Bioreaction Kinetics 2

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Reference

- **Chemistry and the Living Organism**, 6th edition, Molly M. Bloomfield, Lawrence J. Stephens, John Wiley & Sons, Inc.

Enzymes

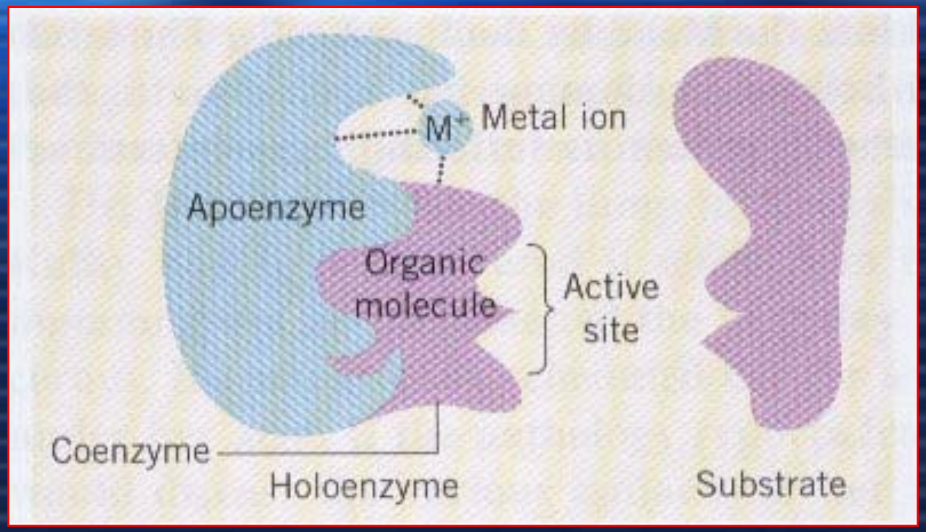
1. Most enzymes are protein catalysts in biological systems.
2. For an enzyme to catalyze a reaction, the substrate must attach to the surface of the enzyme in a region called the active site.
3. Enzyme activity can be inhibited in several ways.
4. Metabolic processes are the result of sequences of enzyme-catalyzed reactions called multienzyme systems.
5. Enzyme activity in an organism is regulated at many levels, both inside and outside the cell.

What Is Metabolism?

- Metabolism : all the enzyme-catalyzed reactions in the cell.
- Two types
  - Catabolic reactions : Molecules are broken down to produce smaller molecules and energy.
  - Anabolic reactions (Biosynthetic reaction) : The cell uses energy to produce the molecules it needs for growth and repair.

Enzymes (Biological catalysts

- The largest and most highly specialized class of proteins.
- Water-soluble globular proteins that carry out their functions in body fluids or bound to the membranes of the cell.
- Molecular weight from 12,000 to over 1 million.
- A simple single polypeptide chain or a more complex molecule composed of several polypeptide chains and other nonprotein parts.
- Examples
  - Ribonuclease : A single polypeptide chain of 124 aa
    Molecular mass: About 13,700 daltons(1 dalton = 1 amu)
  - Pyruvate dehydrogenase : Macromolecular complex containing 42 individual molecules
    Molecular mass: About 10 million daltons



Important terms used in the study of enzymes.

A computer model of The substrate ATP binding To the enzyme Phosphoglycerate kinase.

Terms about Enzyme

1. Apoenzyme
   - The protein part of the enzyme molecule
2. Cofactor
   - Additional chemical groups that are required for enzyme activity.
   - Cofactors may consists of metal ions or complex organic molecules.
   - Some enzymes require both types of cofactors.
3. Coenzyme
   - When the cofactor is a complex organic molecule other than a protein, the cofactor is called a coenzyme.
4. Prosthetic group
   - If the cofactor is a permanent part of the enzyme, bound to the protein portion with covalent bonds, it is called a prosthetic group.

Terms about Enzyme

5. Holoenzyme
   - An entire active enzyme, which consists of an apoenzyme and one or more cofactors.
6. Proenzyme or zymogen
   - An enzyme in its inactive form.
   - Enzymes(especially digestive enzymes) are often synthesized in an inactive form, transported to the place where activity is desired, and then converted to their active forms.
7. Substrate
   - The chemical substance or substances on which the enzyme acts
8. Active site
   - The specific area of the enzyme to which the substrate attaches during the reaction.
   - An enzyme molecule can have several active sites.

Enzyme Nomenclature and Classification

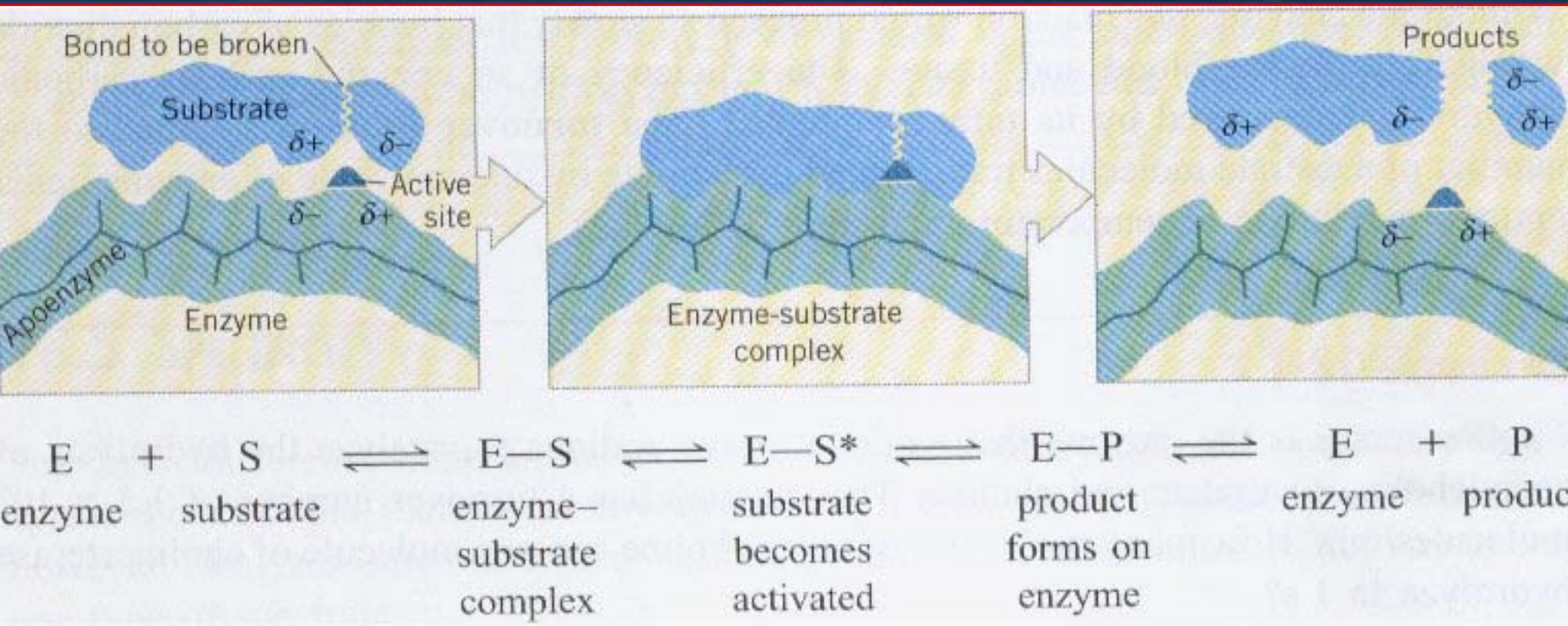
- In 1961, the Commission on Enzymes of the International Union of Biochemistry.

Table 18.1 Classes of Enzymes

Example	*Reaction Catalyzed*
Hydrolases: Enzymes that catalyze hydrolysis reactions	
Carbohydrases	Polysaccharides $\xrightarrow{+H_2O}$ Monosaccharides and dissaccharides
Esterases	Ester $\xrightarrow{+H_2O}$ Acid + alcohol
Proteases	Protein $\xrightarrow{+H_2O}$ Peptides and amino acids
Nucleases	Nucleic acids $\xrightarrow{+H_2O}$ Pyrimidines + purines + sugars + phosphoric acid
Oxidoreductases: Enzymes that catalyze oxidation–reduction reactions	
Oxidases	Addition of oxygen to a substrate
Dehydrogenases	Removal of hydrogen from a substrate
Transferases: Enzymes that catalyze reactions involved in the transfer of functional groups	
Transaminases	Transfer of $-NH_2$
Transmethylases	Transfer of $-CH_3$
Transacylases	Transfer of $-\overset{\overset{O}{\|}}{C}-R$
Transphosphatases (Kinases)	Transfer of $-O-\overset{\overset{O}{\|}}{\underset{\underset{OH}{\|}}{P}}-OH$
Lyases: Enzymes that catalyze the elimination of groups to form double bonds	
Isomerases: Enzymes that catalyze the interconversion of isomers	
Ligases: Enzymes that, in conjunction with ATP, catalyze the formation of new bonds	

Method of Enzyme Actio

- Enzyme catalyze reactions in cells by lowering the activation energy.



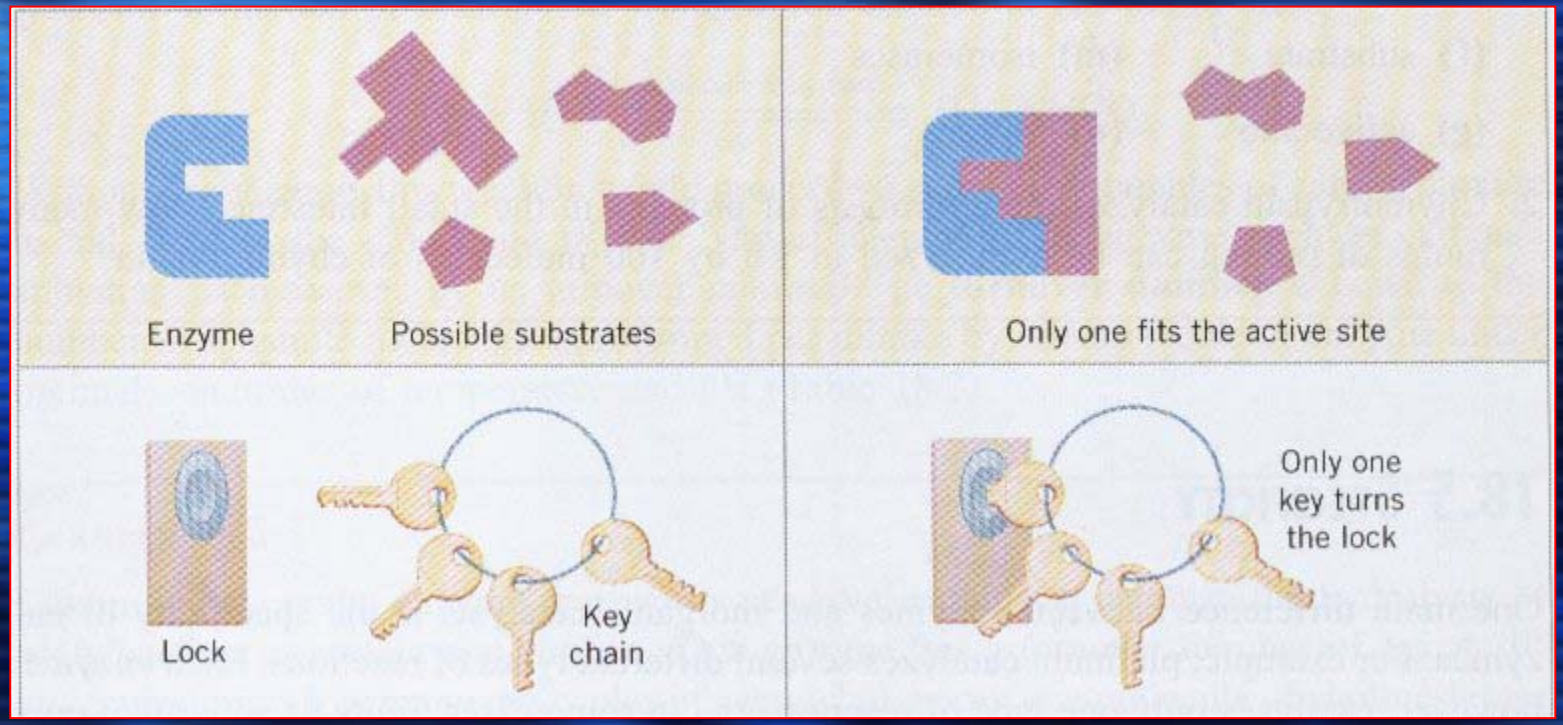
- **Turnover number : The number of substrate molecules transformed per minute by one molecule of enzyme under optimal conditions of temperature and pH.**

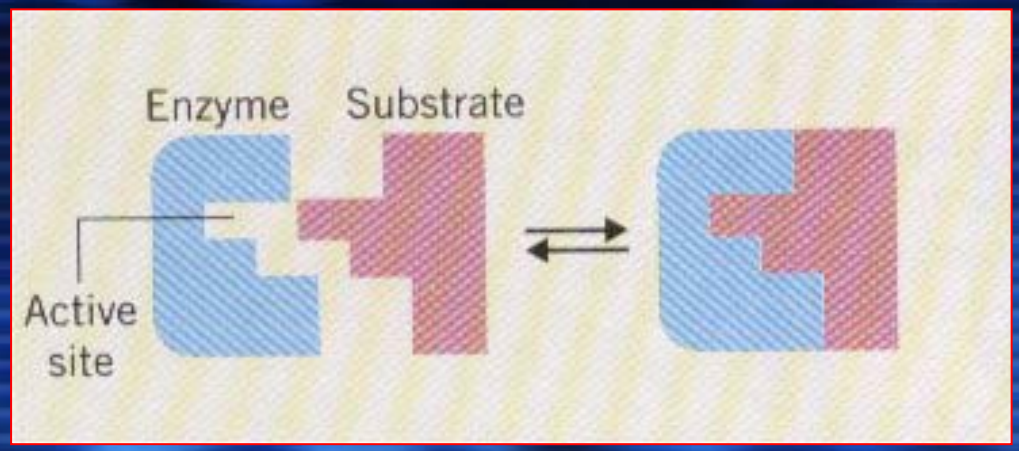
Table 18.2 **Turnover Numbers of Enzymes**

Enzyme	*Turnover Number (molecules of substrate per minute)*
Carbonic anhydrase	36,000,000
α-Glucosidase	1,000,000
Glutamate dehydrogenase	30,000
Phosphoglucomutase	1,240
Chymotrypsin	100
DNA polymerase	15

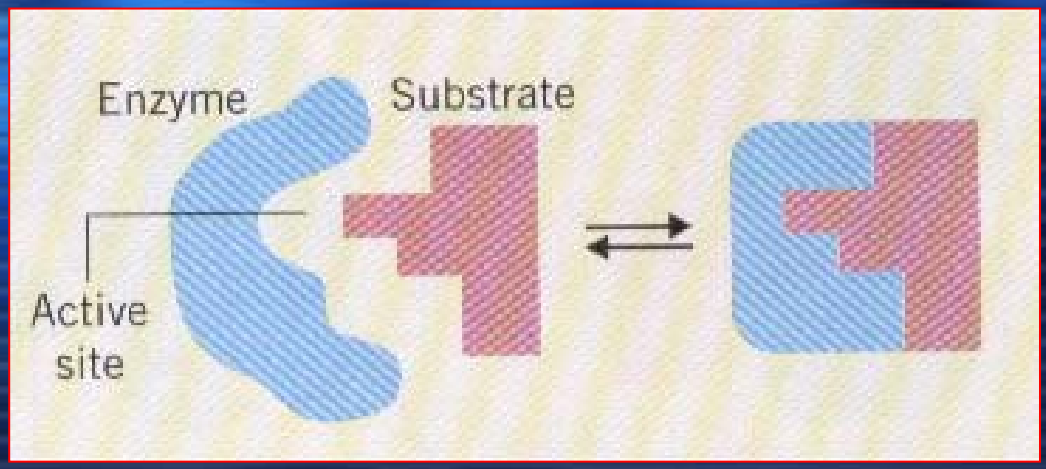
Specificity

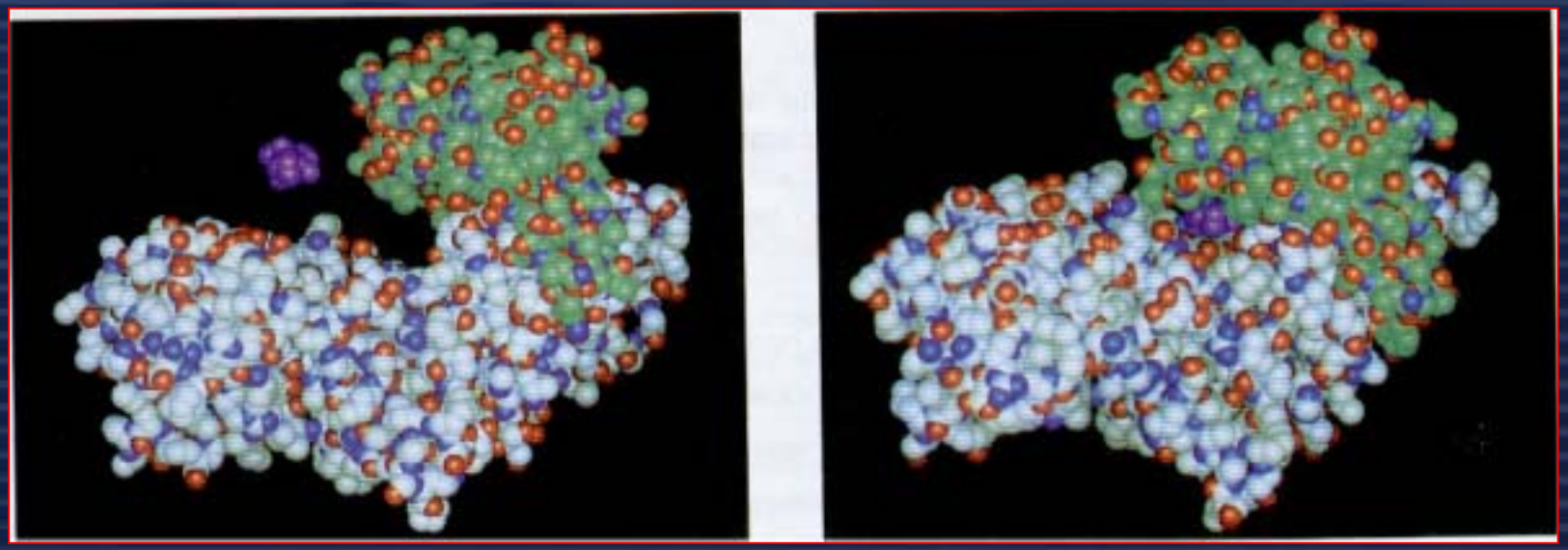
- Each enzyme catalyzes only one type of reaction.
- Lock and Key Theory
  - The shape, or configuration, of the aa R groups around the active site is especially designed for a specific substrate. Enzymes are formed from L-aa, therefore the active sites are asymmetrical.
  - D-aa does not fit in the active site.
  - Because the configuration of the active site is determined by the aa sequence of the enzyme, the native configuration of the entire enzyme molecule must be intact for the active site to have the correct shape.
  - The shape of the lock is specific for only one key.


Enzyme
Possible substrates
Only one fits the active site
Lock
Key chain
Only one key turns the lock


Enzyme
Substrate
Active site

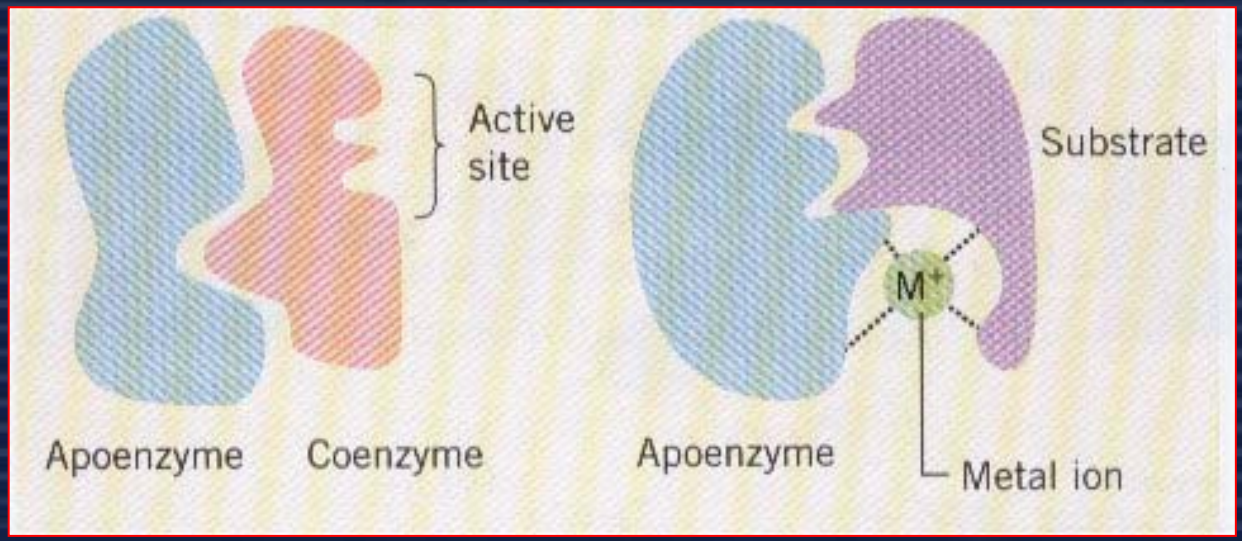
- **Induced-Fit Theory**
  - **Enzyme molecules are flexible.**
  - **Although the active site of some enzymes may not initially match the substrate, the substrate itself, as it is drawn to the enzyme, may induce the enzyme to take on a shape that matches the substrate.**





These two space-filling models of the yeast enzyme hexokinase and the complex of the enzyme with glucose (purple) illustrate the induced-fit theory.

- Enzymes that are secreated within the body as proenzymes have their active sites blocked. To activate the enzyme, these sites must be unblocked by the hydrolysis of part of the molecule.
- In other enzymes, cofactors must be present for the enzyme to be active because the cofactors provide the arrangement of molecules necessary for the active site to form a bridge between the substrate and the enzyme.

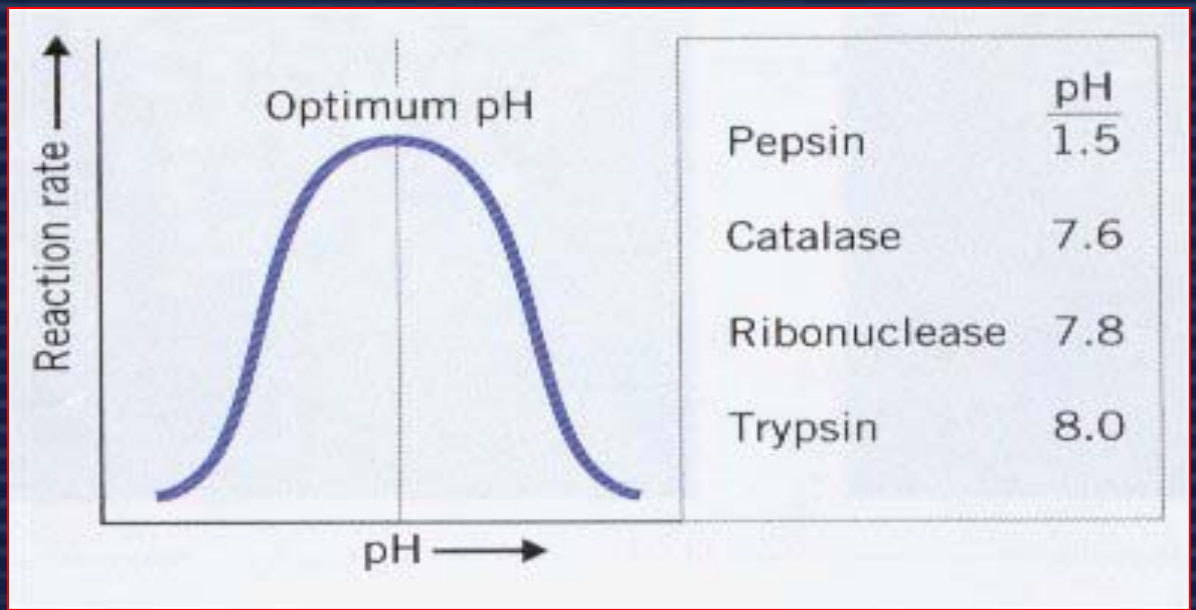


Factors Affecting Enzyme Activity

- Factors : pH, temperature, solvents, salt concentrations.
- Such factors can change the structure of a protein and have an effect on the activity levels of enzymes.

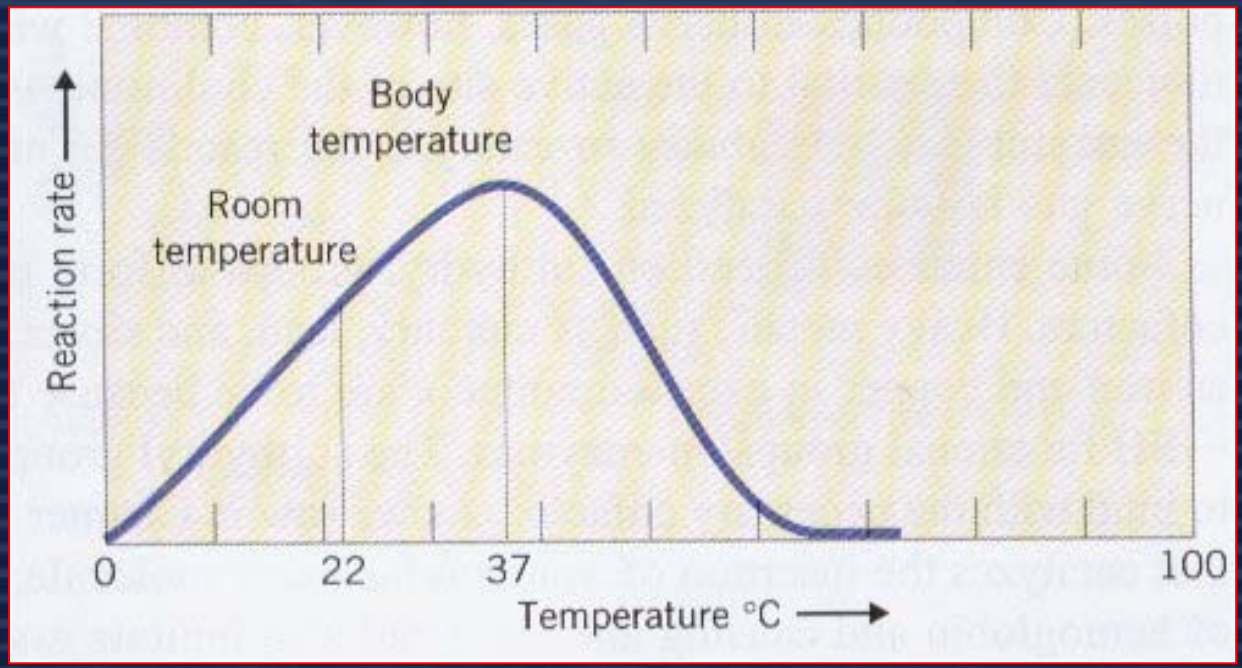
pH

- Changes in the pH of the surrounding medium can change the secondary or tertiary structure of an enzyme ; this may alter the geometry of the active site or the surrounding charge distribution. Each enzyme has an optimum pH at which it is most active.
- Extreme changes in pH denature all enzymes. However, body fluids contain buffer systems to protect against such changes.



Temperature

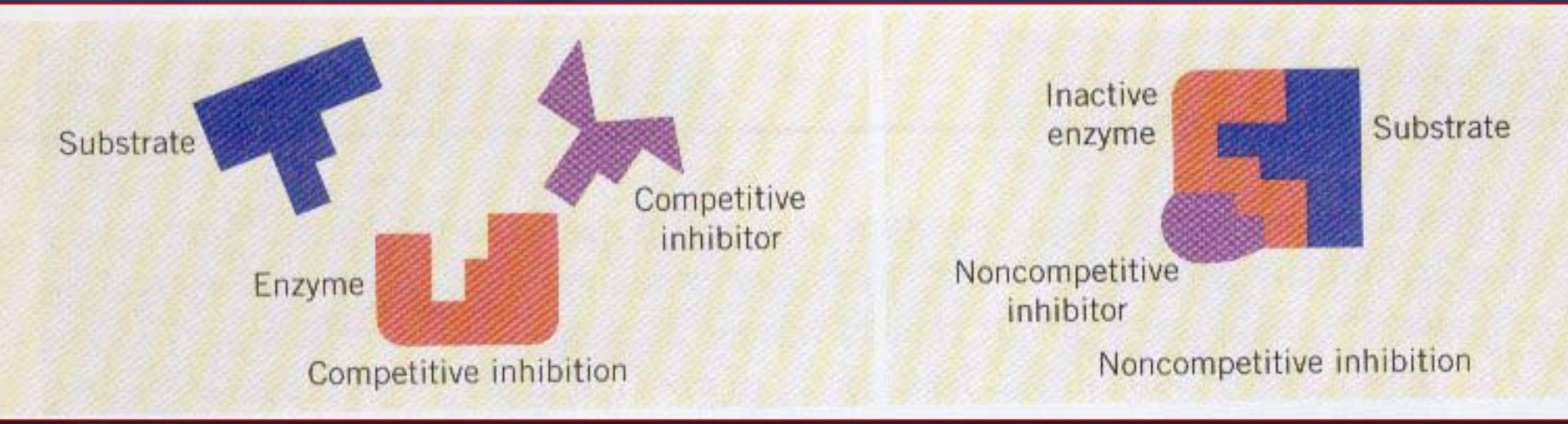
- Temperature affects the rate of enzyme-catalyzed reactions. Most body enzymes have their highest activity at temperatures from 35℃ to 45℃. When you run a high fever, you feel ill because your high body temperature slows enzyme activity.



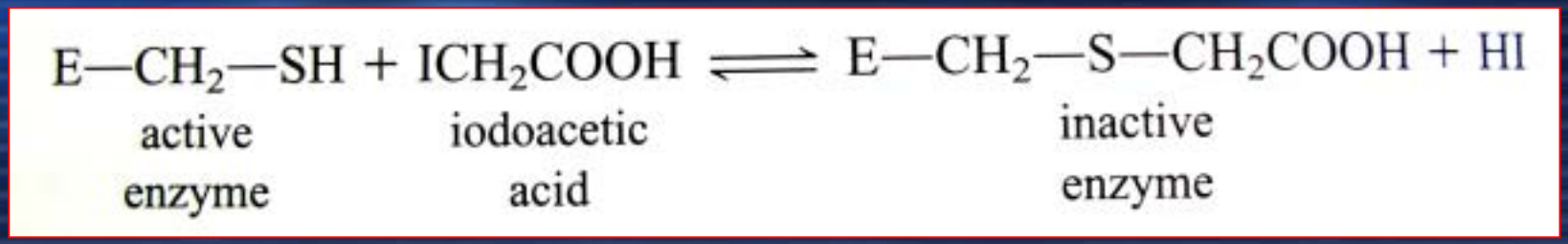
Inhibition of Enzyme Activity

- **Irreversible Inhibition**
  - The activity of enzymes can be inhibited in several ways. The action of many poisons and drugs is due to their ability to inhibit specific enzymes. Irreversible inhibition of enzyme activity occurs when a functional group in the active site or a cofactor required for the activity of the enzyme is destroyed or modified.
  - Ex) Heavy metals, such as mercury, lead, and silver (and even such essential metals as iron and copper in excess amounts), are toxic because they bind irreversibly with free -SH functional groups on enzyme. The sulfhydryl groups, then, are no longer available to bind with the necessary cofactor.

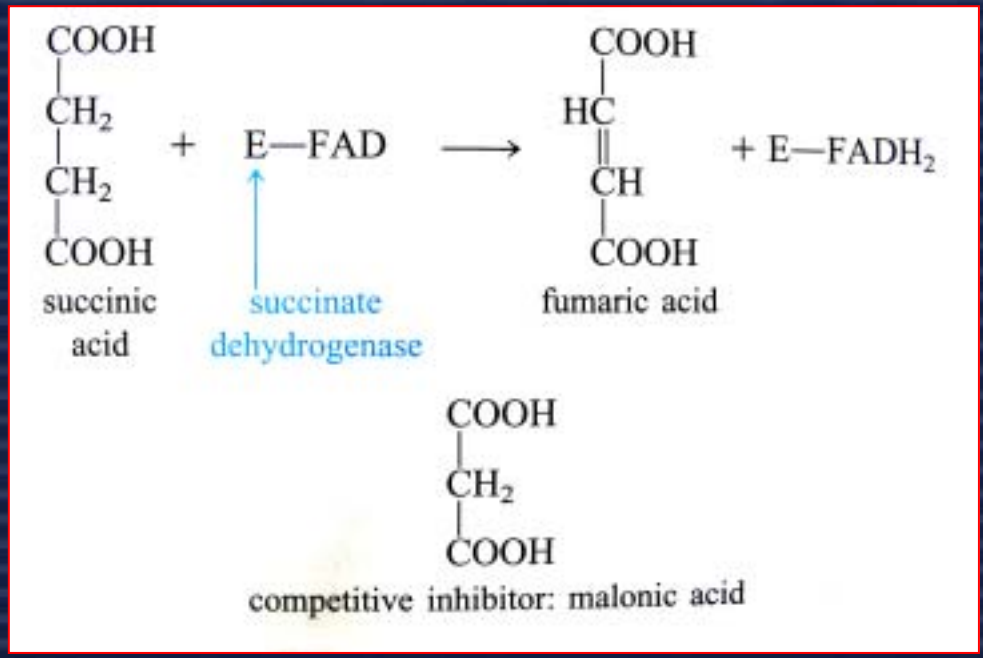
- **Reversible Inhibition**
  - **Reversible inhibition of enzyme activity occurs in two ways : noncompetitive inhibition and competitive inhibition.**



o Noncompetitive inhibition - The inhibitor combines reversibly with some portion of the enzyme (other than the active site) that is essential to enzyme function.



o Competitive inhibition - A compound with a structure very similar to the substrate competes with the substrate for the active site on the enzyme. When such inhibitors become bound to active sites, fewer enzyme molecules are available to the substrate. Enzyme activity therefore decreases.



Regulatory Enzymes

- Living systems can not only function but also control their functioning.

  The control mechanisms of the living system involve the control of enzyme activity and the control of enzyme concentrations.

Multi Enzyme Systems

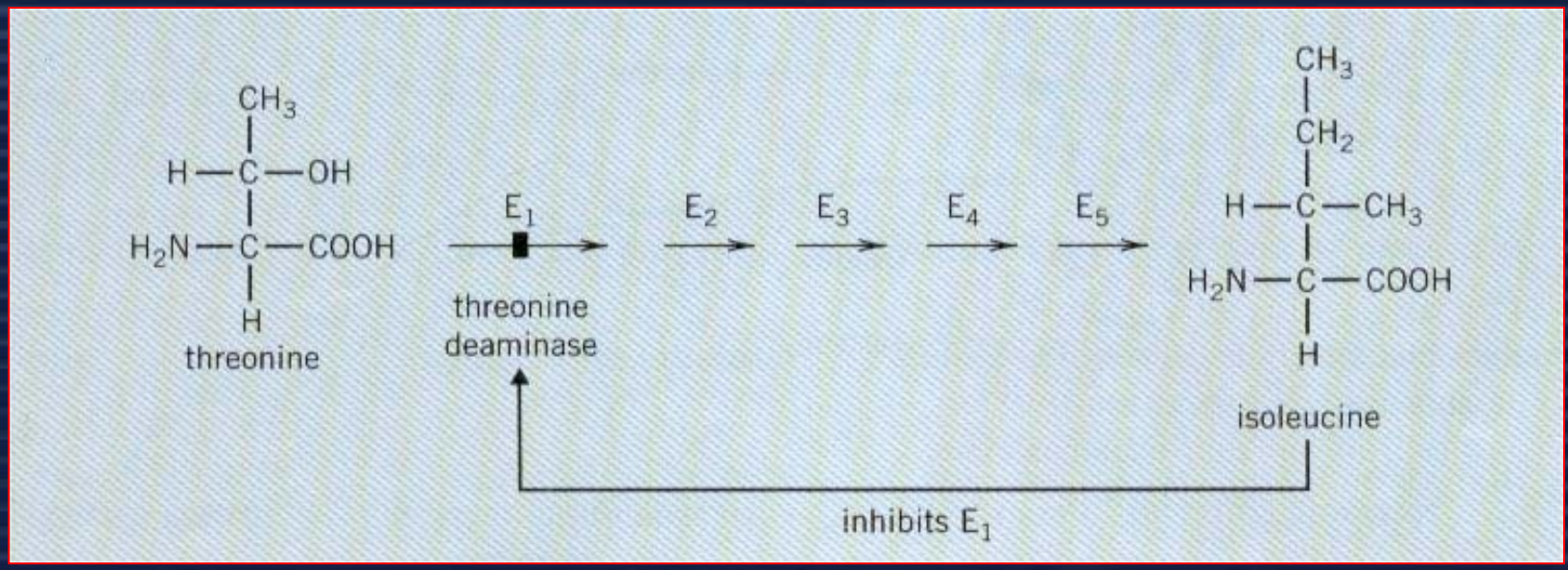
- Most biological chemical reactions occur in a sequence of reactions that eventually produces a specific metabolic end product.
- A separate enzyme catalyzes such reaction in such a sequence.

Regulatory or allosteric enzyme

- In most multienzyme systems, the enzyme that catalyzes the first reaction of the series.
- This enzyme controls the rate of the entire process.
- They are usually complex, high-molecular-weight molecules containing several polypeptide chains and cofactors.
- They usually have more than one site for the attachment of molecules (Regulatory or allosteric site) ; one or more for the substrate, and one or more for regulatory molecules.
- The regulatory molecule itself can either inhibit or increase the activity of the enzyme. Such an allosteric enzyme is flexible molecule, and the regulatory molecule causes a slight change in the shape of the enzyme, thus changing the shape of the active site and making it either more or less receptive to the substrate.

Feedback Inhibition

- A process that many regulatory enzymes are inhibited by the end product of the multienzyme system.
- E1 is the regulatory enzyme for the process, and it is inhibited by high concentrations of product G.



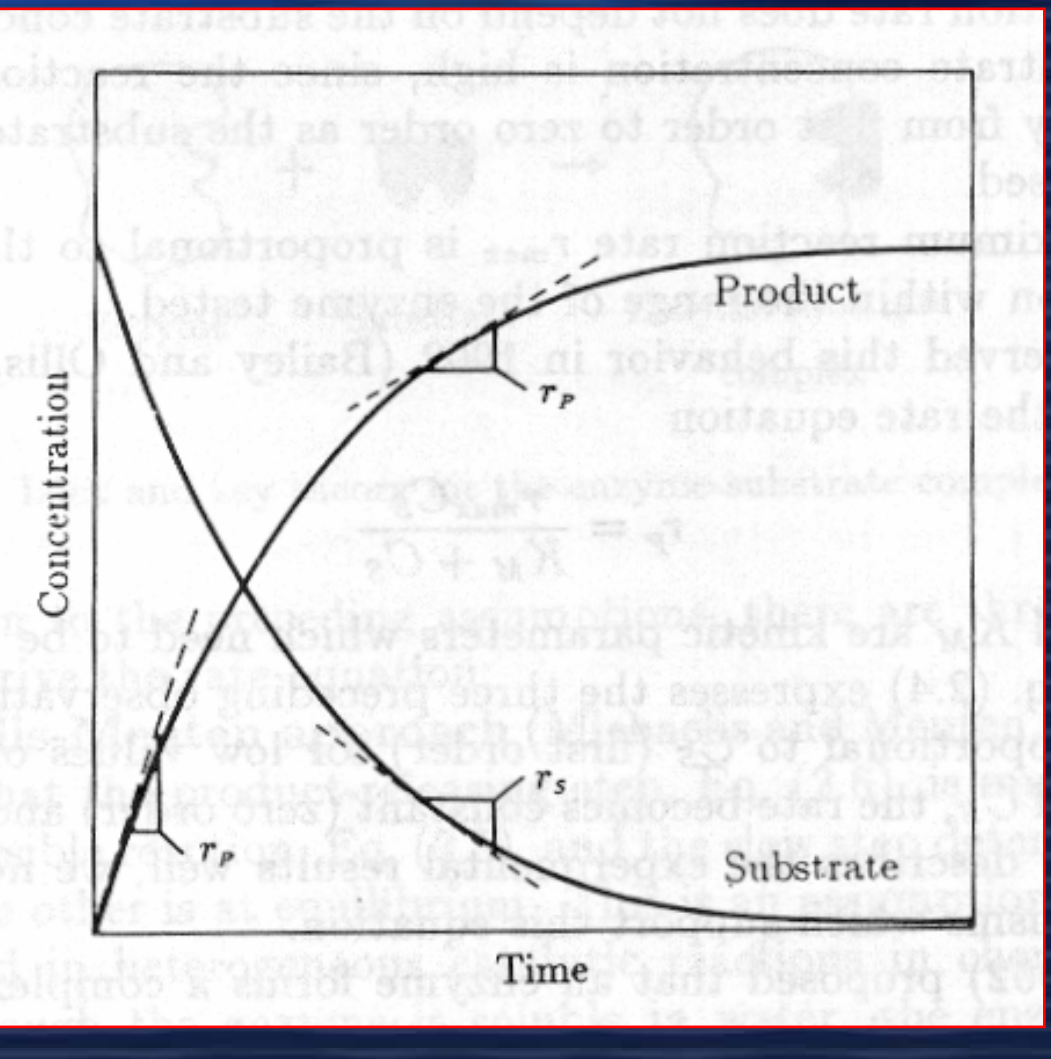
- Metabolic processes are controlled not only by the level of enzyme activity but also by the concentration of each enzyme. The enzyme concentration is determined by the rate of synthesis and the rate of breakdown of the enzyme.

Enzyme Kinetic

- **It deals with the rate of enzyme reaction and how it is affected by various chemical and physical conditions.**
- **Kinetic studies of enzymatic reactions provide information about the basic mechanism of the enzyme reaction and other parameters that characterize the properties of the enzyme.**
- **The rate equations can be applied in calculating reaction time, yields, and optimum economic condition.**

Where, S : substrate
P : product
E : Enzyme

The change of Product & Substrat Concentrations with respect to Tim

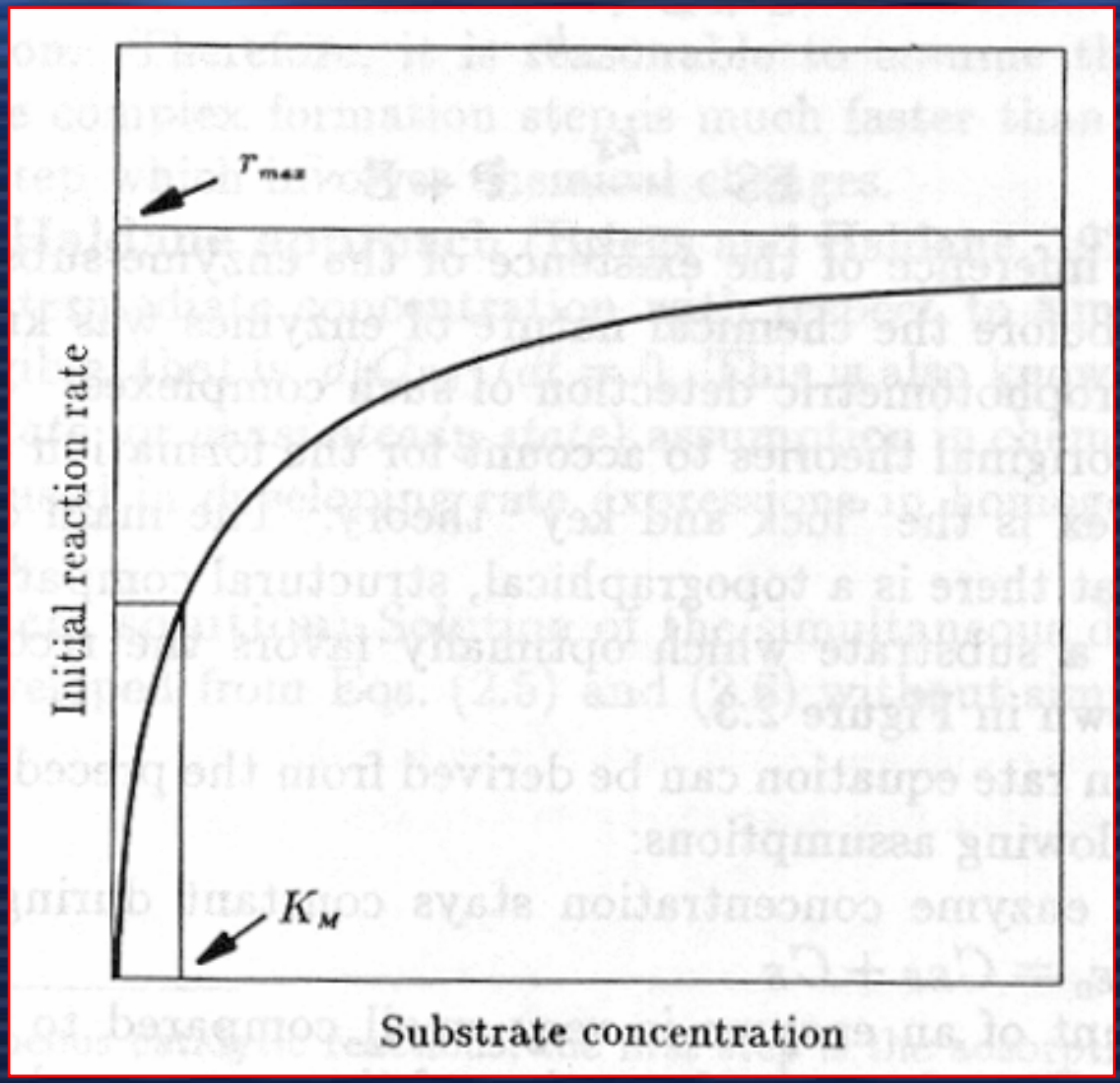


Reaction rate :

$$r_s = -\frac{dCs}{dt} \text{ or } r_p = \frac{dCp}{dt}$$

(the slopes of the two curves

The Effect of Substrate Concentration on the Initial Reaction Rat



Reaction Rate

- The reaction rate is proportional to the substrate concentration(1st order rex.) when the substrate conc. is in the low range.
- The reaction rate does not depend on the substrate conc. when the substrate conc. is high, since the reaction rate changes gradually from 1st order to zero order as the substrate conc. is increased.
- The maximum reaction rate r_{max} is proportional to the enzyme conc. within the range of the enzyme tested.

- **Henri(1902) proposed the rate equation,**

$$r_s = \frac{r_{\max} Cs}{K_M + Cs}$$

r_{max} and K_M can be experimentally determined.

- **Brown(1902) proposed that an enzyme forms a complex with its substrate.**

$$S + E \underset{k2}{\overset{k1}{\rightleftharpoons}} ES \qquad \text{---(1)}$$

$$ES \xrightarrow{k3} P + E \qquad \text{---(2)}$$

One of the original theories to account for the formation of the enzyme-substrate complex is the “Lock and Key” theory.

The Reaction Rate Equation

- The reaction rate equation can be derived from the prededing mechanism based on the following assumptions:

1. The total enzyme conc. stays constant during the reaction.

$$C_{E0} = C_{ES} + C_E$$

2. The amount of an enzyme is very small compared to the amount of substrate. Therefore, the formation of the enzyme-substrate complex does not significantly deplete the substrate.

$$C_S \gg C_E$$

3. The product conc. is so low that product inhibition may be considered negligible.