Enzyme Structure, classification and mechanism of action

Learning Objectives:

- By the end of the lecture, the student should be able to :
- Define enzymes and related terms (active site, apoenzyme, holoenzyme, prosthetic group, enzyme specificity).
- Explain the energy of activation.
- Describe the structure of enzymes.
- Know the Mechanism of action
- Explain the Classification of enzymes

Importance

- Enzymes play an important role in Metabolism, Diagnosis, and Therapeutics.
- All biochemical reactions are enzyme catalyzed in the living organism.
- Level of enzyme in blood are of diagnostic importance e.g. it is a good indicator in disease such as myocardial infarction.
- Enzyme can be used therapeutically such as digestive enzymes.

Define enzymes

(Enzymes as Biological Catalysts)

- **Enzymes** are proteins that increase the rate of reaction by lowering the energy of activation
- They catalyze nearly all the chemical reactions taking place in the cells of the body.
- Not altered or consumed during reaction.
- Reusable

Intro. to Enzymes

All living organisms must be able to self-replicate and catalyze chemical reactions efficiently and selectively.

Enzymes (from the Greek *enzymos*, "leavened") are the chemical catalysts of biological systems. Enzymes have extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts.

They have a high degree of specificity for their substrates and they accelerate chemical reactions tremendously.

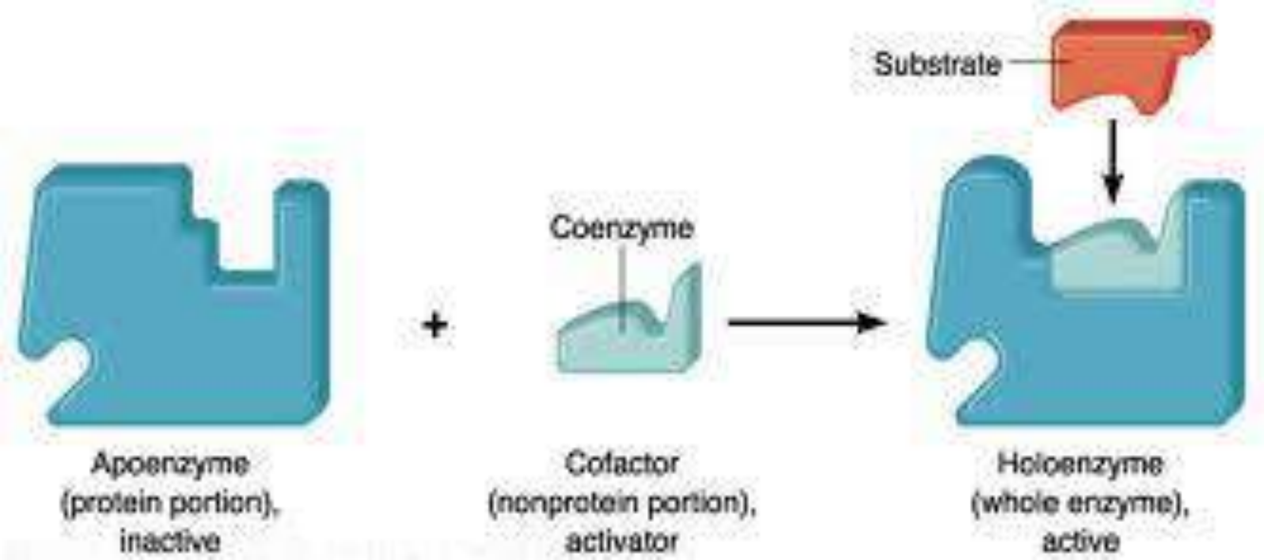
They function in aqueous solutions under very mild conditions of temperature and pH, unlike many catalysts used in organic chemistry.

Enzymes are central to every biochemical process. They catalyze the hundreds of stepwise reactions of metabolism, conserve and transform chemical energy, and make biological macromolecules from simple precursors.

In many diseases, the activity of one or more enzymes is abnormal. Many drugs act via binding to enzymes.

APOENZYME and HOLOENZYME

- The enzyme without its non protein moiety is termed as apoenzyme and it is inactive.
- Holoenzyme is an active enzyme with its non protein component.



Important Terms to Understand Biochemical Nature And Activity of Enzymes

- **Cofactor:**
  - A cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis.
  - Types of Cofactors:
    - Coenzymes.
    - Prosthetic groups.

Types of Cofactors

- **Coenzyme:**
  The non-protein component, loosely bound to apoenzyme by non-covalent bond.
- **Examples :** vitamins or compound derived from vitamins.
- **Prosthetic group**
  The non-protein component, tightly bound to the apoenzyme by covalent bonds is called a Prosthetic group.

Structure of enzymes

Enzymes

Complex or holoenzymes (protein part and nonprotein part – cofactor)

Simple (only protein)

Apoenzyme (protein part)

Cofactor

Prosthetic groups

-usually small inorganic molecule or atom;

-usually tightly bound to apoenzyme

Coenzyme

-large organic molecule

-loosely bound to apoenzyme

Chemical Features of Enzymes (I)

With the exception of a small group of catalytically active RNA molecules (Chap. 26), all enzymes are proteins. Their catalytic activity depends on the integrity of their native protein conformation. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component called a cofactor. Cofactors can be inorganic ions (Table 6-1), or complex organic or metalloorganic molecules called coenzymes (Table 6-2, next slide).

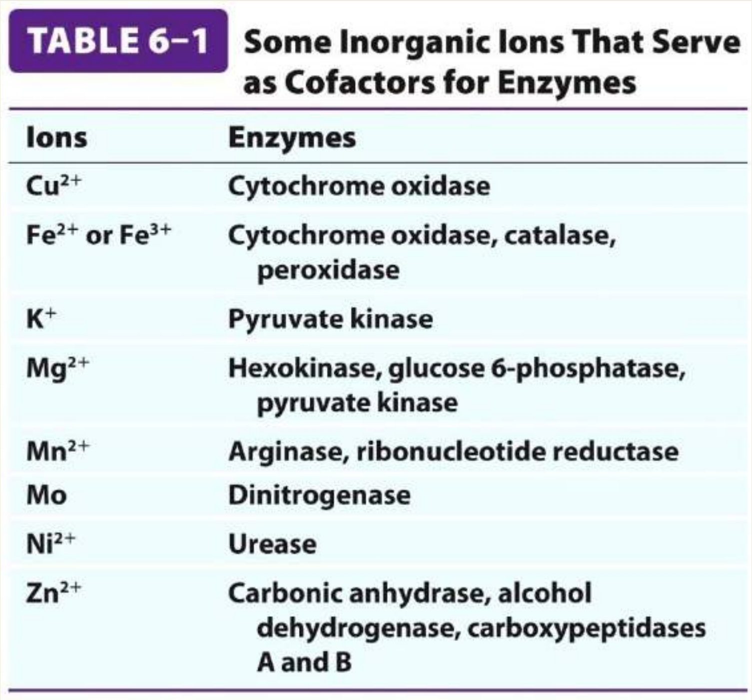


TABLE 6–1 **Some Inorganic Ions That Serve as Cofactors for Enzymes**

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Chemical Features of Enzymes (II)

- Coenzymes usually act as transient carriers of specific functional groups (Table 6-2). Most are derived from vitamins, which are organic nutrients that are required in small amounts in the diet. Some enzymes require both a coenzyme and one or more metal ions for activity. A coenzyme or metal ion that is very tightly or even covalently bound to an enzyme protein is called a prosthetic group. A complete, catalytically active enzyme together with its bound coenzyme and/or metal ion is called a holoenzyme. The protein part of such an enzyme is called the apoenzyme or apoprotein. Many enzymes are modified by phosphorylation or other processes. Modifications often are used to regulate enzyme activity.

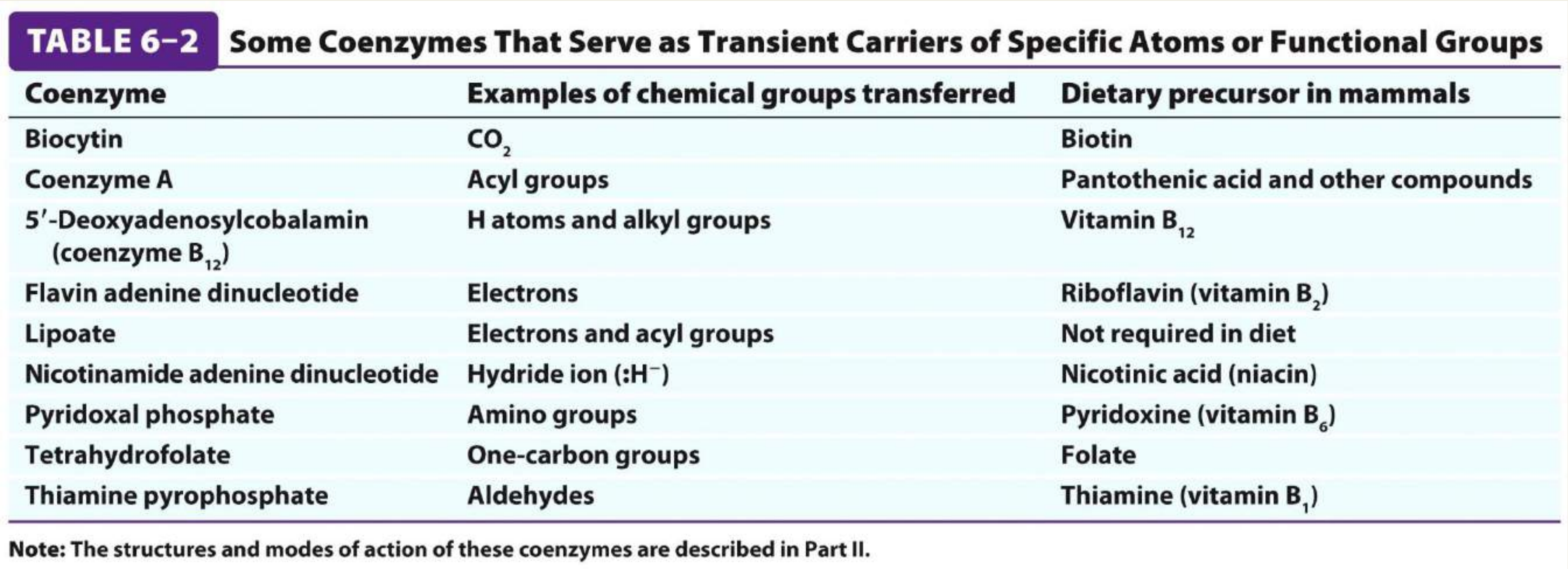


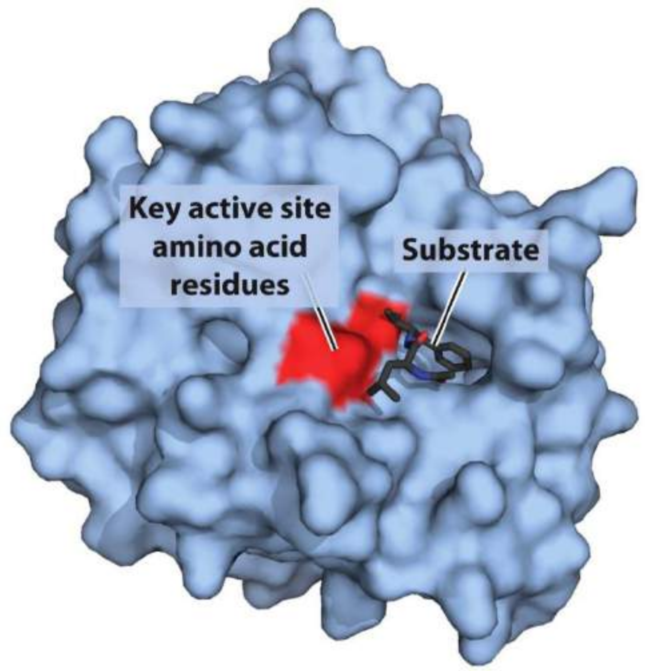
TABLE 6–2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Note: The structures and modes of action of these coenzymes are described in Part II.

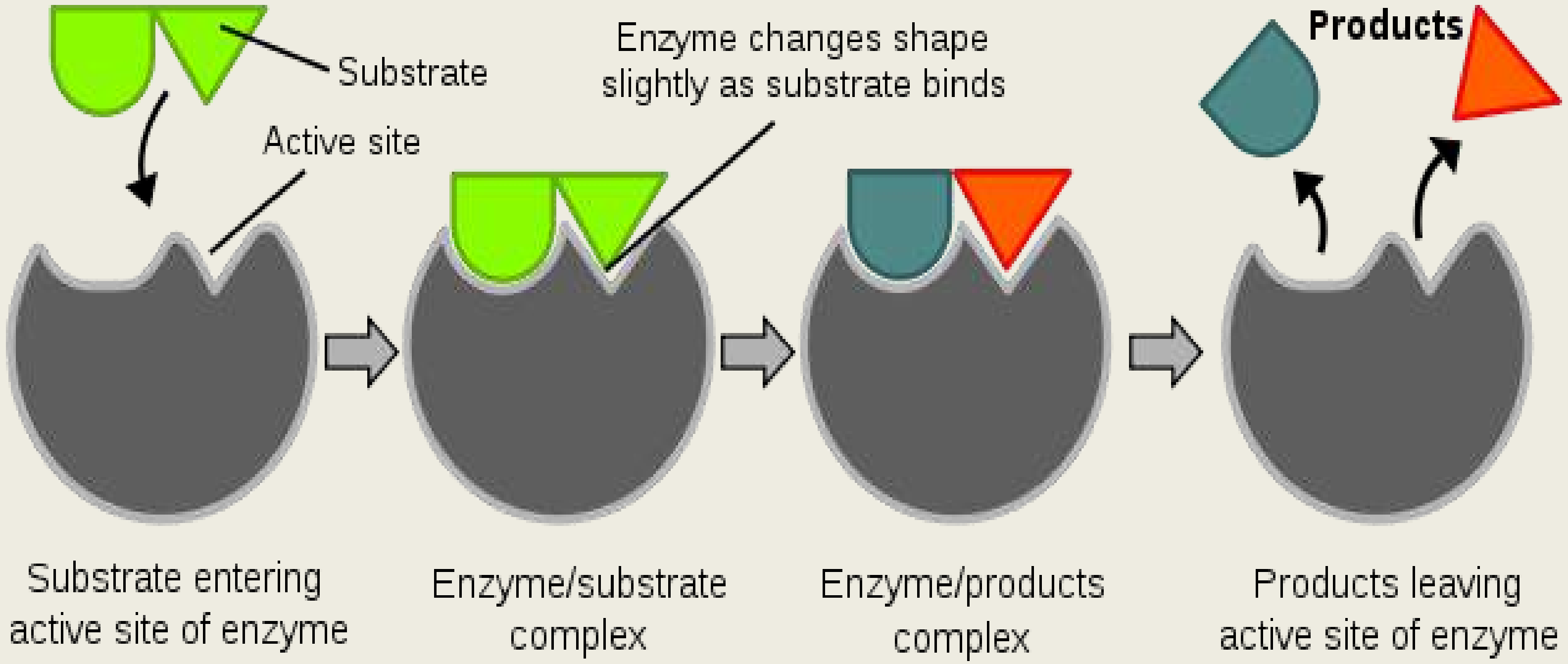
Enzyme Active Sites

Under biologically relevant conditions, uncatalyzed reactions tend to be slow because most biological molecules are quite stable in the neutral-pH, mild-temperature, aqueous environment inside cells. Enzymes greatly increase the rates of biological reactions by providing a specific environment within which a reaction can occur more rapidly. Enzyme-catalyzed reactions take place within the confines of a pocket on the enzyme called the active site. The reactant molecule is referred to as the substrate. The surface of the active site is lined with amino acid residues with substituent groups that bind to the substrate and catalyze its chemical transformation. Often, the active site encloses the substrate, sequestering it from solution. The active site of the enzyme chymotrypsin is highlighted in Fig.



ACTIVE SITES

- Enzyme molecules contain a special pocket or cleft called the active sites.



Enzyme Specificity

- Enzymes have varying degrees of **specificity** for substrates
- Enzymes may recognize and catalyze:
  - a single substrate
  - a group of similar substrates
  - a particular type of bond

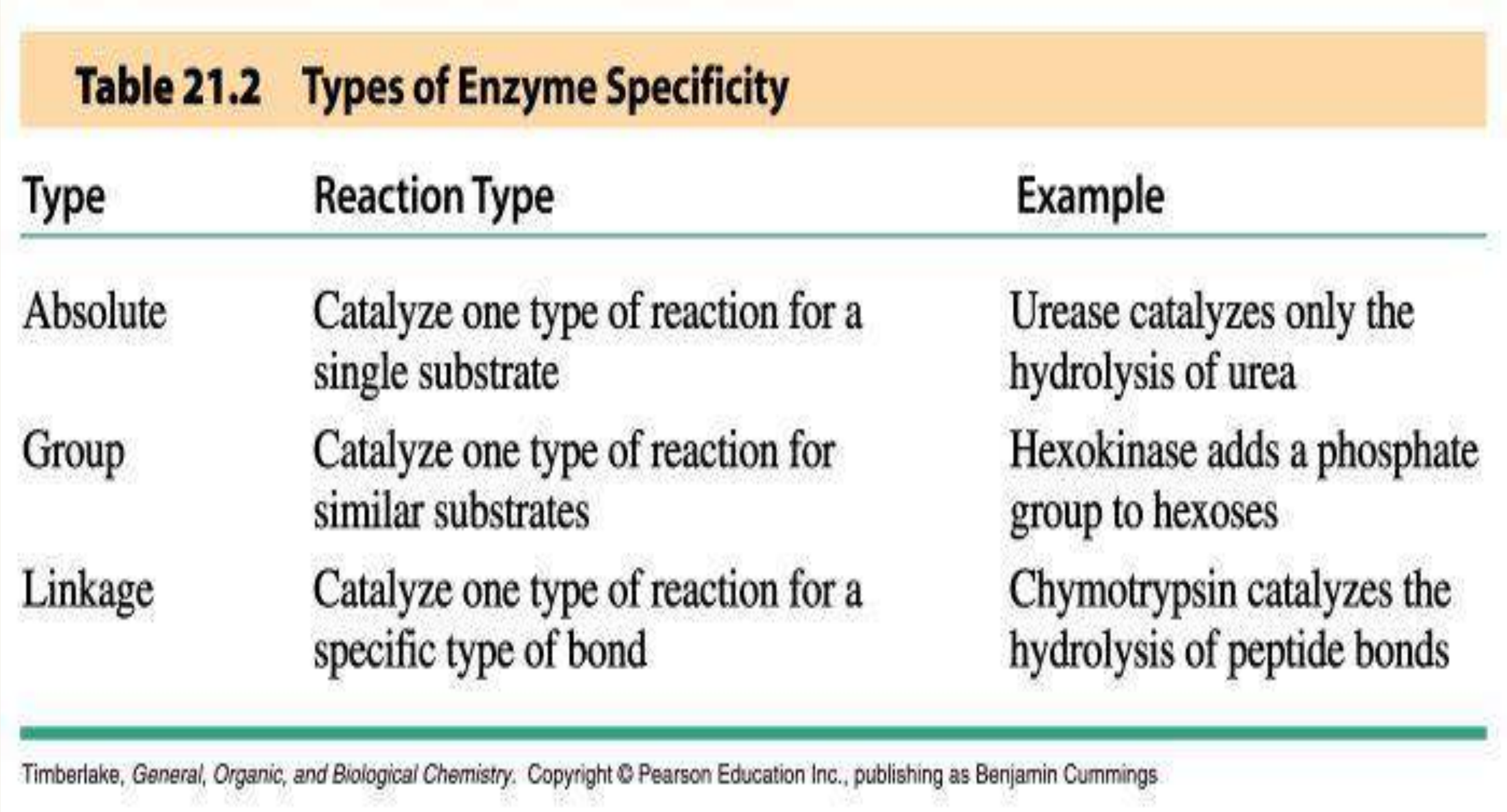


Table 21.2 Types of Enzyme Specificity

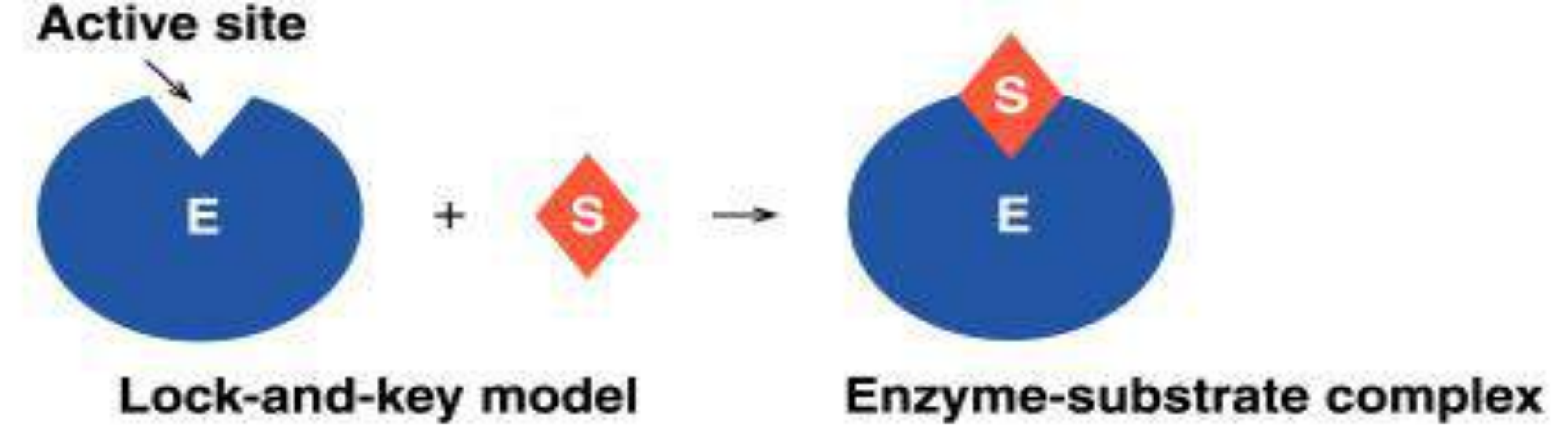
Type	Reaction Type	Example
Absolute	Catalyze one type of reaction for a single substrate	Urease catalyzes only the hydrolysis of urea
Group	Catalyze one type of reaction for similar substrates	Hexokinase adds a phosphate group to hexoses
Linkage	Catalyze one type of reaction for a specific type of bond	Chymotrypsin catalyzes the hydrolysis of peptide bonds

Mechanism of Action of Enzymes

- **Enzymes increase reaction rates by decreasing the Activation energy:**
- **Enzyme-Substrate Interactions:**
  - **Formation of Enzyme substrate complex by:**
  - Lock-and-Key Model
  - Induced Fit Model

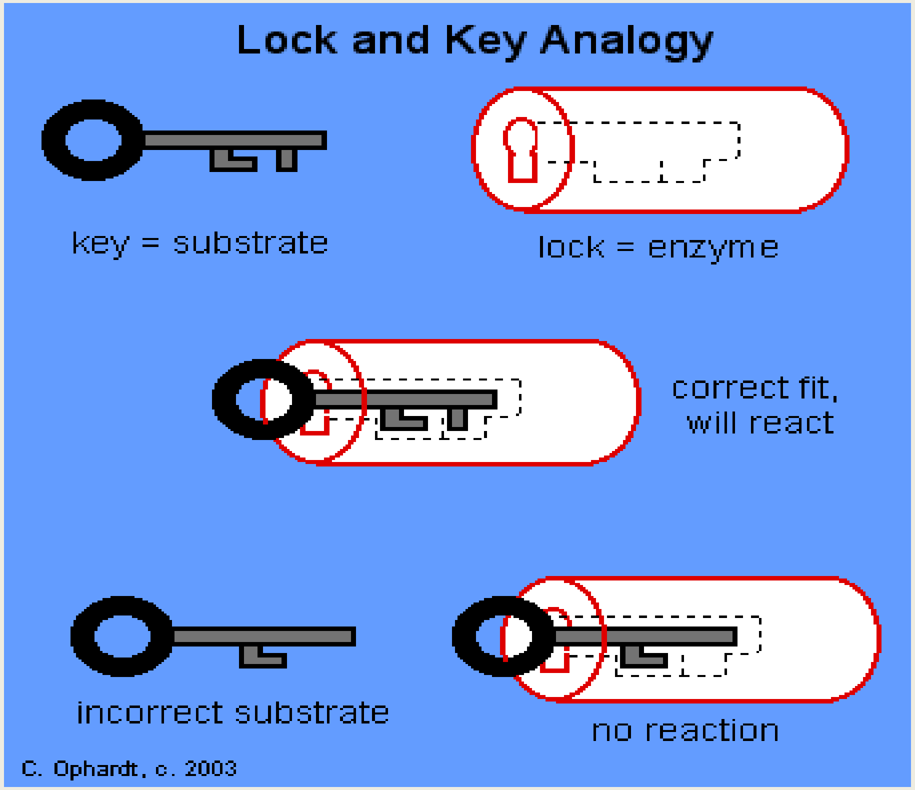
Lock-and-Key Model

- In the **lock-and-key model** of enzyme action:
  - the active site has a rigid shape
  - only substrates with the matching shape can fit
  - the substrate is a key that fits the lock of the active site
- This is an older model, however, and does not work for all enzymes



Lock-and-Key Model

- In the lock-and-key model of enzyme action:
  - the active site has a rigid shape
  - only substrates with the matching shape can fit
  - the substrate is a key that fits the lock of the active site



This explains enzyme specificity

This explains the loss of activity when enzymes denature

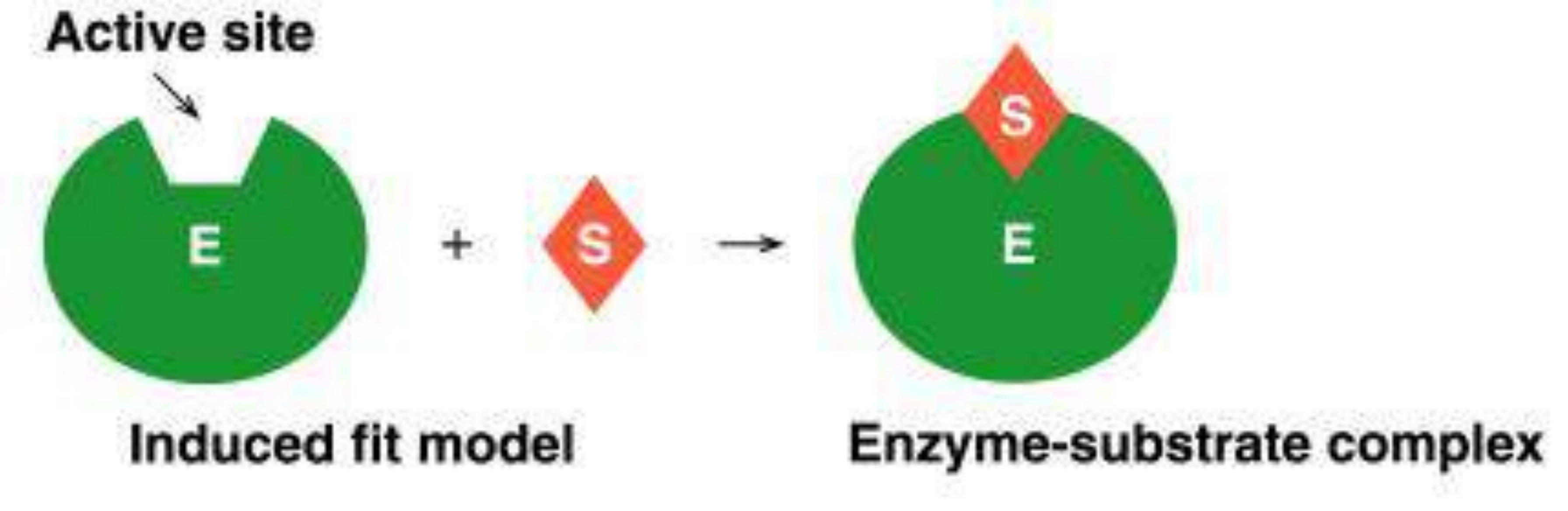
Induced Fit Model

- In the **induced-fit model** of enzyme action:

  - the active site is flexible, not rigid

  - the shapes of the enzyme, active site, and substrate adjust to maximumize the fit, which improves catalysis

  - there is a greater range of substrate specificity
- This model is more consistent with a wider range of enzymes



Important Terms to Understand Biochemical Nature And Activity of Enzymes

Activation energy or Energy of Activation:

All chemical reactions require some amount of energy to get them started.
OR
It is First push to start reaction.

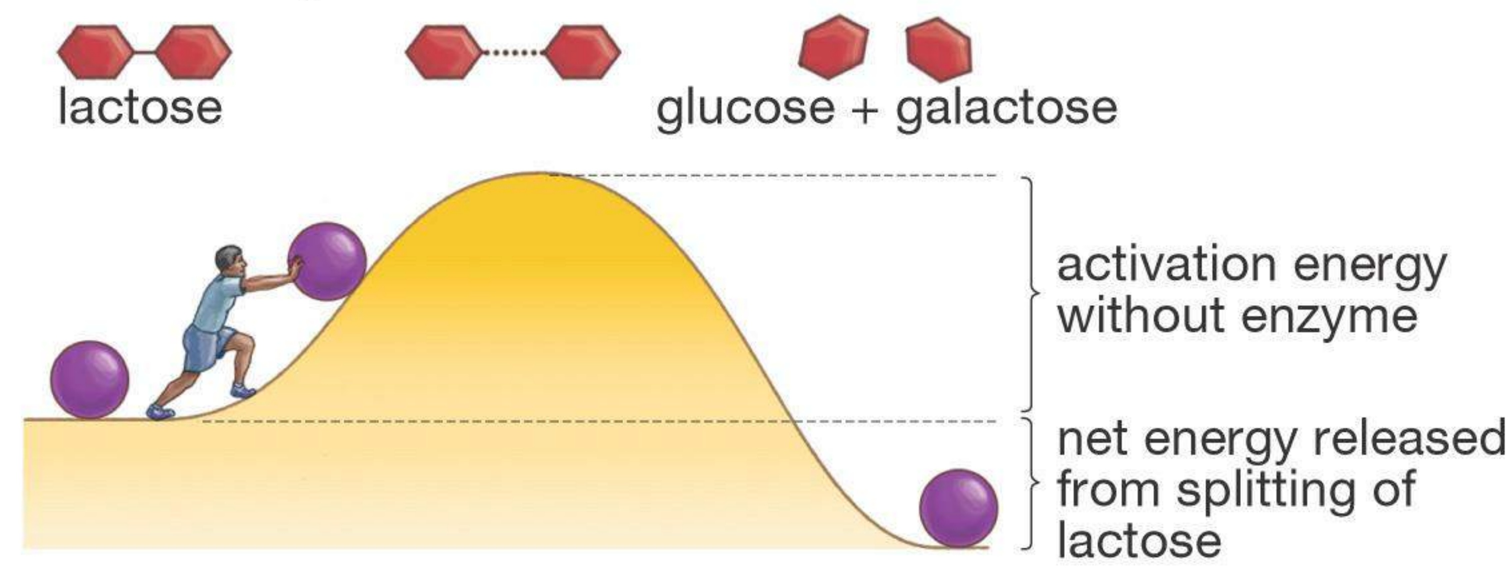
This energy is called activation energy.

Activation energy may also be defined as the minimum energy required to start a chemical reaction.

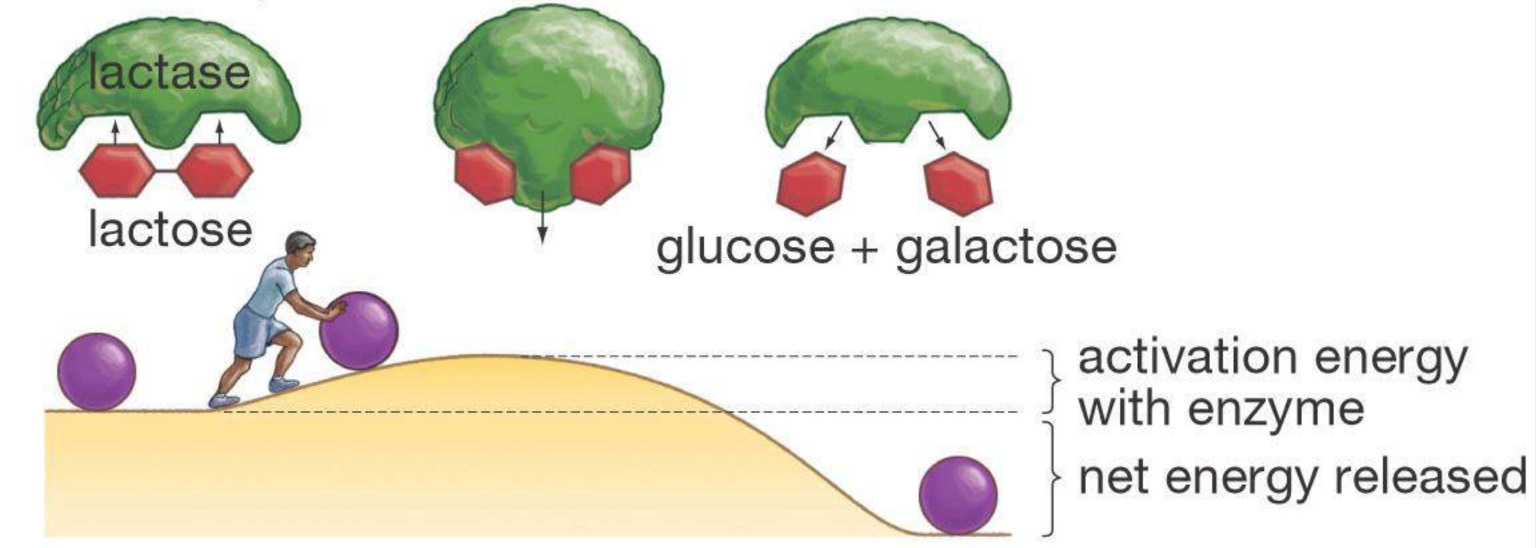
The activation energy of a reaction is usually denoted by E_a and given in units of kilojoules per mole (kJ/mol) or kilocalories per mole (kcal/mol).

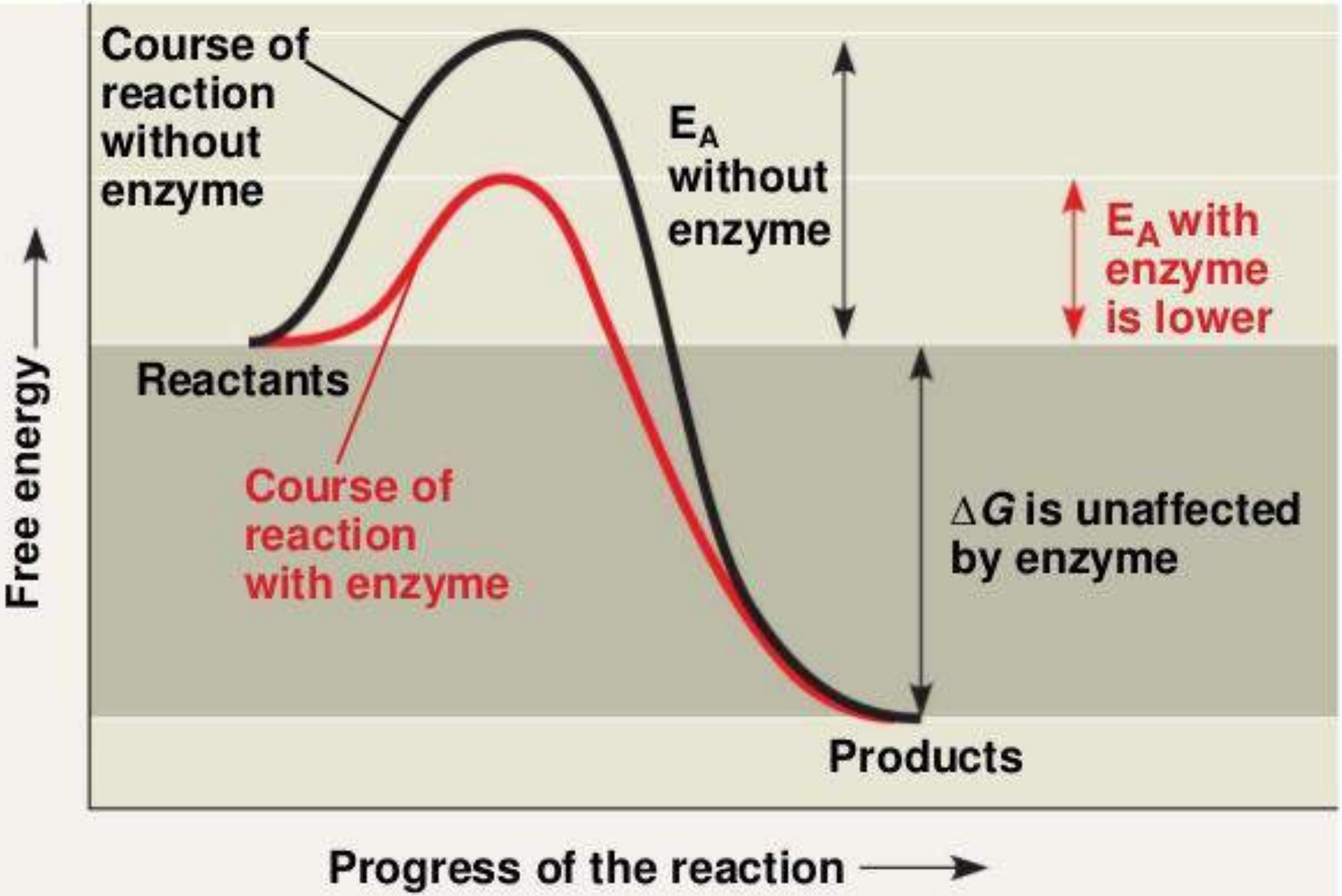
Enzymes Lower a Reaction's Activation Energy

(a) Without enzyme



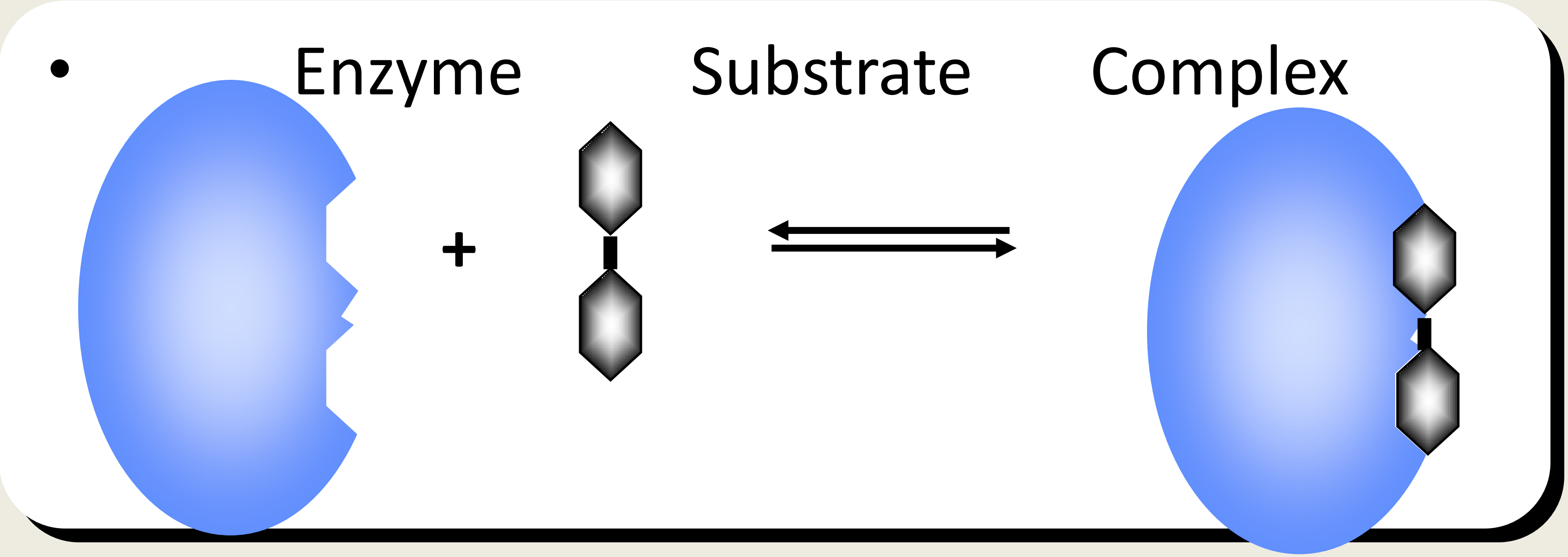
(b) With enzyme




Course of reaction without enzyme
E_A without enzyme
E_A with enzyme is lower
Reactants
Course of reaction with enzyme
ΔG is unaffected by enzyme
Products
Free energy
Progress of the reaction

Enzyme-substrate complex

- Step 1:
- Enzyme and substrate combine to form complex
- $E + S \rightleftharpoons ES$

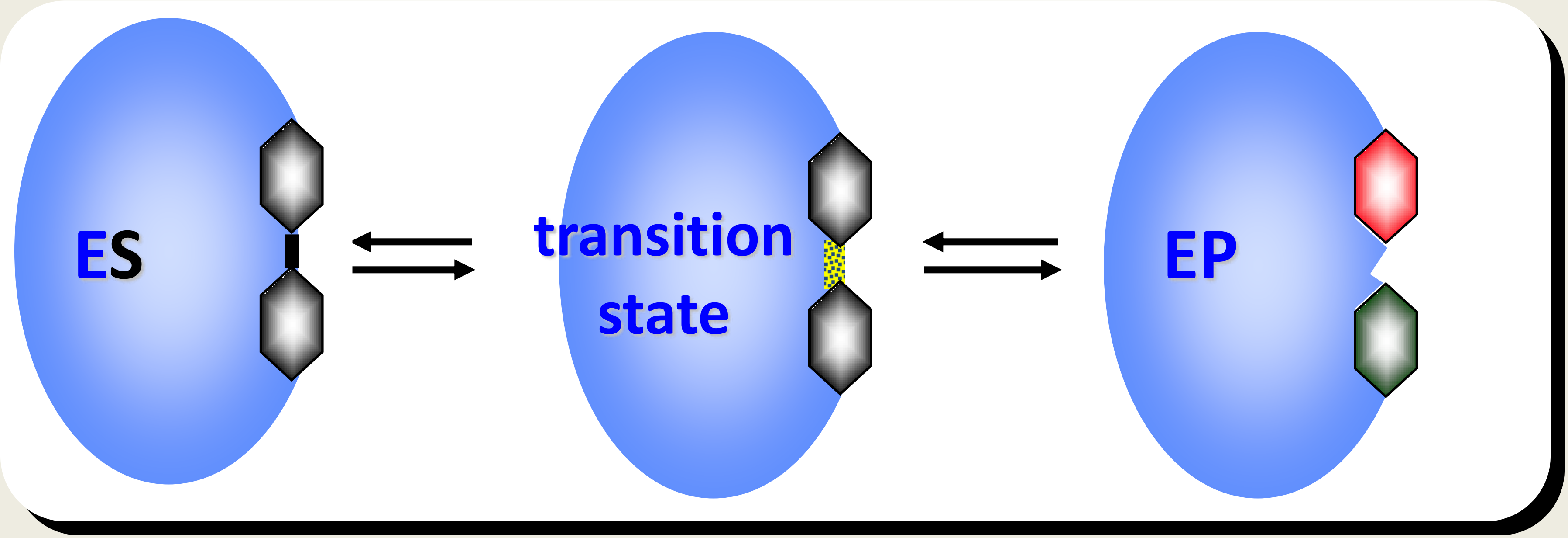


- Enzyme Substrate Complex

Enzyme-product complex

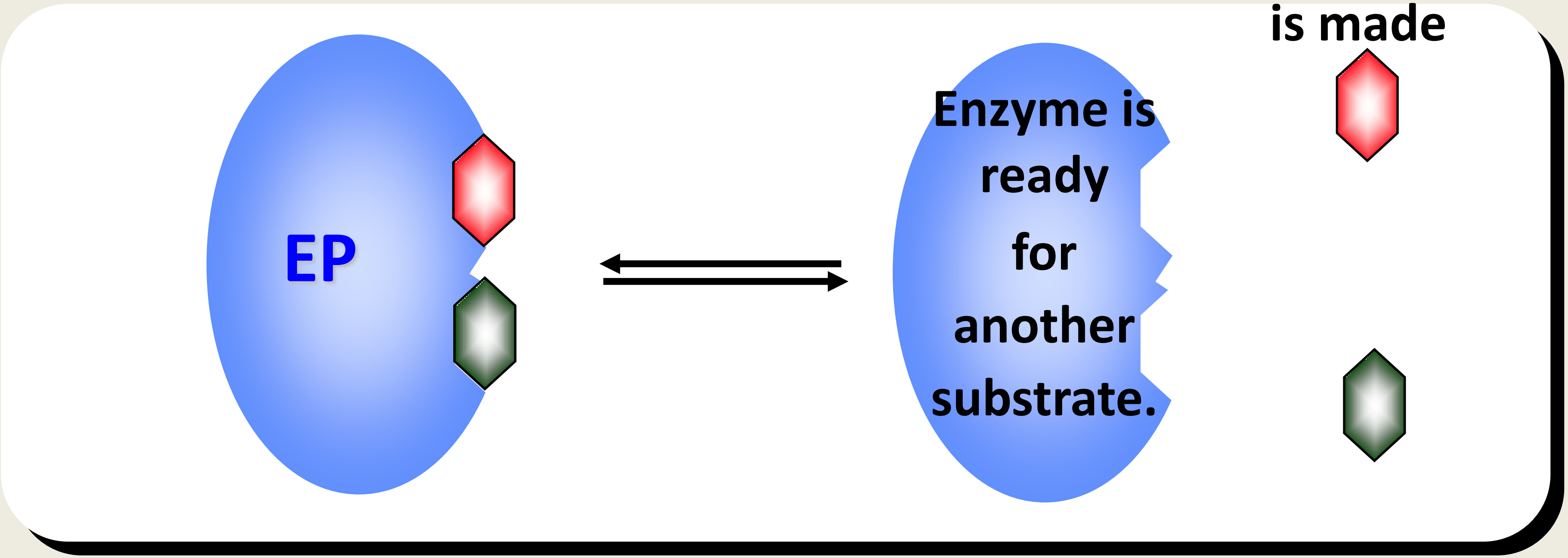
- Step 2:
- An enzyme-product complex is formed.

- ES $\leftrightarrows$ EP



Product

- The enzyme and product separate
  - $EP \rightleftharpoons E + P$



The product is made

Enzyme is ready for another substrate.

What Affects Enzyme Activity?

- **Three factors:**

  1. **Environmental Conditions**

  2. **Cofactors and Coenzymes**

  3. **Enzyme Inhibitors**

1. Environmental Conditions

1. Extreme Temperature are the most dangerous

- high temps may denature (unfold) the enzyme.

2. pH (most like 6 - 8 pH near neutral)

3. substrate concentration .

2. Cofactors and Coenzymes

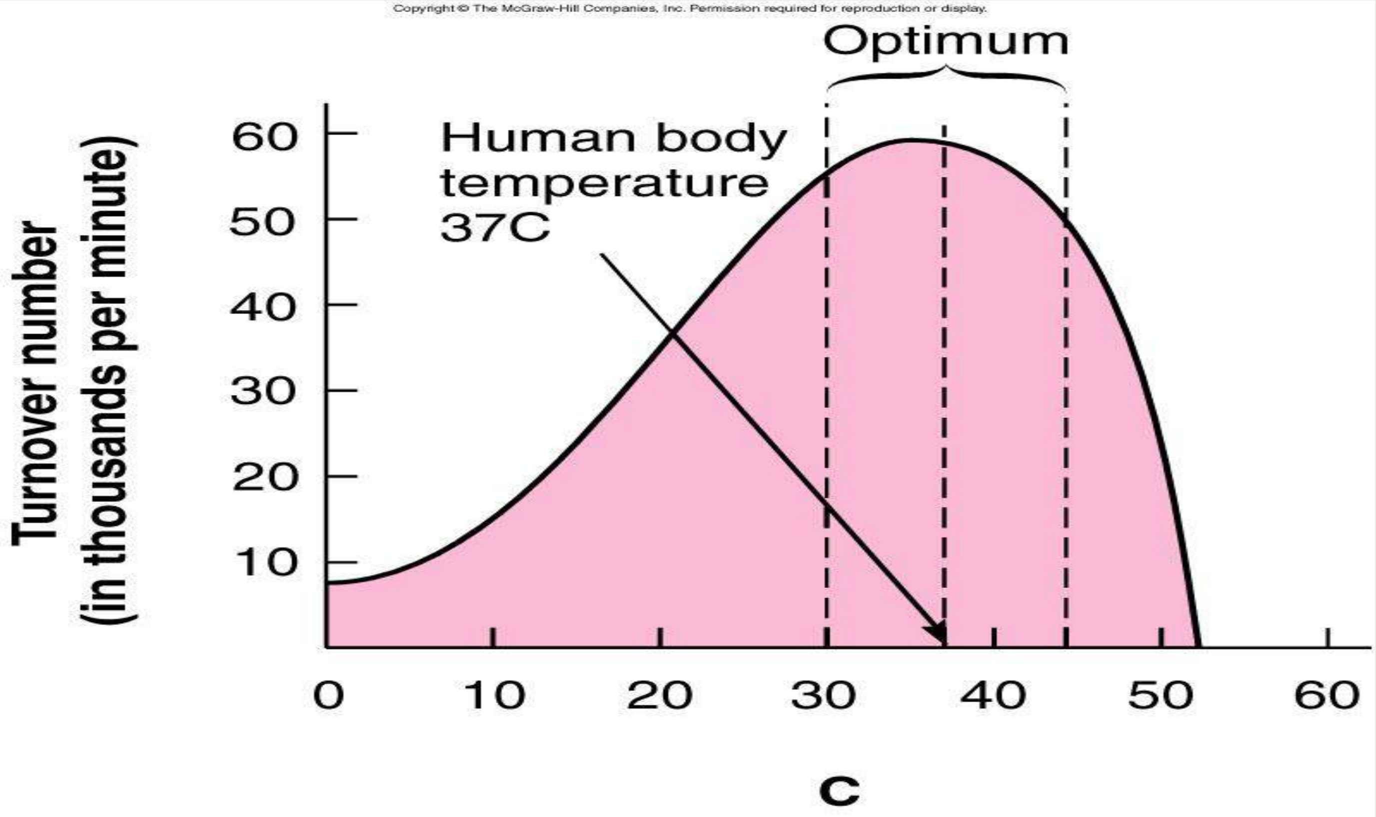
- **Inorganic substances (zinc, iron) and vitamins (respectively) are sometimes need for proper enzymatic activity.**

- **Example:**

  Iron must be present in the quaternary structure - hemoglobin in order for it to pick up oxygen.

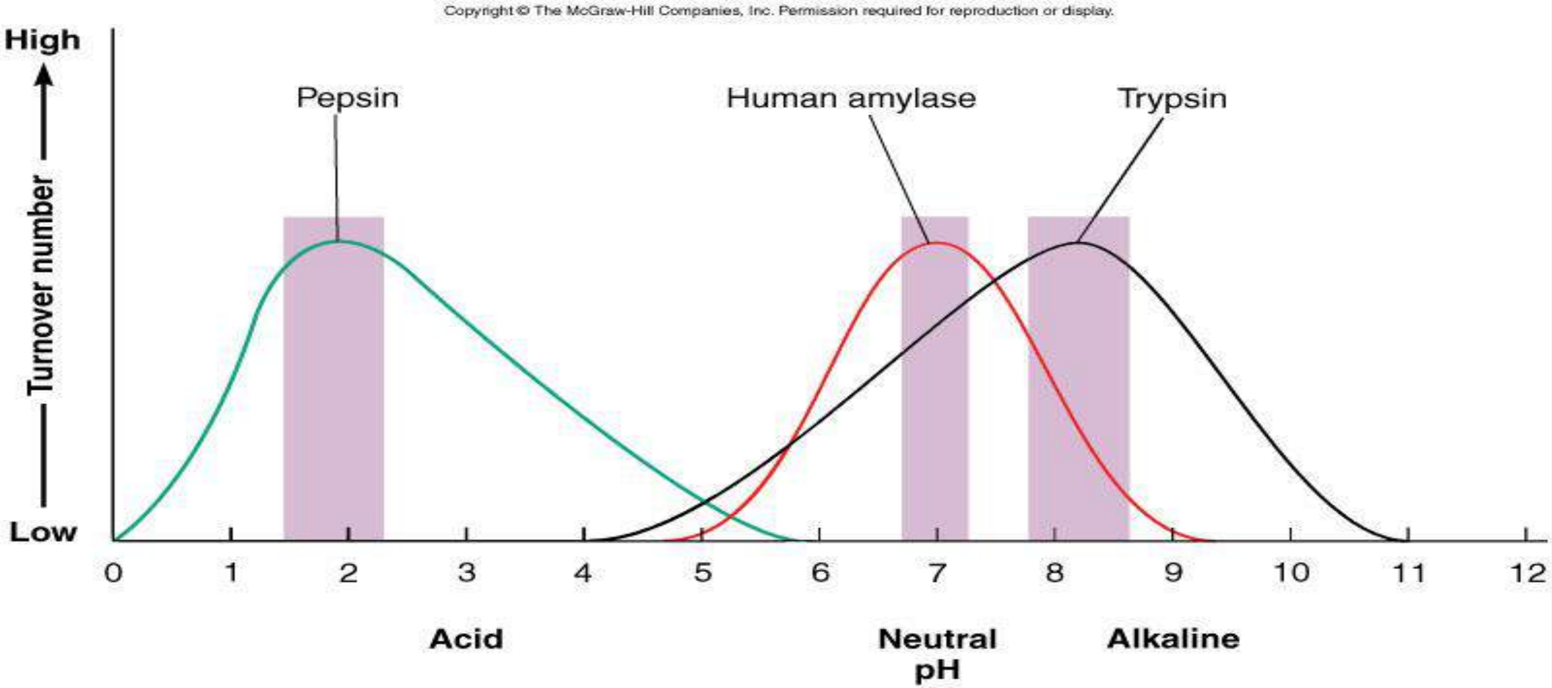
Environmental factors

- ***Optimum temperature*** The temp at which enzymatic reaction occur fastest.



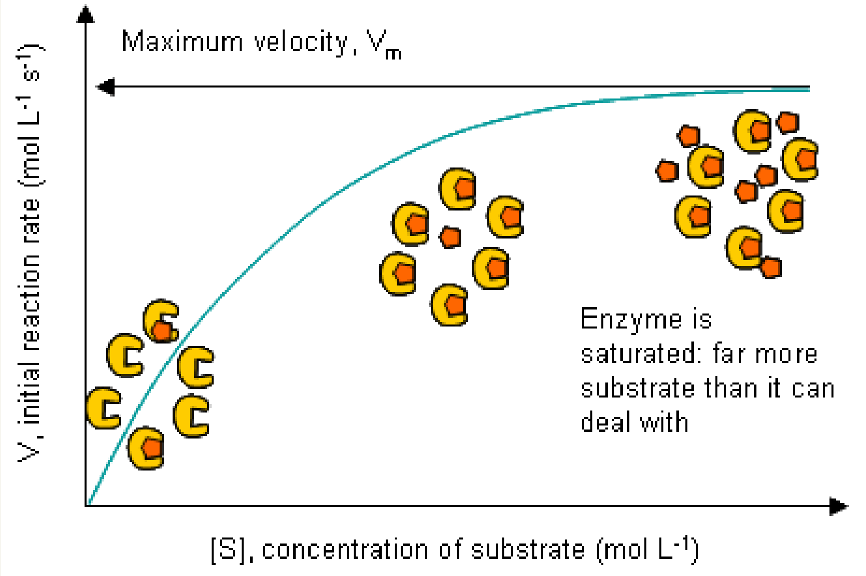
Environmental factors

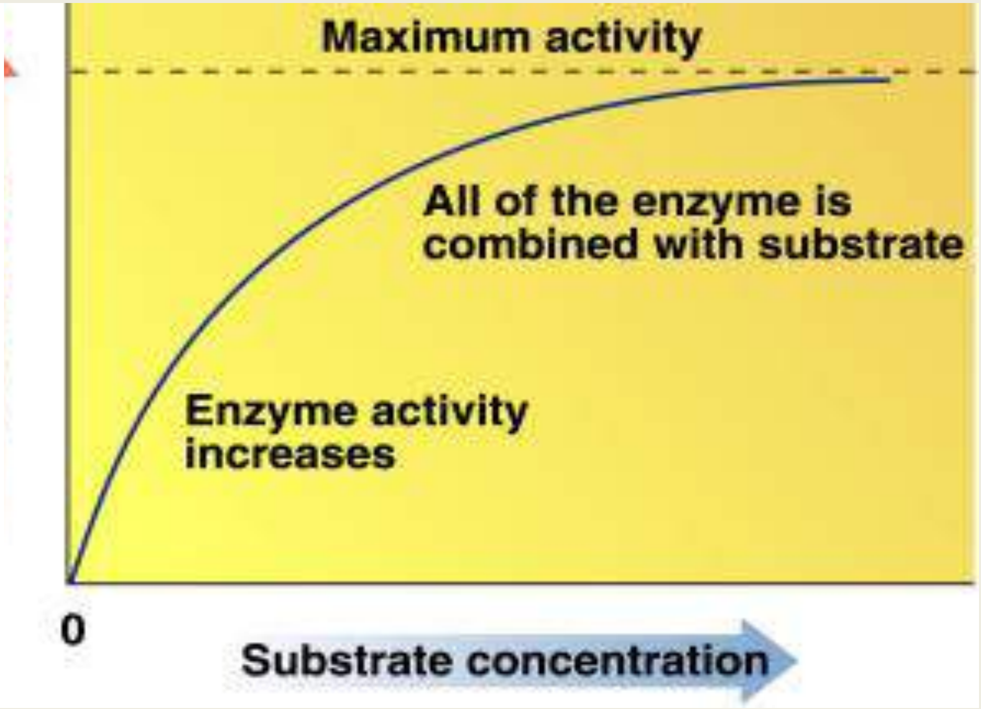
- pH also affects the rate of enzyme-substrate complexes
  - Most enzymes have an optimum pH of around 7 (neutral)
    - However, some prefer acidic or basic conditions



Substrate Concentration and Reaction Rate

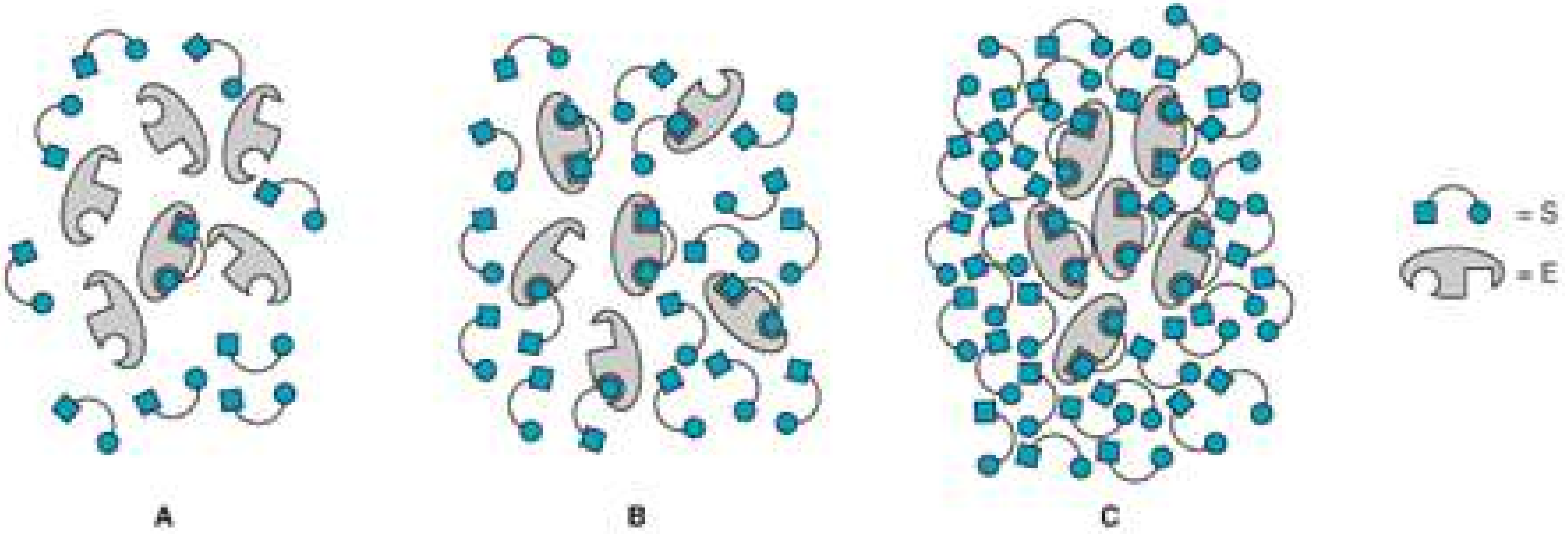
- The rate of reaction increases as substrate concentration increases (at constant enzyme concentration)
- Maximum activity occurs when the enzyme is saturated (when all enzymes are binding substrate)





EFFECTS OF SUBSTRATE CONCENTRATION

with rise in substrate concentration initial velocity rises untill it reaches maximum later no increase in velocity of reaction.



Source: Murray RK, Granner DK, Rodwell VW: *Harper's Illustrated Biochemistry*, 27th Edition: http://www.accessmedicine.com

Classification of Enzymes

Enzymes Are Classified into six functional Classes (EC number Classification) by the International Union of Biochemists (I.U.B.).

on the Basis of the Types of Reactions That They Catalyze

- EC 1. Oxidoreductases
- EC 2. Transferases
- EC 3. Hydrolases
- EC 4. Lyases
- EC 5. Isomerases
- EC 6. Ligases

Principle of the international classification

Each enzyme has **classification number** consisting of four digits:

Example, **EC:** (2.7.**1.**1) **HEXOKINASE**

- EC: (2.7.1.1) these components indicate the following groups of enzymes:

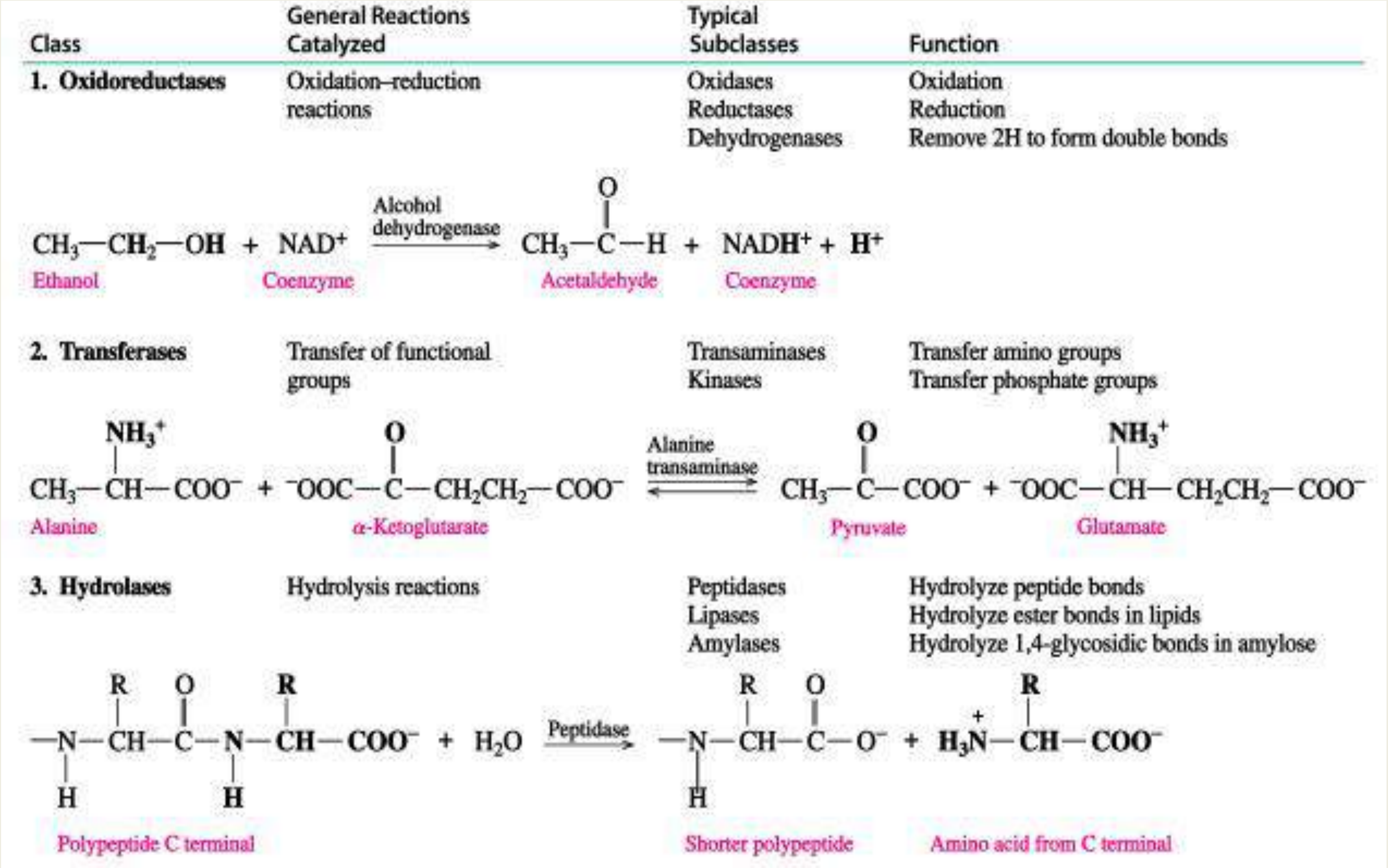
- 2. IS CLASS (TRANSFERASE)

- 7. IS SUBCLASS (TRANSFER OF PHOSPHATE)

- 1. IS SUB-SUB CLASS (ALCOHOL IS PHOSPHATE ACCEPTOR)
- 1. SPECIFIC NAME

  ATP,D-HEXOSE-6-PHOSPHOTRANSFERASE (Hexokinase)

Oxidoreductases, Transferases and Hydrolases



Class	General Reactions Catalyzed	Typical Subclasses	Function
1. Oxidoreductases	Oxidation–reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Remove 2H to form double bonds
2. Transferases	Transfer of functional groups	Transaminases Kinases	Transfer amino groups Transfer phosphate groups
3. Hydrolases	Hydrolysis reactions	Peptidases Lipases Amylases	Hydrolyze peptide bonds Hydrolyze ester bonds in lipids Hydrolyze 1,4-glycosidic bonds in amylose

$$CH_3-CH_2-OH + NAD^+ \xrightarrow{\text{Alcohol dehydrogenase}} CH_3-\overset{\overset{O}{|}}{C}-H + NADH^+ + H^+$$

Ethanol, Coenzyme, Acetaldehyde, Coenzyme

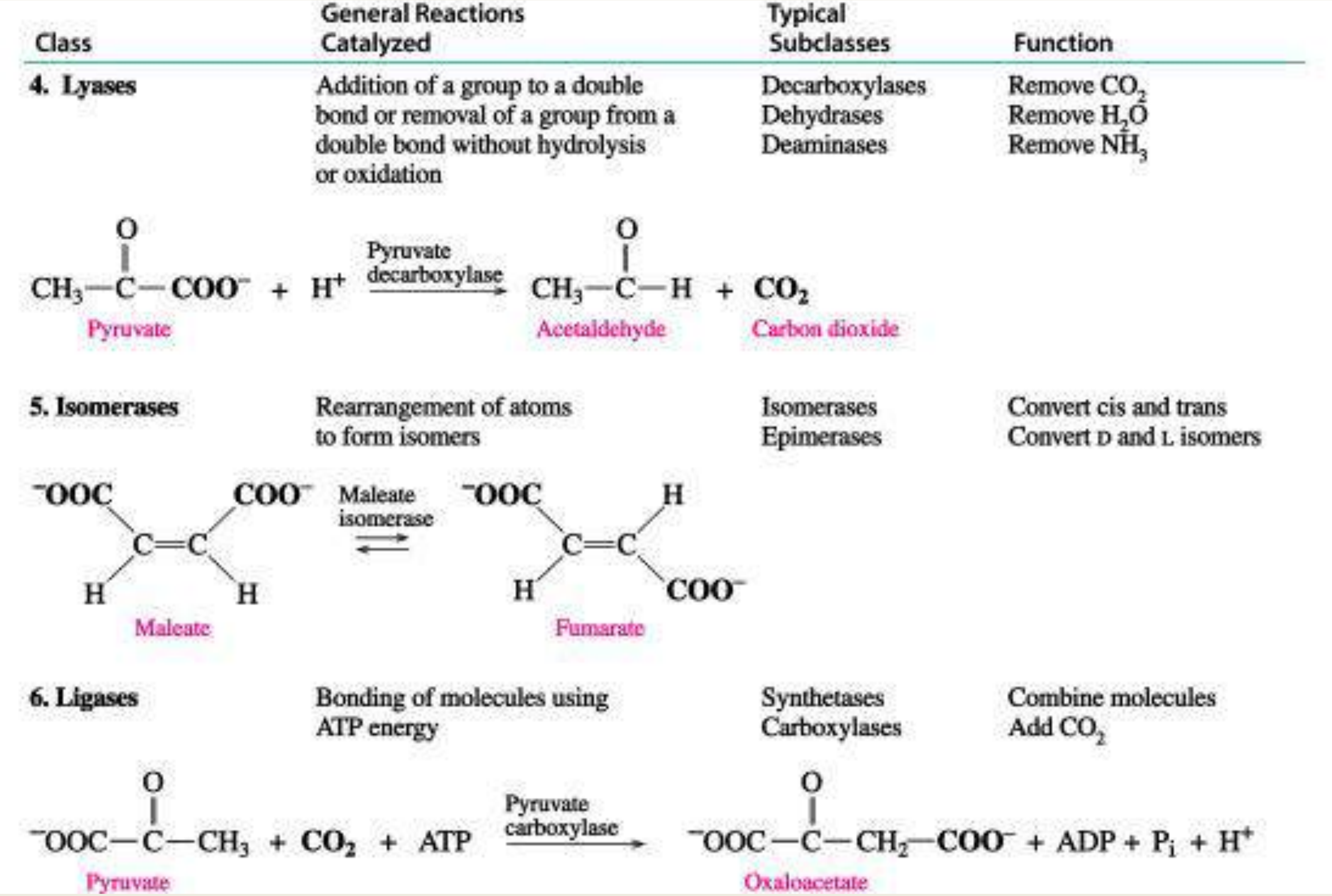
$$CH_3-\overset{\overset{NH_3^+}{|}}{CH}-COO^- + {}^-OOC-\overset{\overset{O}{\|}}{C}-CH_2CH_2-COO^- \overset{\text{Alanine transaminase}}{\rightleftharpoons} CH_3-\overset{\overset{O}{\|}}{C}-COO^- + {}^-OOC-\overset{\overset{NH_3^+}{|}}{CH}-CH_2CH_2-COO^-$$

Alanine, α-Ketoglutarate, Pyruvate, Glutamate

$$-\underset{\underset{H}{|}}{N}-\overset{\overset{R}{|}}{CH}-\overset{\overset{O}{\|}}{C}-\underset{\underset{H}{|}}{N}-\overset{\overset{R}{|}}{CH}-COO^- + H_2O \xrightarrow{\text{Peptidase}} -\underset{\underset{H}{|}}{N}-\overset{\overset{R}{|}}{CH}-\overset{\overset{O}{\|}}{C}-O^- + H_3\overset{+}{N}-\overset{\overset{R}{|}}{CH}-COO^-$$

Polypeptide C terminal, Shorter polypeptide, Amino acid from C terminal

Lyases, Isomerases and Ligases



Class	General Reactions Catalyzed	Typical Subclasses	Function
4. Lyases	Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation	Decarboxylases Dehydrases Deaminases	Remove CO_2 Remove H_2O Remove NH_3
5. Isomerases	Rearrangement of atoms to form isomers	Isomerases Epimerases	Convert cis and trans Convert D and L isomers
6. Ligases	Bonding of molecules using ATP energy	Synthetases Carboxylases	Combine molecules Add CO_2

$$CH_3-\overset{O}{\overset{|}{C}}-COO^- + H^+ \xrightarrow{\text{Pyruvate decarboxylase}} CH_3-\overset{O}{\overset{|}{C}}-H + CO_2$$

Pyruvate — Acetaldehyde — Carbon dioxide

Maleate — Fumarate

$${}^-OOC-\overset{O}{\overset{|}{C}}-CH_3 + CO_2 + ATP \xrightarrow{\text{Pyruvate carboxylase}} {}^-OOC-\overset{O}{\overset{|}{C}}-CH_2-COO^- + ADP + P_i + H^+$$

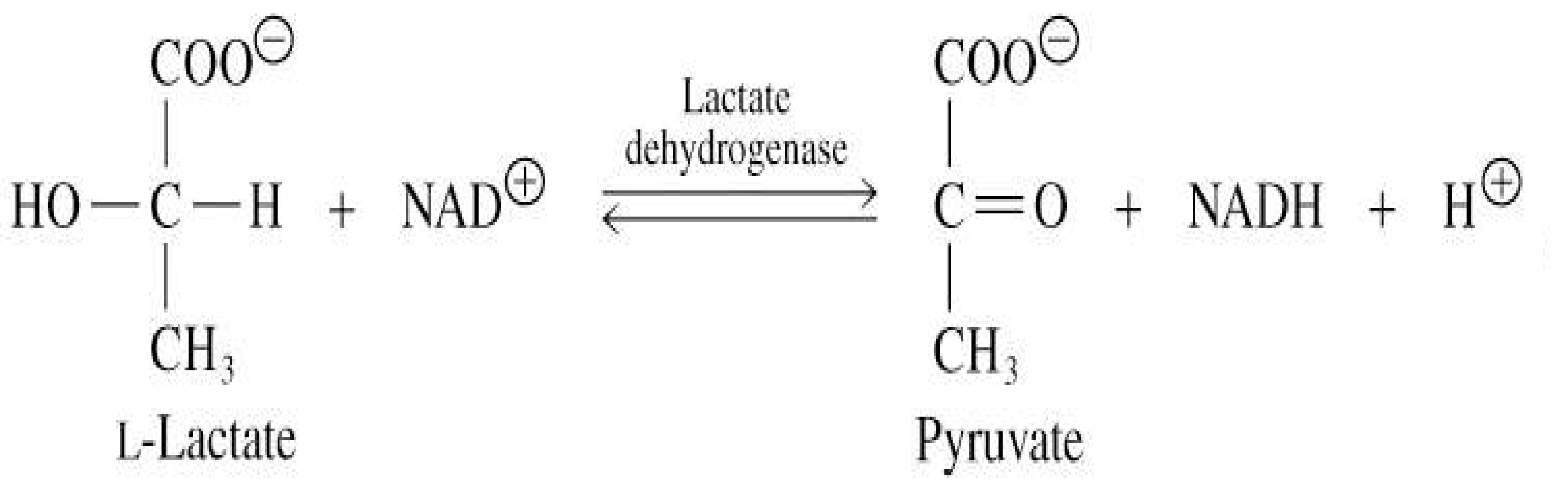
Pyruvate — Oxaloacetate

EC 1. Oxidoreductases

- **Biochemical Activity:**
  - **Catalyse Oxidation/Reduction Reactions Act on many chemical groupings to add or remove hydrogen atoms.**
- **Examples:**
  - **Lactate dehydrogenase.**
  - **Glucose Oxidase.**
  - **Peroxidase.**
  - **Catalase.**
  - **Phenylalanine hydroxylase.**

1. Oxidoreductases

- Catalyze oxidation-reduction reactions



$$HO-\overset{\overset{\displaystyle COO^{\ominus}}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C}}-H + NAD^{\oplus} \xrightleftharpoons[]{\text{Lactate dehydrogenase}} \overset{\overset{\displaystyle COO^{\ominus}}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C}}=O + NADH + H^{\oplus}$$

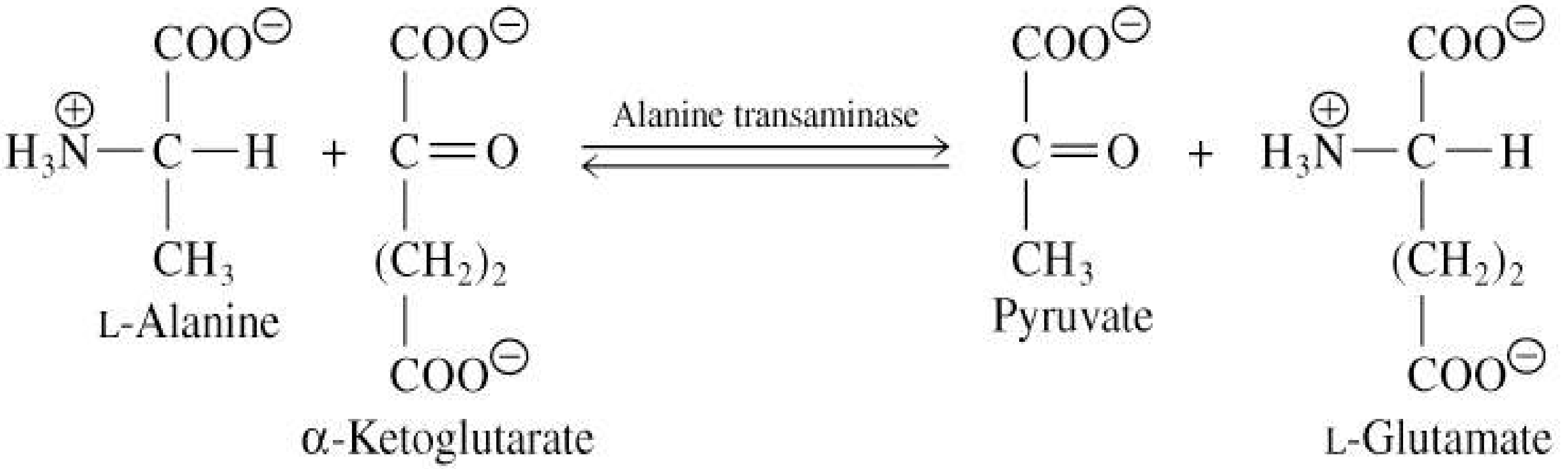
- oxidases
- peroxidases
- dehydrogenases

EC 2. Transferases

- Biochemical Activity:
  - Transfer a functional groups (e.g. methyl or phosphate) between donor and acceptor molecules.
- Examples:
  - Transaminases (ALT & AST).
  - Phosphotransferases (Kinases).
  - Transmethylases.
  - Transpeptidases.
  - Transacylases.

2. Transferases

- Catalyze group transfer reactions



$$H_3\overset{\oplus}{N}-\underset{\underset{CH_3}{|}}{\overset{\overset{COO^{\ominus}}{|}}{C}}-H + \underset{\underset{\underset{\underset{COO^{\ominus}}{|}}{(CH_2)_2}}{|}}{\overset{\overset{COO^{\ominus}}{|}}{C}}=O \xrightleftharpoons{\text{Alanine transaminase}} \underset{\underset{CH_3}{|}}{\overset{\overset{COO^{\ominus}}{|}}{C}}=O + H_3\overset{\oplus}{N}-\underset{\underset{\underset{\underset{COO^{\ominus}}{|}}{(CH_2)_2}}{|}}{\overset{\overset{COO^{\ominus}}{|}}{C}}-H$$

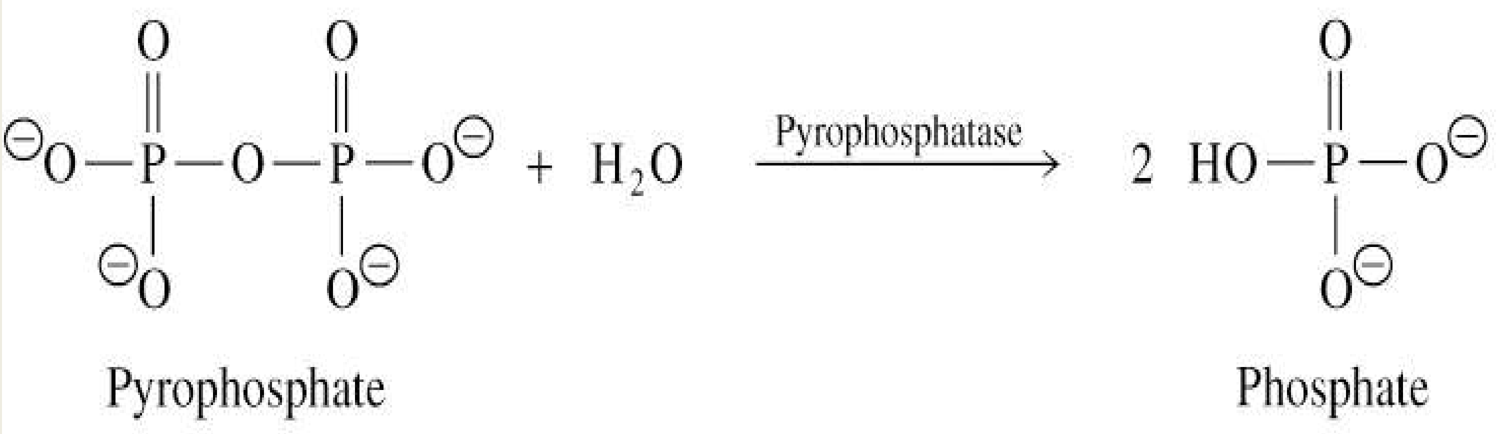
(5.2)

EC 3. Hydrolases

- **Biochemical Activity:**
  - **Catalyse the hydrolysis of various bonds Add water across a bond.**
- **Examples:**
  - **Protein hydrolyzing enzymes (Peptidases).**
  - **Carbohydrases (Amylase, Maltase, Lactase).**
  - **Lipid hydrolyzing enzymes (Lipase).**
  - **Deaminases.**
  - **Phosphatases.**

3. Hydrolases

- Catalyze hydrolysis reactions where water is the acceptor of the transferred group



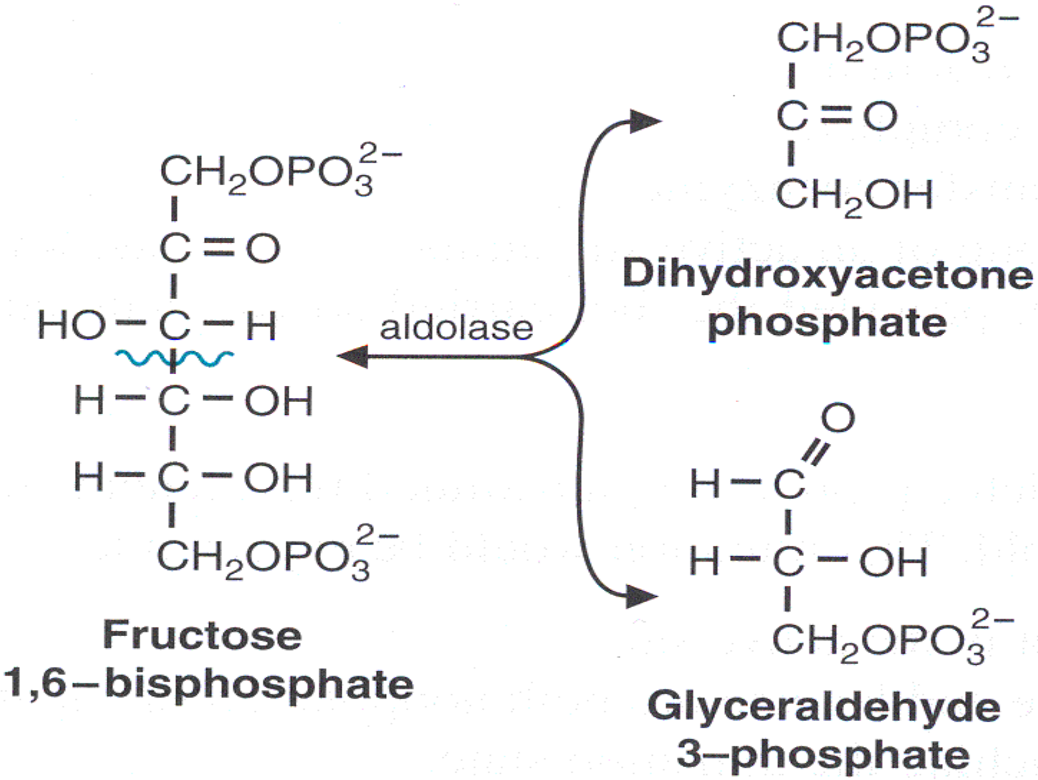
$$^{\ominus}O-\overset{\overset{O}{\|}}{\underset{\underset{^{\ominus}O}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O^{\ominus}}{|}}{P}}-O^{\ominus} + H_2O \xrightarrow{\text{Pyrophosphatase}} 2\ HO-\overset{\overset{O}{\|}}{\underset{\underset{O^{\ominus}}{|}}{P}}-O^{\ominus} \qquad (5.3)$$

- esterases
- peptidases
- glycosidases

EC 4. Lyases

- Biochemical Activity:
  - Cleave various bonds by means other than hydrolysis and oxidation.
  - Add Water, Ammonia or Carbon dioxide across double bonds, or remove these elements to produce double bonds.
- Examples:
  - Fumarase.
  - Carbonic anhydrase.

4. Lyases



Fructose 1,6–bisphosphate

Dihydroxyacetone phosphate

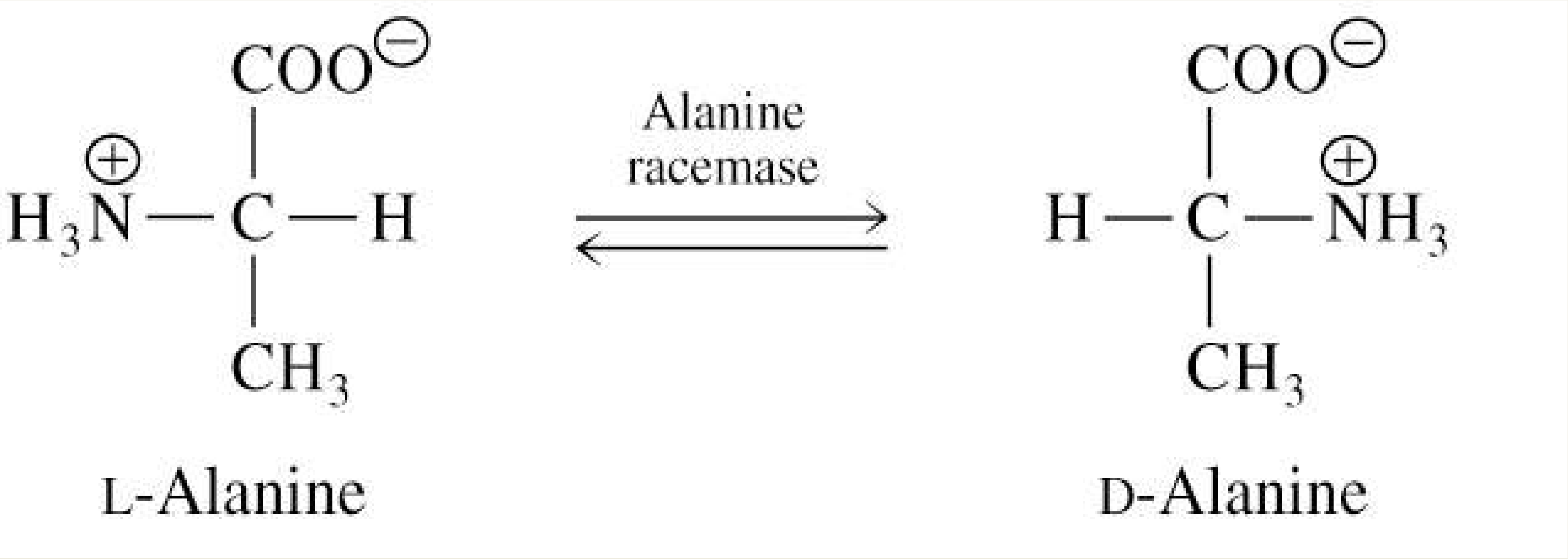
Glyceraldehyde 3–phosphate

EC 5. Isomerases

- **Biochemical Activity:**
  - **Catalyse isomerization changes within a single molecule.**
  - **Carry out many kinds of isomerization:**
    - **L to D isomerizations.**
    - **Mutase reactions (Shifts of chemical groups).**
- **Examples:**
  - **Isomerase.**
  - **Mutase.**

5. Isomerases

- Catalyze isomerization reactions

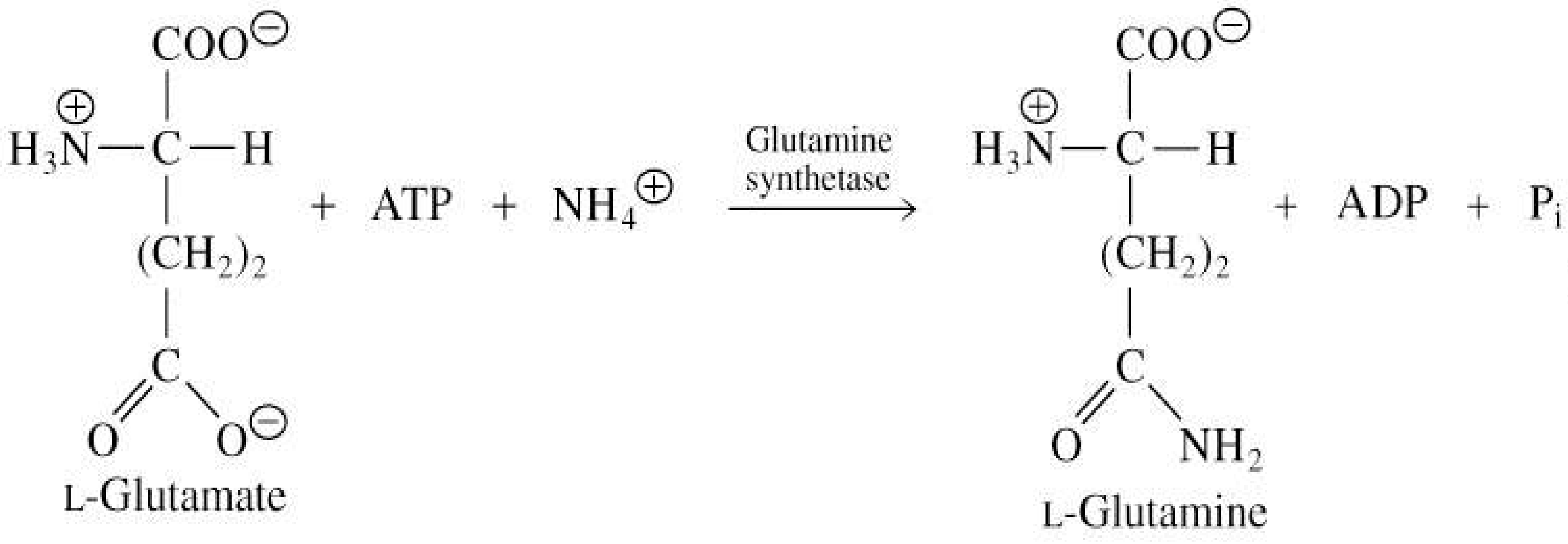


EC 6. Ligases

- Biochemical Activity:
  - Join two molecules with covalent bonds Catalyse reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP.
- Examples:
  - Acetyl~CoA Carboxylase.
  - Glutamine synthetase

6. Ligases (synthetases)

- Catalyze ligation, or joining of two substrates
- Require chemical energy (e.g. ATP)



(5.6)

Thank you