Introduction

- **Enzymes are biological catalysts responsible for supporting almost all of the chemical reactions**
- **In these reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, the products.**
- **Almost all processes in a biological cell need enzymes in order to occur at significant rates.**

Enzyme Terminology

- **Enzyme**
  - **Biological catalyst (most are protein, some RNAribozymes) acts on substrate through binding, conversion, and release of product**
- **Substrate**
  - **Starting material of an enzyme catalyzed reaction**
- **Product**
  - **Substance to which the substrate is converted**
- **Active site**
  - **Region on enzyme to which substrate binds**

Introduction

As Biological Catalysts enzymes

- **Permit reactions to be carried out at conditions that the body can tolerate**
  - **Lowers the activation energy**
  - **Increases the rate of reaction**
  - **Does not change the free energy of the reaction**
- **Are typically are very large proteins**
  - **Activity lost if denatured**
- **May contain cofactors such as metal ions or organic (vitamins)**

Properties of enzymes

- **High reaction rates**
  - **rates of enzymatically catalyzed reactions are typically 10^6 to 10^{12} times greater than the uncatalyzed reactions**
- **Mild reaction conditions**
  - **temperatures below 100°C, atmospheric pressure, nearly neutral pH**
- **Specificity**
  - **enzymes have a high degree of specificity for their substrates (reactants) and their products**
- **Regulation**
  - **the catalytic activity of many enzymes is modulated by concentrations of substances other than their products**

High Reaction Rates

Enzyme	Substrate	Product	Rate without Enzyme µmoles/L per min	Rate with Enzyme µmoles/L per min	Acceleration due to Enzyme
Hexokinase Glucose	Glucose	6-Phosphate	<.0000001	1300	> 13 billion
Phosphorylase	Glucose	6-Phosphate	<.000000005	1600	> 320 billion
Alcohol Dehydrogenase	Ethanol	Acetaldehyde	<.000006	2700	> 450 million
Creatine Kinase	Creatine	Creatine Phosphate	<.003	40	> 13, 000

Mild Reaction Conditions

- Consider that biochemistry takes place at about 37^0C in water and contrast that to typical reaction conditions in organic chemistry.
  - For example, to hydrolyze (saponify) fats we boil them with concentrated sodium hydroxide solution for a few hours.
  - Enzymes called lipases do the same thing at body temperature in minutes.
  - Without enzymes, our body chemistry would not occur, and life would not exist.
- This illustrates the impressive power of enzymes as catalysts.

Specificity of Enzymes

Enzymes have a high degree of specificity for their substrates (reactants) and their products

- **Absolute specificity**
  - **the enzyme will catalyze only one reaction.**
- **Group specificity**
  - **the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.**
- **Linkage specificity**
  - **the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.**
- **Stereochemical specificity**
  - **the enzyme will act on a particular steric or optical isomer.**

Specificity of Enzymes

Sulfuric acid as a source of H^+ will catalyze the formation of any ester from the appropriate alcohol and carboxylic acid,

- Many enzymes are so specialized that they will catalyze a reaction of one molecule, but will leave untouched a very similar molecule.
  - **Amylase, a digestive enzyme, will hydrolyze starch, but not cellulose. Both molecules are polymers of glucose.**
    - They differ in the orientation of one bond at the junction of glucose units. Other enzymes can work effectively on a broader range of substrates (the molecule whose reaction is being catalyzed.

Metabolic Pathways & Enzymes

Cellular reactions are usually part of a *metabolic pathway*, a series of linked reactions, illustrated as follows:

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} E \xrightarrow{E_5} F \xrightarrow{E_6} G$$

The letters A-G represent *substrates* and *products*

The alpha numeric E_1-E_6 represent *enzymes*.

Nomenclature

- Many enzymes have been named by adding the suffix **"-ase"** to the name of their substrate or to a word or phrase describing their activity.

Substrate	Enzyme
Lipid	Lipase
Urea	Urease
Maltose	Maltase
Ribonucleic acid	Ribonuclease
DNA Synthesis	DNA polymerase

- **Common names of digestion enzymes still use – *in***
  - **pepsin, trypsin**

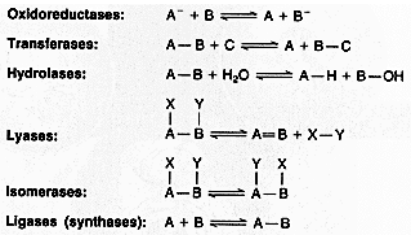
Classification

- Traditionally, enzymes were simply assigned names by the investigator who discovered the enzyme.
- As knowledge expanded, systems of enzyme classification became more comprehensive and complex.
- Currently enzymes are grouped into six functional classes by the International Union of Biochemists (I.U.B.).

Classification

Number	Classification	Biochemical Properties
1	**Oxidoreductases**	Act on many chemical groupings to add or remove hydrogen atoms.
2	**Transferases**	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules.
3	**Hydrolases**	Add water across a bond, hydrolyzing it.
4	**Lyases**	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds.
5	**Isomerases**	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others.
6	**Ligases**	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP.

Classification



Classification

International Enzyme Commission

- **4 digit Numbering System [1.2.3.4.]**
  - **1st #… Major Class of Enzyme Activity**
  - **2nd #… a subclass (type of bond acted upon)**
  - **3rd #... a subclass (group acted upon, cofactor required, etc...)**
  - **4th #... serial number … order in which enzyme was added to list**

Classification

1. **Oxidoreductases**
   - **Alcohol dehydrogenase [EC 1.1.1.1]**
2. **Transferases**
   - **Hexokinase [EC 2.7.1.2]**
3. **Hydrolases**
   - **Carboxypeptidase A [EC 3.4.17.1]**
4. **Lyases**
   - **Pyruvate decarboxylase [EC 4.1.1.1]**
5. **Isomerases**
   - **Maleate isomerase [EC 5.2.1.1]**
6. **Ligases**
   - **Pyruvate Carboxylase [EC 6.4.1.1]**

Classification

Enzymes are also classified on the basis of their composition.

- Enzymes composed wholly of protein are known as **Simple Enzymes**
- Enzymes which are composed of protein plus a relatively small organic molecule are known as **Complex Enzyme**
  - Complex enzymes are also known as **Holoenzymes**.
  - In this terminology the protein component is known as the **Apoenzyme**
  - while the non- protein component is known as the **Coenzyme** or **prosthetic group**
    - where prosthetic group describes a complex in which the small organic molecule is bound to the apoenzyme by covalent bonds.

Cofactors

Enzymes are often composed of only protein. In this case only Amino Acid side chains are used for catalysis.

- Some enzymes require additives for assisting with catalysis.
- Additives like vitamins often provide functional groups not available to the enzyme among the side chains of the amino acids.
- In these cases the protein of the enzyme binds:
  - Organic cofactors (Vitamins = organic cofactors)
  - Metal ions (e.g. Mg2+)
  - Nucleotides (even RNA)

Cofactors

- **Coenzyme** - a **non-protein** organic substance which is dialyzable, thermo stable and loosely attached to the protein part.
  - They do not form a permanent part of the enzymes' structures
  - They do not affect the catalytic activity, but may influence enzyme stability or solubility
- **Prosthetic group** - an organic substance which is dialyzable and thermo stable which is firmly attached to the protein or apoenzyme portion.
  - Prosthetic groups are a subset of cofactors and differ from coenzymes in that they bind permanently to the enzyme as opposed to temporarily for coenzymes.
  - In enzymes, prosthetic groups are involved in the active site in some way
- **Metal-ion-activator** - these include K^{+}, Fe^{++}, Fe^{+++}, Cu^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, Ca^{++} and Mo^{+++}.

Cofactors

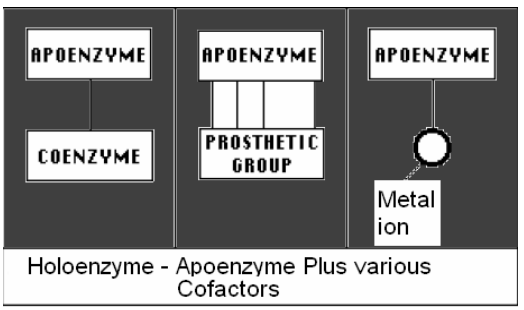
- The Common Cofactors (Enzyme Additives):
  - **Biotin** aids in carboxylation reactions (carbon dioxide fixation).
  - **Cobaltamine** (vitamin B-12) aids in alkylation reactions
  - **Coenzyme A** aids in acyl transfers like in the tricarboxylic acid cycle.
  - **Flavin** (vitamin B-2) aids in oxidation-reduction reactions (e.g. nitrate reductase).
  - **Lipoic acid** aids in acyl transfers via oxidation-reduction processes.
  - **Nicotinamide** coenzymes like NAD+ act as independent co-substrates.
  - **Pyridoxal** (vitamin B-6) aids in amino group transfers (provides aldehyde functional group).
  - **Tetrahydrofolate** aids in one-carbon transfers.
  - **Thiamin** (vitamin B-1) aids in aldehyde transfers and alpha-keto-acids decarboxylations

Complex Enzymes

- The complex of protein and additive is called **Holo-Enzyme**.
- When the additive is removed from the enzyme, the remaining protein part of the enzyme is called the **Apo-Enzyme**.

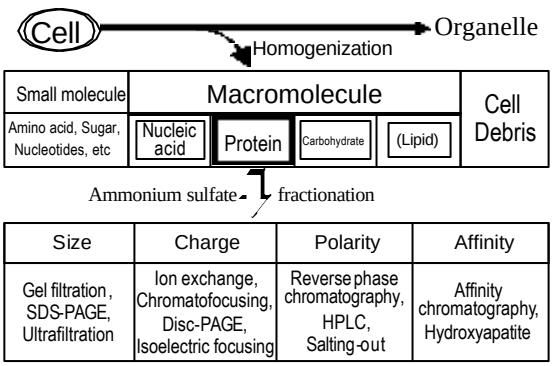
Apo-Enzyme (inactive) + Additive = Holo-Enzyme (active)

Complex Enzymes



Holoenzyme - Apoenzyme Plus various Cofactors

Basic Principles of Enzyme Purification



Size	Charge	Polarity	Affinity
Gel filtration, SDS-PAGE, Ultrafiltration	Ion exchange, Chromatofocusing, Disc-PAGE, Isoelectric focusing	Reverse phase chromatography, HPLC, Salting-out	Affinity chromatography, Hydroxyapatite

Principles of Catalysis

Analogous reactions found in organic chemistry are observed in enzymology

- **Acid Base Catalysis** - Donation or abstraction of protons
- **Covalent Catalysis** - Covalent enzyme-substrate intermediate
- **Metal Ion Catalysis -** Substrates and metals positioned for reaction
- **Electrostatic Considerations -** Compliment of charges with transition state
- **Proximity and Orientation** - Substrates aligned for reaction
- **Transition state stabilization** – Activation energy reduced

Principles of Catalysis

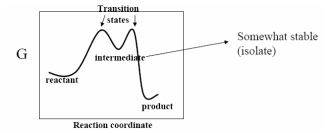
Acid-Base Catalysis

- General acids transfer protons
- General bases abstract protons
- Specific acid or base catalysis is when the or hydroxide ion is the catalyst (organic)

Principles of Catalysis

Covalent Catalysis

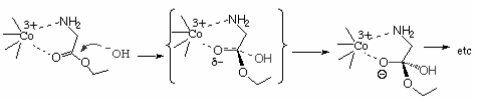
- A covalent bond is formed between the enzyme and its substrate during the formation of the transition state
- Covalent bond is initiated by an electron rich group in the active site
- Covalent catalysis involves a two part reaction process containing two energy barriers in the reaction coordinate diagram



Principles of Catalysis

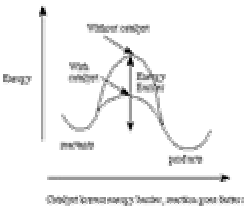
Metal Ion Catalysis

- A specific type of electrostatic catalysis
- Employs the positively charged metal ion to stabilize negative charges for increased catalysis (also called Electrophilic catalysis)
- Coordination of the cobalt complex increases the ability of a base to catalyze the hydrolysis of glycine ester two million fold



Mechanism of Action

- **Catalysts increase the rate of a reaction, but are not themselves consumed or produced by the reaction.**
- **They do not change the equilibrium constant of a reaction.**
  - **This means that any catalyst which catalyzes a reaction in one direction (e.g., esterification) also catalyzes the reverse (e.g., ester hydrolysis) reaction.**
- **To say these things another way, catalysts do not change the energy balance between reactants and products; catalysts do lower the energy barrier between reactants and products.**
  - **These statements are true of enzymes as well as other types of catalysts.**



Mode of Action

- **Enzymes can act in several ways, all of which lower ΔG:**
  - **Lowering the activation energy**
    - by creating an environment in which the transition state is stabilized (e.g. straining the shape of a substrate - by binding the transition-state conformation of the substrate/product molecules, the enzyme distorts the bound substrate(s) into their transition state form, thereby reducing the amount of energy required to complete the transition).
  - **Providing an alternative pathway**
    - (e.g. temporarily reacting with the substrate to form an intermediate ES Complex which would be impossible in the absence of the enzyme).
  - **Reducing the reaction entropy change**
    - by bringing substrates together in the correct orientation to react.

Enzyme Substrate Interactions

Substrate Binding and Enzyme Action

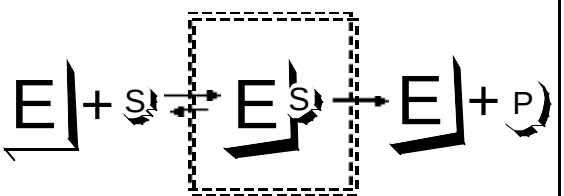
- The first step in a enzyme catalyzed reaction is the formation of the enzyme-substrate complex. This is represented by the equation:

E + S = ES = E + P.

- The region of the enzyme where the substrate binds is called as the **active site**. This consists of a substrate binding site and the catalytic site.
- The active site is usually a cleft or pocket created by the unique tertiary structure of the enzyme protein
- Enzyme specificity is due to specificity of substrate binding driven by substrate and enzyme 3D structure
- The ES complex is stabilized in the transition state by non-covalent interactions between substrate the amino acid in the active site.

Essential of Enzyme Kinetics

Steady State Theory



The reaction ES=E + P determines catalytic rate
The reaction ES=E + P is irreversible
ES is in equilibrium. Formation = Removal

In steady state, the production and consumption of the transition state proceed at the same rate. So the concentration of transition state keeps a constant.