BCH 3000
PRINCIPLES OF BIOCHEMISTRY
(Semester 2 - 2014/15)
Enzymology - 1
Definition of Enzymes

Enzyme - a generic term for a biological catalyst

Unless otherwise indicated, all enzymes are proteins and will exhibit all of the properties of a protein in solution. Exceptions to this are so-called "Ribozymes" or catalytic ribonucleic acids which have been invoked as catalysts in RNA processing.
Properties of a Catalyst

1. Required in only trace amounts compared to reactants.

2. Unchanged by the net reaction.

3. Enhances rates but will not affect the equilibrium.

4. Catalysts facilitate both forward and reverse reactions.

5. Catalysts generally show specificity in the type or nature of the enzyme reactions which they accelerate.
Properties of Enzymes

1. Definitions to know
   a. active site
   b. substrate
   c. coenzyme or cofactor
   d. rate or velocity (produce or reactant)
   e. activator
   f. inhibitor
Properties of Enzymes

1. All enzymes are proteins
   a. molecular weight range 15 kd - 1000 kd
   b. enzymes show the same physical and chemical properties as all proteins.

1. denaturation
2. precipitation
3. sensitivity to proteases.
Properties of Enzymes

3. Enzymes are extremely efficient biological catalysts which must operate at 37° C or below and at pH values found in living cells (mild conditions).

In spite of this, they are capable of rate increases which exceed those seen with non-enzymatic catalysts.

4. Enzymes are highly specific in their catalysis. This is because they must bind (form a complex with) substrate. This allows enzymes to select for overall structure as well as configuration in one or more of the cargons in its substrate.
Properties of Enzymes

5. Enzymes may not only allow reactions to take place under physiological conditions, but they also allow the regulation of reactions through activation or inhibition of the enzyme by effectors **. Because of this, virtually all biological reactions are found to be enzyme catalyzed, even if they would proceed to a degree even without catalysis. In addition, enzymes may control reactions by varying enzyme concentration or modifying the enzyme structure.
The Transition State and Activation Energy

\[ A + B \rightleftharpoons C + D \]

- **Free Energy**
  - Ground State
  - Transition State
  - Activation Energy

\[ \Delta G \]

\[ \Delta G_0 \]

- **Course of Reaction**
  - Products

### Diagram Description:
- The diagram illustrates the transition state and activation energy in a reaction.
- The ground state (A+B) and activated state (AB) are shown with their corresponding energy changes (\( \Delta G_0 \)).
- The transition state (AB) is marked with an asterisk (*) and the activation energy (\( \Delta G \)).
The Transition State and Activation Energy

1. Molecular Collision must occur.

2. Reactants must have sufficient energy to reach the transition state.

3. Generally reactants must be oriented correctly.

4. In many cases, bond angles and lengths are distorted towards the those found in product.
Enzymology -2
Catalysis and Enzymology

• Introduction to catalysis
  – Free energy profiles
  – What enzymes do

• Chemical kinetics and reaction order
  – First-order kinetics
  – Other reaction orders

• Enzyme kinetics
  – Binding and saturation
What is a catalyst?

“A catalyst is a compound which enhances the rate of a chemical reaction without being destroyed or incorporated in the product” (IUPAC)

- Example:

\[ \text{HOCH}_2-\text{C}-\text{OH} + \text{C}_2\text{H}_5\text{OH} \xrightleftharpoons{H^+} \text{HOCH}_2-\text{C}-\text{OC}_2\text{H}_5 + \text{H}_2\text{O} \]

Features of a catalyst:

- Makes an alternative reaction path in which less activation energy (\(\Delta G^\ddagger\)) is needed
- Equally increases the rate of the back and forth reaction \(\Rightarrow\) catalysis has no effect on equilibrium position!
Gibbs free energy

Reaction coordinate

$X^\dagger$ (T.S.)

$\Delta G^\ddagger$

S

P
Two ways to decrease $\Delta G^\ddagger$:

- **a** By lowering the energy content of the transition state
- **b** By ground state destabilisation
What kinds of catalysts are there around?

- Organic
- Inorganic
- Biological (biocatalyst)

Other way of subdivision:

- Homogeneous = freely dissolved in solution
  - organic catalyst
  - organometallic complex
  - enzyme in water
- Heterogeneous = solid, in liquid or gaseous environment
  - inorganic catalyst (e.g. zeolite)
  - immobilised enzyme
  - enzyme in an organic solvent
All Enzymes are not Proteins: Ribozymes

- Ribozymes defined as RNA catalysts
- Function as phosphodiester transferases
  - Sequence specific
  - Kinetically like enzymes
  - Efficiently lower $\Delta G^*$
  - Require native 3D-structure
- Function in RNA splicing
How Enzymes Work

- Enzymes are protein catalysts: they increase the velocity of the reaction, but are not themselves altered.
- Enzymes cannot change $K_{eq}/DGo$.
- Enzymes do change $DG^*/k$
  - Energy of transition state decreased or
  - Energy of ground state increased.
Simple Kinetics: A First-order Reaction

A $\rightarrow$ B

\[ v = -\frac{d[A]}{dt} = \frac{d[B]}{dt} \quad \text{units: M/time} \]

\[ v = k[A] \quad \text{Empirical rate law} \]
\[ (M \times \text{time}^{-1}) = k(M) \quad \text{units of k: time}^{-1} \]

Reaction order: exponent of concentration term on which rate depends. Above rxn is 1\text{st} order in reactant A.
1<sup>st</sup>-Order Rate Equation

\[ v = -\frac{d[A]}{dt} = k[A] \]

or \[ \frac{d[A]}{[A]} = d \ln[A] = -k dt \]

so, \[ \ln[A] = \ln[A_0] - kt \quad \text{and} \quad [A] = [A_0]e^{-kt} \]

\[ [A] = [A_0]e^{-kt}; \quad \text{exponential decay} \]

Slope = -k
Other Reaction Orders

- **Zero order reaction** independent of [A]
  - $v = k$, units of $k$: M x time$^{-1}$

- **Second order reaction**: $A + B \rightarrow P$
  - $v = k[A][B]$
  - $k$: unit of $M^{-1}\text{time}^{-1}$
  - 1st order in A
  - 1st order in B; second order overall
  - For $2A \rightarrow P$; $v = k[A]^2$; 2nd order in A
The graph depicts the linear relationship between $\frac{1}{V_o}$ and $\frac{1}{[S]}$, where $V_o$ is the observed velocity and $[S]$ is the substrate concentration. The slope of this line is given by:

$$\text{SLOPE} = \frac{K_m}{V_{\text{max}}},$$

where $K_m$ is the Michaelis constant and $V_{\text{max}}$ is the maximum velocity. The y-intercept is $\frac{-1}{K_m}$ and the x-intercept is $\frac{1}{V_{\text{max}}}$.
Order in Enzyme Catalysis depends on [S]

\[ S \rightarrow P \]

\[ v_0 = \text{initial velocity}; \text{ measure at several [S] but constant [E]} \]
Michaelis-Menten Kinetics. I.

At infinite \([S]\), \(v_0 = V_{\text{max}}\)

\(K_m\) defined as \([S]\) at which \(v_0 = \frac{1}{2} V_{\text{max}}\)

M-M Equation:

\[v_0 = \frac{(V_{\text{max}} [S])}{(K_m + [S])}\]
Michaelis-Menten Kinetics. II

\[
\begin{align*}
\text{if } [S] &\ll K_m \\
V_o &= \left(\frac{V_{\text{max}}}{K_m}\right)[S] \\
\text{if } [S] &= K_m \\
V_o &= 0.5 \times V_{\text{max}} \\
\text{if } [S] &\gg K_m \\
V_o &= V_{\text{max}}
\end{align*}
\]
The reaction rate $v$ can be described by the Michaelis-Menten equation:

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

where $V_{\text{max}}$ is the maximum reaction rate, $K_m$ is the Michaelis constant, and $[S]$ is the substrate concentration.

The enzyme kinetics are depicted in the graph, showing the relationship between substrate concentration and reaction rate. The $x$-axis represents the substrate concentration ([S], mM), and the $y$-axis represents the reaction rate (v, µmol/min). The graph illustrates the Michaelis-Menten curve, with 0.5 $V_{\text{max}}$ corresponding to the substrate concentration $K_m$. The concentration - $K_m$, $V_{\text{max}}$ is also indicated.
Classification of Enzymes

1. **Oxidoreductases** - Catalyze a variety of oxidation-reduction reactions and frequently employ coenzymes such as NADH, NADPH or FADH$_2$. Common trivial names of this class of enzymes include: dehydrogenases, oxidases, peroxidases and reductases.

2. **Transferases** - Catalyze transfers of groups such as amino, carboxyl, carbonyl, methyl, acyl, glycosyl, or phosphoryl. Kinases catalyze the transfer of phosphoryl groups from adenosine triphosphate (ATP) or other nucleotide triphosphates. Common trivial names include: aminotransferases (transaminases), carnitine acyl transferase, and transcarboxylase.
Classification of Enzymes

3. *Hydrolases* - Catalyze cleavage of bonds between a carbon atom and some other atom by addition of water. Common trivial names include: esterases, peptidases, amylases, phosphatases, urease, proteases (i.e. trypsin, chymotrypsin, pepsin, collagenase).


5. *Isomerases* - Catalyze racemization of optical isomers and certain intramolecular oxidation-reduction reactions. Common trivial names include: epimerases, racemases and mutases.
Classification of Enzymes

6. *Ligases* - Catalyze the formation of bonds between carbon and oxygen, sulfur, nitrogen, and other atoms. The energy for bond formation is frequently derived from the hydrolysis of ATP; the term synthase is reserved for this group. Common trivial names include: thiokinases and carboxylases.
Enzymology -3
Amino Acids, Proteins, and Enzymes

Enzymes
Enzyme Action
Factors Affecting Enzyme Action
Enzyme Inhibition
Enzymes

- Catalysts for biological reactions
- Most are proteins
- Lower the activation energy
- Increase the rate of reaction
- Activity lost if denatured
- May be simple proteins
- May contain cofactors such as metal ions or organic (vitamins)
Name of Enzymes

- End in –ase
- Identifies a reacting substance
  - sucrase – reacts sucrose
  - lipase - reacts lipid
- Describes function of enzyme
  - oxidase – catalyzes oxidation
  - hydrolase – catalyzes hydrolysis
- Common names of digestion enzymes still use –*in*
  - pepsin, trypsin
Classification of Enzymes

Class
• Oxidoreductoases: oxidation-reduction
• Transferases: transfer group of atoms
• Hydrolases: hydrolysis
• Lyases: add/remove atoms to/from a double bond
• Isomerases: rearrange atoms
• Ligases: combine molecules using ATP
Examples of Classification of Enzymes

- **Oxidoreductoases**
  - oxidases - oxidize, reductases – reduce

- **Transferases**
  - transaminases – transfer amino groups
  - kinases – transfer phosphate groups

- **Hydrolases**
  - proteases - hydrolyze peptide bonds
  - lipases – hydrolyze lipid ester bonds

- **Lyases**
  - carboxylases – add CO₂
  - hydrolases – add H₂O
Enzyme Action: 
Lock and Key Model

• An enzyme binds a substrate in a region called the active site
• Only certain substrates can fit the active site
• Amino acid R groups in the active site help substrate bind
• Enzyme-substrate complex forms
• Substrate reacts to form product
• Product is released
Lock and Key Model

E + S → ES complex → E + P
Enzyme Action: Induced Fit Model

- Enzyme structure flexible, not rigid
- Enzyme and active site adjust shape to bind substrate
- Increases range of substrate specificity
- Shape changes also improve catalysis during reaction
Enzyme Action: Induced Fit Model

$E + S \rightarrow ES$ complex $\rightarrow E + P$
Factors Affecting Enzyme Action: Temperature

- Little activity at low temperature
- Rate increases with temperature
- Most active at optimum temperatures (usually 37°C in humans)
- Activity lost with denaturation at high temperatures
Factors Affecting Enzyme Action

Optimum temperature

Reaction Rate

Low Temperature  High Temperature
Factors Affecting Enzyme Action: Substrate Concentration

- Increasing substrate concentration increases the rate of reaction (enzyme concentration is constant)
- Maximum activity reached when all of enzyme combines with substrate
Factors Affecting Enzyme Action

- Maximum activity
- Reaction Rate
- Substrate concentration
Factors Affecting Enzyme Action: pH

- Maximum activity at *optimum pH*
- R groups of amino acids have proper charge
- Tertiary structure of enzyme is correct
- Narrow range of activity
- Most lose activity in low or high pH
Factors Affecting Enzyme Action

- Reaction Rate
- Optimum pH

Optimum pH

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<th>Reaction Rate</th>
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Enzyme Inhibition

Inhibitors

• cause a loss of catalytic activity
• Change the protein structure of an enzyme
• May be competitive or noncompetitive
• Some effects are irreversible
Competitive Inhibition

A competitive inhibitor

• Has a structure similar to substrate
• Occupies active site
• Competes with substrate for active site
• Has effect reversed by increasing substrate concentration
Noncompetitive Inhibition

A noncompetitive inhibitor

- Does not have a structure like substrate
- Binds to the enzyme but not active site
- Changes the shape of enzyme and active site
- Substrate cannot fit altered active site
- No reaction occurs
- Effect is not reversed by adding substrate
Enzymology - 4
• The Michaelis-Menten Equation
• Louis Michaelis and Maude Menten's theory
• It assumes the formation of an enzyme-substrate complex
• It assumes that the ES complex is in rapid equilibrium with free enzyme
• Breakdown of ES to form products is assumed to be slower than 1) formation of ES and 2) breakdown of ES to re-form E and S
Enzymes

- Enzymes endow cells with the remarkable capacity to exert kinetic control over thermodynamic potentiality.
- Enzymes are the agents of metabolic function.
Specificity

- Enzymes selectively recognize proper substrates over other molecules
- Enzymes produce products in very high yields - often much greater than 95%
- Specificity is controlled by structure - the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield
What Enzymes Do....

- Enzymes accelerate reactions by lowering the free energy of activation
- Enzymes do this by binding the transition state of the reaction better than the substrate
- Much more of this in Chapter 16!
The Michaelis-Menten Equation

You should be able to derive this!

- Louis Michaelis and Maude Menten's theory
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**The Michaelis-Menten Equation**

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Understanding $K_m$

The "kinetic activator constant"

- $K_m$ is a constant
- $K_m$ is a constant derived from rate constants
- $K_m$ is, under true Michaelis-Menten conditions, an estimate of the dissociation constant of E from S
- Small $K_m$ means tight binding; high $K_m$ means weak binding
Understanding $V_{\text{max}}$

The theoretical maximal velocity

- $V_{\text{max}}$ is a constant
- $V_{\text{max}}$ is the theoretical maximal rate of the reaction - but it is NEVER achieved in reality
- To reach $V_{\text{max}}$ would require that ALL enzyme molecules are tightly bound with substrate
- $V_{\text{max}}$ is asymptotically approached as substrate is increased
The dual nature of the Michaelis-Menten equation

Combination of 0-order and 1st-order kinetics

- When $S$ is low, the equation for rate is 1st order in $S$
- When $S$ is high, the equation for rate is 0-order in $S$
- The Michaelis-Menten equation describes a rectangular hyperbolic dependence of $v$ on $S$!
Linear Plots of the Michaelis-Menten Equation

Be able to derive these equations!

- Lineweaver-Burk
- Hanes-Woolf
- Hanes-Woolf is best - why?
- Smaller and more consistent errors across the plot
\[ \frac{1}{v} = \frac{K_m}{V_{max}} \left( \frac{1}{[S]} \right) + \frac{1}{V_{max}} \]

- **x-intercept** = \(-\frac{1}{K_m}\)
- **y-intercept** = \(\frac{1}{V_{max}}\)

Slope = \(\frac{K_m}{V_{max}}\)
\[
\frac{[S]}{v} = \left( \frac{1}{V_{\text{max}}} \right) [S] + \frac{K_m}{V_{\text{max}}}
\]

**x-intercept** = \( -K_m \)

**y-intercept** = \( \frac{K_m}{V_{\text{max}}} \)

Slope = \( \frac{1}{V_{\text{max}}} \)
The graph illustrates the pH optimum for various enzymes:

- Pepsin: Optimum pH 1.5
- Catalase: Optimum pH 7.6
- Trypsin: Optimum pH 7.7
- Cholinesterase: Optimum pH 8
- Fumarase: Optimum pH 7.8
- Ribonuclease: Optimum pH 7.8
- Arginase: Optimum pH 9.7
Enzyme Inhibitors

Reversible versus Irreversible

- Reversible inhibitors interact with an enzyme via noncovalent associations
- Irreversible inhibitors interact with an enzyme via covalent associations
Classes of Inhibition

Two real, one hypothetical

- Competitive inhibition - inhibitor (I) binds only to E, not to ES
- Noncompetitive inhibition - inhibitor (I) binds either to E and/or to ES
- Uncompetitive inhibition - inhibitor (I) binds only to ES, not to E. This is a hypothetical case that has never been documented for a real enzyme, but which makes a useful contrast to competitive inhibition
Double-reciprocal form of the rate equation:

\[ \frac{1}{v} = \frac{K_m^A}{V_{max}} \left( \frac{1}{[A]} \right) + \left(1 + \frac{K_m^B}{[B]} \right) \left( \frac{1}{V_{max}} \right) \]

- **y-intercepts** are \( \frac{1}{V_{max}} \left(1 + \frac{K_m^B}{[B]} \right) \)
- **x-intercepts** are \( -\frac{1}{K_m^A} \left(1 + \frac{K_m^B}{[B]} \right) \)

Increasing concentration of B:
- [B]
- 2[B]
- 3[B]

Slope is constant:

\[ \frac{K_m^A}{V_{max}} \]
Garrett & Grisham: Biochemistry, 2/e
Figure 14.15

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

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

Slope = \( \frac{K_m}{V_{\text{max}}} \)
• Enzymes are regulated:
• at genetic level (transcription, translation);
• by concentration of substrate and product;
• allosterically.
Enzymology -5
Co-enzymes & Cofactors
Cofactors and Coenzymes

**Terminology:**

Enzyme with bound cofactor or coenzyme - **holoenzyme**

Enzyme without bound cofactor or coenzyme - **apoenzyme**

**Examples:**

1. Metals ($\text{Mg}^{+2}$, $\text{Mn}^{+2}$, $\text{Zn}^{+2}$, $\text{Cu}^{+2}$, $\text{Fe}^{+2}$ or $\text{Fe}^{+3}$) may stabilize the enzyme structure or participate in catalysis.
Cofactors are exogenous molecules that associate with proteins to yield full activity. In the absence of cofactor, protein is an apoprotein.

Co-enzymes are soluble and associate transiently with enzyme during catalytic cycle. An example is vitamin K in activation of blood clotting enzymes.

Prosthetic groups are covalently attached to the protein. Examples are heme, in hemoglobin, and riboflavin, in flavoproteins.
Some Cofactors

- Thiamin Diphosphate
- Biotin
- Pyridoxal Phosphates
- FAD
- FMN
- Heme
- Coenzyme A (CoA)
- Vitamin B12
- Folic Acid
- Glutathione
- Lipoic Acid
Pyridoxal Phosphate  Vitamin B6 Derivative

Forms Schiff bases with many amine containing compounds. These covalent intermediates are important in amino acid metabolism.
Major electron carrier / acceptor: Electrons from metabolic oxidation are transferred to pyridine nucleotides or flavins. Reduced forms of these carriers transfer their high potential electrons to the electron transport chain and ultimately to $O_2$ as part of oxidative phosphorylation.
Flavin Mononucleotide (FMN)

FAD (FMN)

Oxidized Isoalloxazine

FADH₂ (FMNH₂)

Electron-Carrier = Flavin Mononucleotide. Flavins are stronger oxidizing agents than NAD⁺
Some Cofactors May Be Organometallic Compounds

Heme structure contains ferrous iron chelated to the pyrrole nitrogens

Additional Examples: cobalamin, chlorophyll
Thiamin Dependent Reactions

**Pyruvate Decarboxylase**

\[
\text{H}_3\text{C}-\text{C}-\text{CO}_2\text{H} \xrightarrow{\text{thiamin}} \text{H}_3\text{C}-\text{C}-\text{H} + \text{CO}_2
\]

**Acetolactate synthase**

\[
2\text{H}_3\text{C}-\text{C}-\text{CO}_2\text{H} \xrightarrow{\text{thiamin}} \text{H}_3\text{C}-\text{C}-\text{C}-\text{CO}_2\text{H} + \text{CO}_2
\]

**Pyruvate dehydrogenase:** pyruvate decarboxylase, dihydrolipoyl transacetylase, dihydrolipoyl dehydrogenase,

\[
\text{H}_3\text{C}-\text{C}-\text{CO}_2\text{H} + \text{thiamin} + \text{lipoic acid} + \text{CoA-SH} \rightarrow \text{Acetyl-CoA} + \text{CO}_2
\]

\[
(\text{CH}_3\text{COS-CoA})
\]

Thiamin Diphosphate (pyrophosphate)
Pyridoxal Phosphate (PLP) dependent enzymes (Vitamin B₆) (Bugg, Chapt. 9, pp 186-198)

Involved in amino acid biosynthesis, metabolism and catabolism