Protein Functions

Structural - keratin, collagen, fibroin

Enzyme - ________________________________
  - ________________________________
  - ________________________________
  - ________________________________

Gas transport/storage - hemoglobin, myoglobin

Nutrient - ovalbumin, main protein in egg white
  - casein, predominant protein in milk

Antibodies - IgG
  - proteins of vertebrates in the immune system for recognizing foreign object, pathogens

Hormones - peptide hormones (oxytocin, vasopressin)
  - protein hormones (insulin)

Mechanical work - myosin, actin (molecular motors)
Enzymes
Enzymes act as catalysts

**Catalyst** - a substance that increases ______________________________ without itself ______________________________.

**Catalysis** - the acceleration of ______________________________.

**Analogy**: crossing a mountain

<table>
<thead>
<tr>
<th>Mode of transportation</th>
<th>speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>walking</td>
<td>several days</td>
</tr>
<tr>
<td>bicycle</td>
<td>one day</td>
</tr>
<tr>
<td>car</td>
<td>&lt; one day</td>
</tr>
</tbody>
</table>

A bicycle would be a ____________

A car would be a ____________
Enzymes

Enzymes are most efficient catalysts known

<table>
<thead>
<tr>
<th>catalyst</th>
<th>reaction rate increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>no catalyst</td>
<td></td>
</tr>
<tr>
<td>enzyme</td>
<td>100,000,000,000,000,000,000</td>
</tr>
<tr>
<td>non enzyme</td>
<td>100 - 10,000</td>
</tr>
</tbody>
</table>

With out enzymes (catalysts) most chemical reactions in biological systems ________________________
__________________________.

3
Enzymes

How do enzymes increase reaction rates?

Reactants → products

- Reactions (rxn) require activation energy ($\Delta G^\ddagger$), ______________

____________________________________________

Analogy: activation energy is similar to amount of effort to push an object to the top of a hill so it can slide down the other side.

- free energy change ($\Delta G^\circ$) of a rxn is the ______________

____________________________________________

Can calculate $\Delta G^\circ$ by knowing the ______________

__________ and the amount ______________ at the end of reaction

- transition state: the maximum point of the rxn curve where ______________ and atom arrangement is ______________.

once reached rxn will ______________

$\Delta G^\ddagger$ can be viewed as the amount of ______________
Enzymes

How do enzymes increase reaction rates?

- enzymes lower the $\Delta G^\ddagger$, ______________________
- enzymes lower $\Delta G^\ddagger$ by changing the rxn __________
  ____________________________________________________________________.
- enzymes find __________________________

- uncatalyzed rxn has ____________________________
i.e. uncatalyzed rxn is _______ because it takes more energy
to get started. Thus, slower rxn rate.

consider oxidation of glucose

Glucose + 6 $\text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O}$

Is a _______________ ($\Delta G^\circ$ is negative, -689 kcal/mol
gives off energy), does not mean ________________.

Thus, rxn is ____________________________
but $\Delta G^\ddagger$ is large and is overcome by ____________
**Enzymes**

How do enzymes increase reaction rates?

- enzymes do not affect the ___________________________

- enzymes can ____________________

The $\Delta G^\circ$ of __________________________ rxn are the same.
Enzymes

How do enzymes increase reaction rates?

Breakdown of hydrogen peroxide to water and oxygen

$$2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$$

This reaction can be catalyzed by 

Table 6.1

Lowering of the Activation Energy of Hydrogen Peroxide Decomposition by Catalysts

<table>
<thead>
<tr>
<th>Reaction Conditions</th>
<th>Activation Free Energy</th>
<th>Relative Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kJ mol(^{-1})</td>
<td>kcal mol(^{-1})</td>
</tr>
<tr>
<td>No catalyst</td>
<td>75.2</td>
<td>18.0</td>
</tr>
<tr>
<td>Platinum surface</td>
<td>48.9</td>
<td>11.7</td>
</tr>
<tr>
<td>Catalase</td>
<td>23.0</td>
<td>5.5</td>
</tr>
</tbody>
</table>
Enzymes

Enzyme - Substrate Binding

**Substrate** - a reactant that forms ________________________________

**Transition-state species** - the rxn intermediate __________________________

**Active site** - an area of the enzyme with aa’s ____________________________
- site of __________________________
- usually located in a __________________________
- is the site of ____________

A simplified model of Enzyme-substrate interaction
Enzymes
Enzyme - Substrate Binding

The interaction -_________________________________

- is highly specific

- substrate interacts with _______________________
  and __________________ of active site amino acids
Enzymes

Enzyme - Substrate Binding

Two models for enzyme - substrate binding
1. ______________
2. ______________

Lock-and-Key Model

Substrate binds active site that has a shape
__________________________
__________________________.

Does not take into account
__________________________
__________________________
__________________________
after substrate binding.
Enzymes
Enzyme - Substrate Binding

Two models for enzyme - substrate binding
1. lock-and-key
2. induced-fit

Induced-Fit Model

Takes into account conformational change in enzyme after __________ ____________.

Favored over __________
____________
Enzymes
Enzyme - Substrate Binding
Induced fit model

Takes into account conformational change

________________________________________

Active site (______________) has different ____________ before substrate binding.

Binding of substrate induces changes in ________________________
_______________________________allowing for full binding of substrate.
Enzymes

Enzyme - Substrate Binding

Comparing the models

\[ E + S \longrightarrow ES \longrightarrow E + P \]

If the ES complex is a perfect fit as in lock-and-key model, ______________
__________________________________________________________________.

By not having a perfect fit of ES complex the ______________
__________________________________________________________________. 
Enzymes
Enzyme - Substrate Binding

Activation energy profile of lock-and-key type of interaction

ES complex will have lower ____________
_________________.

This will give too great a difference between ____
____________________
____________________

In the induced-fit model the ______________
____________________ is not so low.

Thus, difference between
____________________
____________________.
Enzymes
Product Formation

- Once substrate is bound, ________________________________
- substrate is arranged in correct __________________________
  ________________________________
- allows for ________________________________ and product to be formed.
- product is ____________________.
Enzymes

Michaelis-Menten Enzyme Kinetics

Leonor Michaelis  - German biochemist
  - worked at many universities, Berlin, University of Nagoya, Johns Hopkins University, Rockefeller Institute of Medical Research
  - did groundbreaking work on enzyme kinetics at Berlin University in 1913 with:

Maud Menten  - Canadian biochemist,
  - at time women could not get Ph.D. in Canada
  - completed medical doctorate at University of Chicago
  - moved to Berlin University in 1912 to work with Michaelis
  - obtained Ph.D. in 1916

Their work dealt with the kinetics of enzyme-substrate interaction
Enzymes
Michaelis-Menten Enzyme Kinetics

How to measure rates of reactions carried out by enzymes?

- in a test tube mix:
  - ______________
  - ______________
  - ______________

- allow rxn to take place
- take samples at ______________
- measure amount of ____________ units:
  - ______________
  - ______________
  - ______________

- repeat rxn using ______________
- graph results
Enzymes

Michaelis-Menten Enzyme Kinetics

What information can we obtain from a plot of \( V \) vs. \([S]\)?

\[
V = V_0 = \frac{V_{\text{max}}}{V_{\text{max}}} = \text{Enzymes}
\]

At 1/2 of \( V_{\text{max}} \) the \([S]\) is equal to:

K\(_m\) - an inverse measure of the ______

___________________________.

lower the ____ , higher the ______

K\(_m\) - the \([S]\) at which ____________

_____________________________
Problem: how to determine ______________ is theoretically unattainable?

\( V_{\text{max}} \) is asymptotic: never really ______________, reaches at infinity \([S]\).
Enzymes

Lineweaver-Burk double reciprocal plot

Hans Lineweaver - American biochemist
- worked as a graduate student under Burk
- at Department of Agriculture laboratory in Washington, D.C.

Dean Burk - American biochemist
- entered UC Davis at age 15.
- in 1934 published paper with Lineweaver describing how to determine $K_m$ and $V_{max}$
- is most frequently cited paper in biochemistry
Enzymes

Lineweaver-Burk double reciprocal plot

If $1/V$ and $1/[S]$ (reciprocal) are plotted, a ______________________
compared to _________________________ Michaelis-Menten data.

Can determine __________________ from this plot
Enzymes

Lineweaver-Burk double reciprocal plot

<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>Velocity (mM/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.024</td>
</tr>
<tr>
<td>5.0</td>
<td>0.036</td>
</tr>
<tr>
<td>10.0</td>
<td>0.053</td>
</tr>
<tr>
<td>15.0</td>
<td>0.060</td>
</tr>
<tr>
<td>20.0</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Michaelis-Menton plot
### Enzymes

#### Lineweaver-Burk double reciprocal plot

<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>Velocity (mM/sec)</th>
<th>1/[S]</th>
<th>Velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.024</td>
<td>0.400</td>
<td>41.667</td>
</tr>
<tr>
<td>5.0</td>
<td>0.036</td>
<td>0.200</td>
<td>27.778</td>
</tr>
<tr>
<td>10.0</td>
<td>0.053</td>
<td>0.100</td>
<td>18.868</td>
</tr>
<tr>
<td>15.0</td>
<td>0.060</td>
<td>0.067</td>
<td>16.667</td>
</tr>
<tr>
<td>20.0</td>
<td>0.064</td>
<td>0.050</td>
<td>15.625</td>
</tr>
</tbody>
</table>

Y-intercept =

V<sub>max</sub> =

V<sub>max</sub> =

X-intercept =

K<sub>m</sub> =

K<sub>m</sub> =
Enzymes

Practical example of $K_m$

Blood Sugar Levels and Glycolysis

Hexokinase
- an enzyme found in most tissues of the body
- converts ________________________________
- production of G6P is first step in _______________
  (break down of ______________________)

Glucokinase
- a variant of hexokinase found in liver
- G6P in liver is converted to glycogen - a polymer
  of glucose that can be broken down ______________
in times of low blood glucose levels.

Hexokinase $K_m$ for glucose = ____________
liver glucokinase $K_m$ for glucose = ____________

Blood [glucose] = ________________
Blood [glucose] after meal = ________________

Low blood [glucose], ≤5mM

liver
- glucokinase ____________
- glucose not stored ____________

muscle
- hexokinase ______________
- G6P produced ______________

High blood [glucose], ≥10mM
(after meal)

liver
- glucokinase active, ____________
- ________________ as glycogen

muscle
- hexokinase active
- G6P produced for glycolysis
Enzymes
Enzyme Inhibitors

**Enzyme inhibitors**: a substance that ____________________ ___________________________ and slows the reaction.

Two types of inhibitors:

1. **reversible** - can bind to enzyme, ____________________ ___________________________. Enzyme left in original condition

2. **Irreversible** - binds to enzyme making it ____________________ ___________________________. Enzyme will not return ______ ____________________________.
Enzymes
Reversible Enzyme Inhibitors

Two types of reversible inhibitors

**Competitive inhibitors**: - inhibitor compound is ________________
- binds to active site and ________________
- competes for ______________________
- substrate or inhibitor can bind enzyme,
  ________________________________

**Noncompetitive inhibitors**: - inhibitor binds to enzyme _________
- inhibitor binding causes ___________
- substrate can _________________
- but, enzyme inactive due __________
- both __________________________ bind at same time.
Enzymes
Kinetics of Competitive Inhibitors

Affects on enzyme kinetics (based on Lineweaver-Burk plot)

- changes the ________________, subsequently:

- changes __________________________

- does not change ____________________

More substrate is needed to ____________________

Because competitive inhibitor can be overcome with ______

Lineweaver-Burk plot
Enzymes

Kinetics of Competitive Inhibitors

Michaelis-Menten Kinetics

In presence of inhibitor higher amount of ________

_____________________.

[S], mM

V, mM/sec

[S], mM

= no inhibitor

= + inhibitor

= + 2x inhibitor
Enzymes

Kinetics of Noncompetitive Inhibitors

Affects on enzyme kinetics (based on Lineweaver-Burk plot)

- like competitive inhibitor, ____________
  ____________________________ but:

- unlike competitive inhibitor, ________
  _______________________________

- unlike competitive inhibitor, ________
  _______________________________

Substrate can still bind fully to enzyme, inhibitor does not ______
  ________________________________.

Because noncompetitive inhibitor and substrate are not competing for active site, __________
  ________________________________.
Enzymes
Inhibitor Example
Sucrose is broken down into its components, glucose and fructose, by the enzyme invertase.

Based on the data below determine if the inhibitor urea is competitive or noncompetitive

<table>
<thead>
<tr>
<th>[sucrose] (mM)</th>
<th>V, no inhib mM/sec</th>
<th>V, + inhib mM/sec</th>
<th>1/[sucrose] 1/(mM)</th>
<th>1/V, no inhib 1/(mM/sec)</th>
<th>1/V, + inhib 1/(mM/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0292</td>
<td>0.182</td>
<td>0.083</td>
<td>34.24</td>
<td>5.49</td>
<td>12.05</td>
</tr>
<tr>
<td>0.0584</td>
<td>0.265</td>
<td>0.119</td>
<td>17.12</td>
<td>3.77</td>
<td>8.40</td>
</tr>
<tr>
<td>0.0876</td>
<td>0.311</td>
<td>0.154</td>
<td>11.4</td>
<td>3.21</td>
<td>6.49</td>
</tr>
<tr>
<td>0.117</td>
<td>0.330</td>
<td>0.167</td>
<td>8.54</td>
<td>3.03</td>
<td>5.99</td>
</tr>
<tr>
<td>0.175</td>
<td>0.372</td>
<td>0.192</td>
<td>5.71</td>
<td>2.69</td>
<td>5.21</td>
</tr>
</tbody>
</table>