S are mixed in the absence of P, the rate of any back reaction is negligible.
- Therefore, $k_4[E][P] = 0$ as [P] is essentially 0 which gives

$$E + S \overset{k_1}{\longrightarrow} ES \overset{k_2}{\longrightarrow} E + P$$

(1)

4. **Total Enzyme**

- Given such simplification, the system described by equation above is analyzed in order to describe the initial velocity $V$ as a function of [S] and amount of enzyme.
- The total amount of enzyme is fixed and is given by the formula

$$[E]_0 = [E] + [ES]$$

(2)

- The rate of product formation is dependent upon [ES] and $k_3$
\[ v = \frac{d[ES]}{dt} = k_1[ES] \]

[ES] is the difference between the rates of ES formation minus the rates of its disappearance.

\[ \frac{d[ES]}{dt} = k_1[E][S] - k_2[ES] - k_3[ES] \]

- The assumption of steady state gives
  - The rate of [ES] formation = The rate of [ES] dissociation
  - The formation of [ES] = \( k_1[E][S] \)
  - The dissociation of [ES] = \( (k_2 + k_3)[ES] \)

Then
\[ \frac{\partial[ES]}{\partial t} = k_1[E][S] - (k_2 + k_3)[ES] = 0 \quad (3) \]

- The substitution of E from equation (2) into the equation (3) results in

\[ (2) \rightarrow [E]_0 = [E] + [ES] \rightarrow [E] = [E]_0 - [ES] \]

\[ (5) \rightarrow \frac{\partial[ES]}{\partial t} = k_1[S][E] - (k_2 + k_3)[ES] \]

\[ k_1[S][E] = k_1[S]([E]_0 - [ES]) = k_1[S][E]_0 - k_1[S][ES] \]

- Further arrangements of the above equation will result in the Michaelis-Menten Model as shown below (see Appendix for derivation)

\[ V = \frac{V_{max}[S]}{K_M + [S]} \]
Michaelis-Menten Equation

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

Model Parameters

**Vmax**
- Vmax is the maximum rate of reaction or the rate of reaction when all enzyme molecules are saturated with the substrate.

**K_M**
- K_M is the concentration of substrate at V = \( \frac{1}{2} \) Vmax
  - If V = \( \frac{1}{2} \)Vmax for V = Vmax[S]/(K_M+[S]), then
  - \( \frac{1}{2} \)Vmax = Vmax[S]/(K_M+[S])
  - \( \frac{1}{2} (K_M+[S]) = [S] \rightarrow K_M+[S] = 2[S]
  - \( K_M = [S] \)
- \( K_M = \frac{(k_2 + k_3)}{k_1} \) is Michaelis-Menten Constant
The rate-determining step of the reaction is $k_3$, for the formation of product.

So if $k_2 >> k_3 \rightarrow K_M = \frac{k_2}{k_1}$

$k_2/k_1$ is known as a dissociation constant for the ES complex.

$k_2/k_1$ reflects a tendency of ES complex to dissociate to be E and S.

- $K_M = \frac{k_2}{k_1}$ can be used as a relative measure of the affinity of a substrate for an enzyme.

Low $K_M \rightarrow$ high apparent affinity of a substrate for an enzyme.

High $K_M \rightarrow$ low apparent affinity of a substrate for an enzyme.

The best substrate for enzyme is that which has the highest $V_{max}/K_M$.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Vmax</th>
<th>$K_M$</th>
<th>$V_{max}/K_M$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymotrypsin</td>
<td>100</td>
<td>5000</td>
<td>1/50</td>
</tr>
<tr>
<td>Carbonic Anhydrase</td>
<td>600,000</td>
<td>8000</td>
<td>600/8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$K_m$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate carboxylase</td>
<td>pyruvate</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>HCO3</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>60</td>
</tr>
</tbody>
</table>
**Turnover number**

- In enzyme kinetics, we are interested to know the maximum molecules of substrate that can be converted into product per catalytic site of a given concentration of enzyme per unit time.

**Turnover number: \( k_{\text{cat}} = V_{\text{max}}/[E]_0 \)**

The units of Turn over number = (moles of product/sec)/ (moles of enzyme) or sec\(^{-1}\).

This \( k_{\text{cat}} \) is achieved when E is fully saturated

- For example, carbonic anhydrase has a turnover number of \( 4 \times 10^5 - 6 \times 10^5 \text{ s}^{-1} \) (each carbonic anhydrase molecule can produce up to \( 6 \times 10^5 \) molecules of bicarbonate ions per second)

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**IV. Penetuan \( K_M \) dan \( V_{\text{max}} \)**

- Harga \( K_M \) bervariasi sangat besar, tapi dari kebanyakan enzim berkisar diantara \( 10^{-1} - 10^{-6} \) M (Tabel 2.1) tergantung substrat dan lingkungan seperti suhu dan kuantitas ion

- Untuk mendapatkan harga \( K_M \) dan \( V_{\text{max}} \), analisis langsung persamaan diatas dapat dilakukan, tapi cara ini membutuhkan waktu yang lama, dan bantuan komputer sangat penting untuk optimasi harga parameter persamaan dengan cepat.
## Tabel 2.1 Parameter beberapa enzim

<table>
<thead>
<tr>
<th>Enzim</th>
<th>Substrat</th>
<th>( K_m ) (M)</th>
<th>( K_i ) (M)</th>
<th>Inhibitor</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose diphosphate dehydrogenase (E.coli)</td>
<td>D-Glyceraldehyde 3-phosphate</td>
<td>9.10^{-3}</td>
<td>3.10^{-5}</td>
<td>1,3 Diphosphoglycerate</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D-Threon 2,4 diphosphate</td>
<td>NC</td>
</tr>
<tr>
<td>Succinate dehydrogenase (E.coli)</td>
<td>Succinate</td>
<td>1.310^{-3}</td>
<td>4.110^{-5}</td>
<td>Malonate</td>
<td>C</td>
</tr>
<tr>
<td>Alcohol dehydrogenase (E.coli)</td>
<td>Ethanol</td>
<td>1.310^{-2}</td>
<td>6.710^{-4}</td>
<td>Acetaldehyde</td>
<td>NC</td>
</tr>
<tr>
<td>Glucose 6-phosphate dehydrogenase (E.coli)</td>
<td>Glucose 6-phosphate</td>
<td>4.210^{-3}</td>
<td>6.10^{-5}</td>
<td>Citrate</td>
<td>C</td>
</tr>
<tr>
<td>Ribulose diphosphate carboxylase (spinach)</td>
<td>Ribulose diphosphate</td>
<td>1.210^{-4}</td>
<td>4.210^{-6}</td>
<td>Pi</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>HCO_{3}^{-}</td>
<td>2.210^{-2}</td>
<td>9.510^{-4}</td>
<td>3 Phosphoglyceric acid</td>
<td>C</td>
</tr>
<tr>
<td>Fructose 1,6-diphosphate aldolase (E.coli)</td>
<td>Fructose 1,6-diphosphate</td>
<td>3.10^{-8}</td>
<td>2.10^{-9}</td>
<td>L-Sorbose 1 PO_4</td>
<td>C</td>
</tr>
<tr>
<td>Succinyl CoA synthetase (E.coli)</td>
<td>Succinate</td>
<td>5.10^{-4}</td>
<td>2.10^{-5}</td>
<td>Succinyl CoA</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Citrate</td>
<td>7.10^{-3}</td>
<td></td>
<td>Pi</td>
<td>UC</td>
</tr>
</tbody>
</table>

\(^{*}C = \text{Competitive, NC = Non-competitive, UC = Uncompetitive}\)

## PENDEKATAN LAIN

- **Linierisasi persamaan**
  - Modifikasi persamaan ke bentuk linier sehingga dapat dianalisis dengan mudah
  1. Persamaan “double-reciprocal” atau “Linewaever-Burk”
  2. Persamaan “Eadie-Hofstee”
  3. Persamaan “Hanes-Woolf”
Persamaan “double-reciprocal” atau “Lineweaver-Bürk”

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

- Jika ruas kiri dibalik dan demikian juga ruas kanan, maka

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

- Sekarang persamaan ini akan mudah dianalisis dengan metode linier sedehana

- Sekarang

\[ y = \frac{1}{V} ; \ x = \frac{1}{[S]} \]

\[ a = \frac{K_M}{V_{\text{max}}} ; \ b = \frac{1}{V_{\text{max}}} \]

dapat dianalisis dengan \( y = ax + b \)

Jika \( \frac{1}{V} \) dihubungkan dengan \( \frac{1}{[S]} \), suatu garis lurus akan dihasilkan yang memotong sumbu \( y \) pada \( \frac{1}{V_{\text{max}}} \) dan sumbu \( x \) pada \(-\frac{1}{K_M}\) serta membentuk sudut terhadap sumbu \( x \) sebesar \( \frac{K_M}{V_{\text{max}}} \).
Persamaan “Eadie-Hofstee”

\[
V = \frac{V_{\text{max}}[S]}{K_M + [S]} \\
V (K_M + [S]) = V_{\text{max}} [S] \\
V [S] = -VK_M + V_{\text{max}} [S] \\
v = -VK_M + \frac{V_{\text{max}}}{[S]} \\
V = -K_M \frac{v}{[S]} + V_{\text{max}}
\]

- Sekarang
  \( y = V \); \( x = V/[S] \)
  \( a = -K_M \); \( b = V_{\text{max}} \)
  dapat dianalisis dengan \( y = ax + b \)

Jika \( V \) dihubungkan dengan \( V/[S] \), suatu garis lurus akan dihasilkan yang memotong sumbu \( y \) pada \( V_{\text{max}} \) dan sumbu \( x \) pada \( V_{\text{max}}/K_M \) serta membentuk sudut terhadap sumbu \( x \) sebesar \( K_M \).
Persamaan “Hanes-Woolf”

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

\[ V (K_M + [S]) = V_{\text{max}} [S] \]

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

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

• Sekarang
  \( y = [S]/V ; \ x = [S] \)
  \( a = 1/V_{\text{max}}; \ b = K_M/V_{\text{max}} \)
  dapat dianalisis dengan \( y = ax + b \)

• Jika \( [S]/V \) dihubungkan dengan \([S]\), suatu garis lurus akan dihasilkan yang memotong sumbu y pada \( K_M/V_{\text{max}} \) dan sumbu x pada \(-K_M\) serta membentuk sudut terhadap sumbu x sebesar \(-K_M\).
### Weaver-Burk Equation

**Equation:**

\[ y = ax + b \]

**Parameters:**

- \( a = \frac{K_m}{V_{max}} \)
- \( b = \frac{1}{V_{max}} \)

**Table:**

<table>
<thead>
<tr>
<th>S</th>
<th>V</th>
<th>( 1/S )</th>
<th>( 1/V )</th>
<th>( x^2 )</th>
<th>( y^2 )</th>
<th>XY</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>0.05</td>
<td>50</td>
<td>200</td>
<td>2500</td>
<td>40000</td>
<td>10000</td>
</tr>
<tr>
<td>0.6</td>
<td>0.2</td>
<td>1.6667</td>
<td>12.5</td>
<td>27778</td>
<td>15625</td>
<td>20833</td>
</tr>
<tr>
<td>2.5</td>
<td>0.2</td>
<td>0.4</td>
<td>5</td>
<td>0.16</td>
<td>25</td>
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<tr>
<td>10</td>
<td>0.34</td>
<td>0.1</td>
<td>2.9412</td>
<td>0.01</td>
<td>8.6505</td>
<td>0.2941</td>
</tr>
<tr>
<td>15</td>
<td>0.36</td>
<td>0.0667</td>
<td>2.8571</td>
<td>0.0044</td>
<td>8.1633</td>
<td>0.1906</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>52.233</td>
<td>223.3</td>
<td>2503</td>
<td>49198</td>
<td>10023</td>
<td></td>
</tr>
</tbody>
</table>

**Mean:**

- \( b_x \) = 1957.3
- \( a \) = 3.9292
- \( b_y \) = 30226
- \( b \) = 3.6125
- \( b_{xy} \) = 7690.6
- \( V_{max} \) = 0.2768

**Graph:**

- \( y = 3.9292x + 3.6125 \)
- \( R^2 = 0.9997 \)

### Eadie-Hofstee Equation

**Equation:**

\[ y = ax + b \]

**Parameters:**

- \( a = -K_m \)
- \( b = V_{max} \)

**Table:**

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<tr>
<th>S</th>
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<td>10023</td>
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</tbody>
</table>

**Mean:**

- \( b_x \) = 1641.1
- \( a \) = -1.578
- \( b_y \) = 0.0944
- \( b \) = 0.3693
- \( V_{max} \) = 1.5775

**Graph:**

- \( y = -1.5775x + 0.3593 \)
- \( R^2 = 0.9005 \)
### Table

<table>
<thead>
<tr>
<th>S</th>
<th>V0</th>
<th>VE1</th>
<th>VE2</th>
<th>VE1</th>
<th>VE2</th>
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<td>0.2</td>
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</table>

### Graph

- **Equation:**
  - For VE1: \( y = 0.9829x + 0.0093 \)  
  - For VE2: \( y = 0.6875x + 0.0267 \)
- **R²:**
  - For VE1: 0.9946
  - For VE2: 0.9584
HOMEWORK

- Calculate KM and Vmax from the following data

<table>
<thead>
<tr>
<th>S (μ mol)</th>
<th>V (μ mol/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015</td>
<td>0.002</td>
</tr>
<tr>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
</tr>
<tr>
<td>8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

THANK YOU

شكراً

謝謝

Спасибо
1. Pembentukan ES adalah inti dari hipotesis tersebut

\[
E + S \xrightleftharpoons[k_2]{k_1} ES \xrightleftharpoons[k_3]{k_4} E + P \quad (1)
\]

2. Reaksi E dengan S terjadi dengan kecepatan \(k_1\) dan menghasilkan kompleks ES (enzim-substrat)

3. Kompleks ES dapat berubah menjadi E dan S bebas kembali dengan kecepatan \(k_2\), atau menjadi E dan P dengan kecepatan \(k_3\).
4. Jika $k_3 \gg k_4$, maka reaksi bersifat “irreversible”, sehingga produk P tidak ada yang diubah kembali menjadi substrat asal dan $k_4$ dapat diabaikan.

5. Suatu hal penting yang perlu diingat adalah bahwa konstanta $k_1$, $k_2$, $k_3$ dan $k_4$ proporsional dengan $\Delta G$ aktivasi substrat dari reaksi yang bersangkutan.


8. Pada [S] yang tinggi, semua enzim dijenuhi oleh substrat dan karenanya berada dalam bentuk kompleks ES.

9. Jadi enzim dalam suatu reaksi dapat berada dalam keadaan bebas dan terikat dengan substrat, sehingga total enzim secara matematis adalah:

$$[E]_0 = [E]+[ES]$$ (2)
10. Penurunan persamaan Michaelis-Menten tergantung pada asumsi yang disebut "Briggs-Haldane Steady-State"

11. Keadaan "steady state" adalah suatu keadaan dimana konsentrasinya intermediat (perantara) ES tetap konstan, sementara konsentrasinya substrat dan produk berubah

12. Keadaan demikian terjadi apabila kecepatan pembentukan ES sama dengan kecepatan peruraian ES

13. Keadaan "steady" dapat dinyatakan secara matematis seperti dengan persamaan berikut

\[ \frac{\delta [ES]}{\delta t} = 0 \]  \hspace{1cm} (3)

dimana \( t \) = waktu (menit)

14. Pernyataan \( \frac{\delta [ES]}{\delta t} \) dapat ditulis dari sudut konstanta dan konsentrasi pers (1) yaitu
Kecepatan pembentukan ES

\[ ES = k_1[E][S] \]  \hspace{1cm} (4a)
Kecepatan peruraian ES

$$ES = (k_2 + k_3) (ES) \quad (4b)$$

15. Dalam keadaan "steady state" kedua persamaan (4a) dan (4b) adalah sama, sehingga

$$\frac{\delta [ES]}{\delta t} = k_1[E][S] - (k_2+k_3)(ES) = 0 \quad (5)$$

16. Subsitusi E dari pers (2) kedalam pers (5) menghasilkan

(2) \rightarrow [E]_0 = [E]+[ES] \rightarrow [E] = [E]_0-[ES]

(5) \rightarrow \frac{\delta [ES]}{\delta t} = k_1[S][E] - (k_2+k_3)(ES)

$$k_1[S][E] = k_1[S][(E)_0-[ES])]$$

$$= k_1[S][E]_0 - k_1[S][ES]$$

Hence

$$k_1[S][E]_0 - k_1[S][ES] - (k_2+k_3)(ES) = 0$$

$$k_1[S][E]_0 - (k_1[S]+k_2+k_3)[ES]=0 \quad (6)$$
17. Pengaturan persamaan lebih lanjut

\[(k_1[S]+k_2+k_3)[ES] = k_1[S][E]_0\]

\[\text{[ES]} = \frac{k_1[S][E]_0}{k_1[S] + k_2 + k_3}\]  

(7)

18. Persamaan ini dapat dimodifikasi dengan cara ruas kanan dibagi dengan \(k_1[S]\),

\[\text{[ES]} = \frac{[E]_0}{1 + (k_2 + k_3)/k_1[S]}\]  

(8)

19. Karena \(k_1, k_2,\) dan \(k_3\) adalah konstanta, maka ketiga konstanta ini dapat dijadikan satu konstanta yaitu \((k_2 + k_3)/k_1 = K_M\) yang dikenal sebagai konstanta Michaelis-Menten

20. Untuk kebanyakan enzim \(k_3 \ll k_2\), sehingga \(K_M\) akan mendekati \((k_2 + k_1)\), sedang \((k_2 + k_3)/k_1\) adalah \(K_s\) (konstanta dissosiasi kompleks enzim-substrat).

21. Jika \(K_M\), yang merupakan ukuran affinitas enzim akan substrat, disubsitusikan kedalam pers (8), maka

\[\text{[ES]} = \frac{[E]_0}{1 + (K_M /[S])}\]  

(9)

22. Kecepatan reaksi katalisis dapat dinyatakan dengan jumlah produk yang tebentuk per satuan waktu yaitu produk dari konsentrasi kompleks ES dengan kapasitas katalisis enzim \(k_3\) (turnover number).

\[V = \frac{\partial[P]}{\partial t} = k_3[ES]\]  

(10)
23. Subsitusi [ES] dari pers. (10) ke dalam pers (9) memberikan

\[ V = \frac{k_2[E]_o}{1 + (K_M /[S])} \]  

(11)

24. Pada keadaan E dijenuhi S yang berarti semua enzim terikat dengan substrat dalam kompleks ES, maka \( V = V_{max} = k_3[E]_o \). Kemudian persamaan diatas dapat ditulis dalam bentuk berikut.

\[ V = \frac{V_{max}}{1+(K_M /[S])} \]  

atau

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

(12)

25. Persamaan diatas dikenal sebagai persamaan Michaelis-Menten yang digunakan secara luas.

26. Stoikiometri pers (12) didasarkan atas satu substrat dan satu produk (uni-uni), sementara banyak reaksi enzimatis yang melibatkan stoikiometri yang lebih kompleks seperti berikut;

\[ S \rightarrow P1 + P2 \quad (Ui-Bi) \]
\[ S1 + S2 \rightarrow P \quad (Bi-Uni) \]
\[ S1 + S2 \rightarrow P1 + P2 \quad (Bi-Bi) \]

27. Tetapi, persamaan Michaelis-Menten berlaku untuk reaksi yang lebih kompleks sekalipun dengan mekanisme yang berbeda.
FIG. 2. Overall structure of Rubisco from *C. reinhardtii*. The view is down the local 4-fold axis. The L subunits are depicted in two shades of gray, and the S subunits are in yellow. The S subunit A-B loops are shown in red. The inset shows a close-up view of the same region in the Spinacia enzyme with the same color coding. Pictures were produced with MOLSCRIPT (41) modified by Esnouf (42) and rendered with RASTER3D (43). Taylor et al., 2001, J. Biol. Chem., 276: 48159–48164