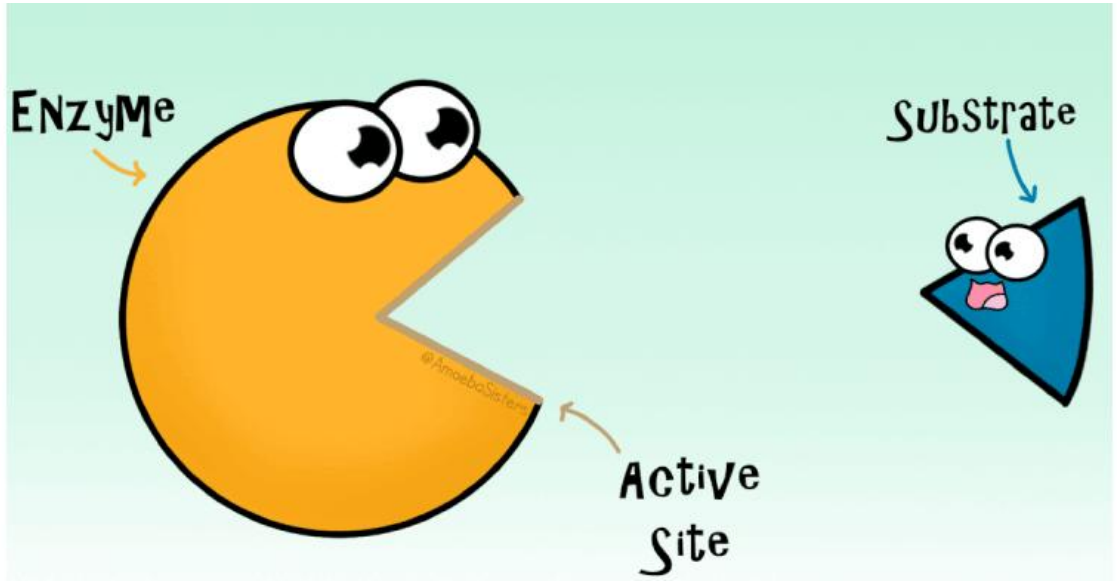
ENZYMES



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2/1/2020

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Enzymes

DEFINITION:

- **Enzymes** are biocatalyst which accelerate rate of chemical reactions but don't change equilibrium.
- The substance upon which an enzyme acts, is called the **substrate**.
- The enzyme will convert the substrate into the **product** or products.

Characteristics of Enzymes

- Almost all enzymes are proteins.
- Enzymes follow the physical and chemical reactions of proteins.
- They are heat labile.
- They are water-soluble.
- They can be precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid).
- They contain 16% weight as nitrogen.

Classification of enzymes

Class **1.** **Oxidoreductases:** Transfer of hydrogen or addition of oxygen; e.g. Lactate dehydrogenase (NAD); Glucose-6-phosphate dehydrogenase (NADP); Succinate dehydrogenase (FAD); di-oxygenases.

Class **2.** **Transferases**: Transfer of groups other than hydrogen. Example, Aminotransferase. (Subclass: Kinase, transfer of phosphoryl group from ATP; e.g. Hexokinase)

Class **3.** **Hydrolases**: Cleave bond and add water; e.g. Acetyl choline esterase; Trypsin

Class **4.** **Lyases**: Cleave without adding water, e.g. Aldolase; HMG CoA lyase; ATP Citrate lyase. (Subclass: Hydratase;add water to a double bond)

Class **5.** **Isomerases**: Intramolecular transfers. They include racemases and epimerases. Example, Triose phosphate isomerase.

Class **6.** **Ligases**: ATP dependent condensation of two molecules, e.g. Acetyl CoA carboxylase; Glutamine synthetase; PRPP synthetase

Co-enzyme

- Enzymes may be simple proteins, or complex enzymes, containing a non-protein part, called the **prosthetic group.**
- The prosthetic group is called the **co-enzyme.**
- The protein part of the enzyme is then named the **apo-enzyme.**
- These two portions combined together is called the **holo-enzyme**

Co- enzymes

- Co-enzymes may be divided into **two groups**

1. Those taking part in reactions catalyzed by **oxidoreductases** by donating or accepting hydrogen atoms or electrons.

2. Those co-enzymes taking part in reactions transferring groups **other than hydrogen**

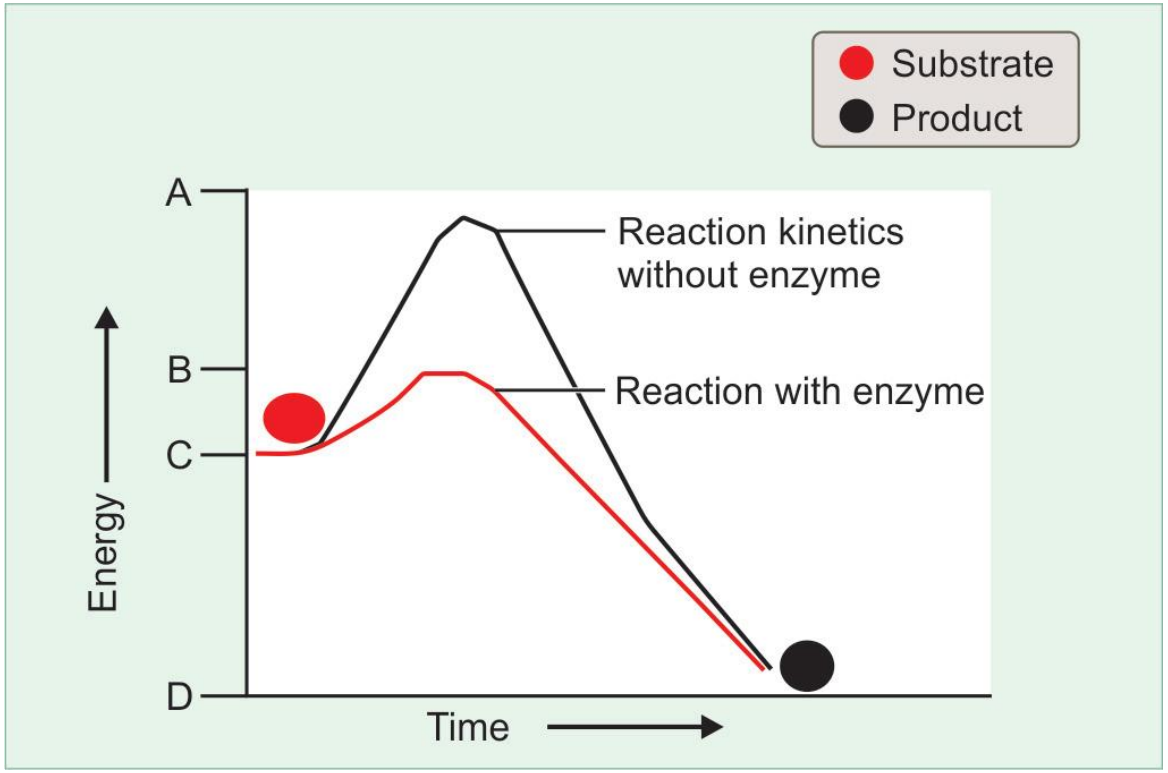
Examples of co-enzymes

Co-enzyme	Group transferred
Thiamine pyrophosphate (TPP)	Hydroxy ethyl
Pyridoxal phosphate (PLP)	Amino group
Biotin	Carbon dioxide
Coenzyme-A (Co-A)	Acyl groups
Tetra hydrofolate (FH_4)	One carbon groups
Adenosine triphosphate (ATP)	Phosphate

Theories Explaining the Mode of Action of Enzymes

1. Lowering of activation energy
2. Acid base catalysis
3. Substrate strain
4. Covalent catalysis
5. Entropy effect
6. Product substrate orientation theory
7. Michaelis-Menten theory
8. Fischer’s template theory
9. Koshland’s induced fit theory

Lowering of activation energy



Lowering of activation energy by enzymes. Red circle = substrate; D = energy level of product. C to A = activation energy in the absence of enzyme; C to B is activation energy in presence of enzyme; B to A = lowering of activation energy by enzyme.

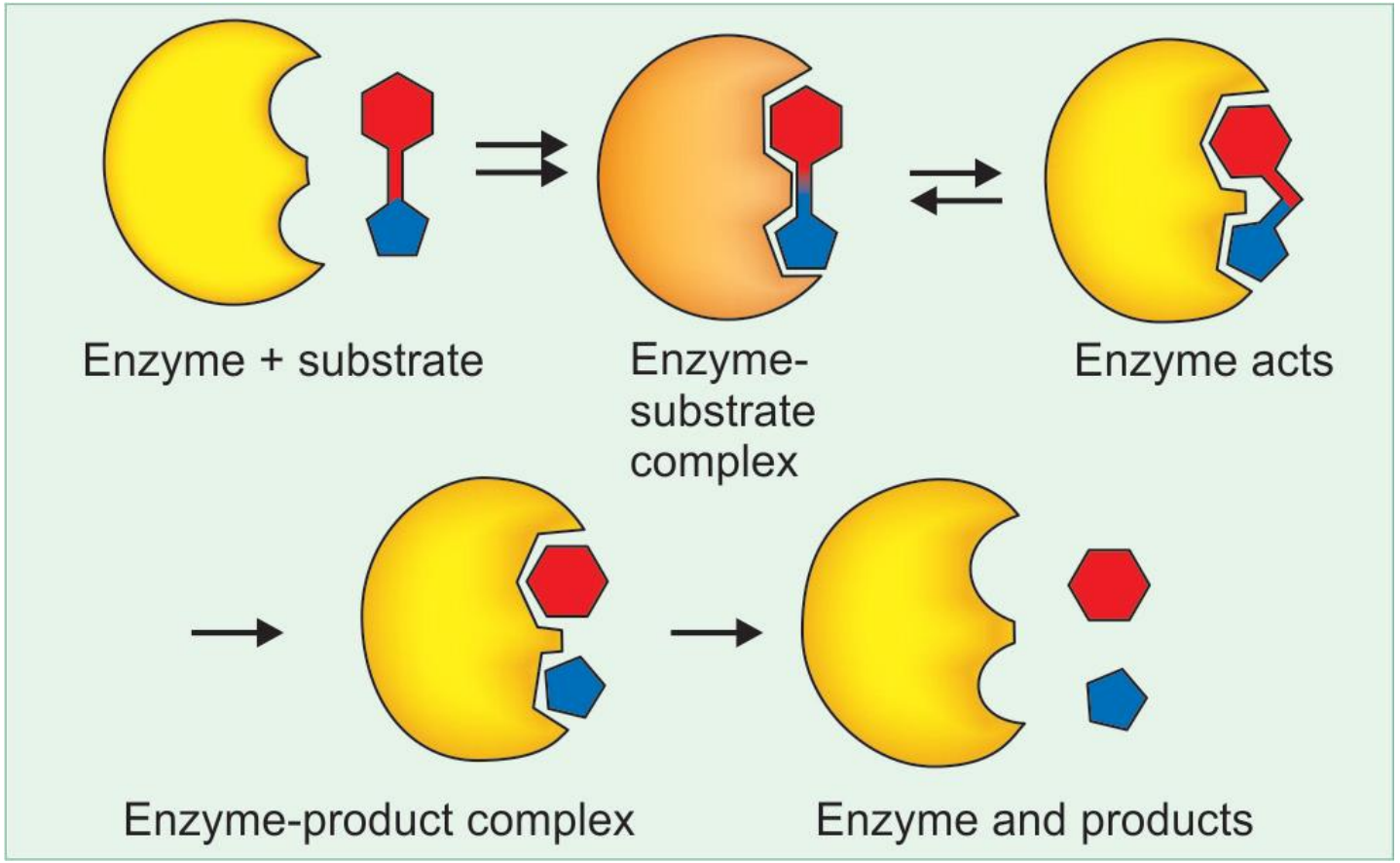
Lowering activation energy

- Enzymes lower the energy of activation.
- **Activation energy** is defined as the energy required to convert all molecules of a reacting substance from the ground state to the transition state.
- Substrates are remaining in an **energy trough**, and are to be placed at a higher energy level, whereupon spontaneous degradation can occur.
- For example, activation energy for acid hydrolysis of sucrose is 26,000 cal/mol,
- while the activation energy is only 9,000 cal/mol when hydrolysed by sucrase.

MICHAELIS–MENTEN THEORY

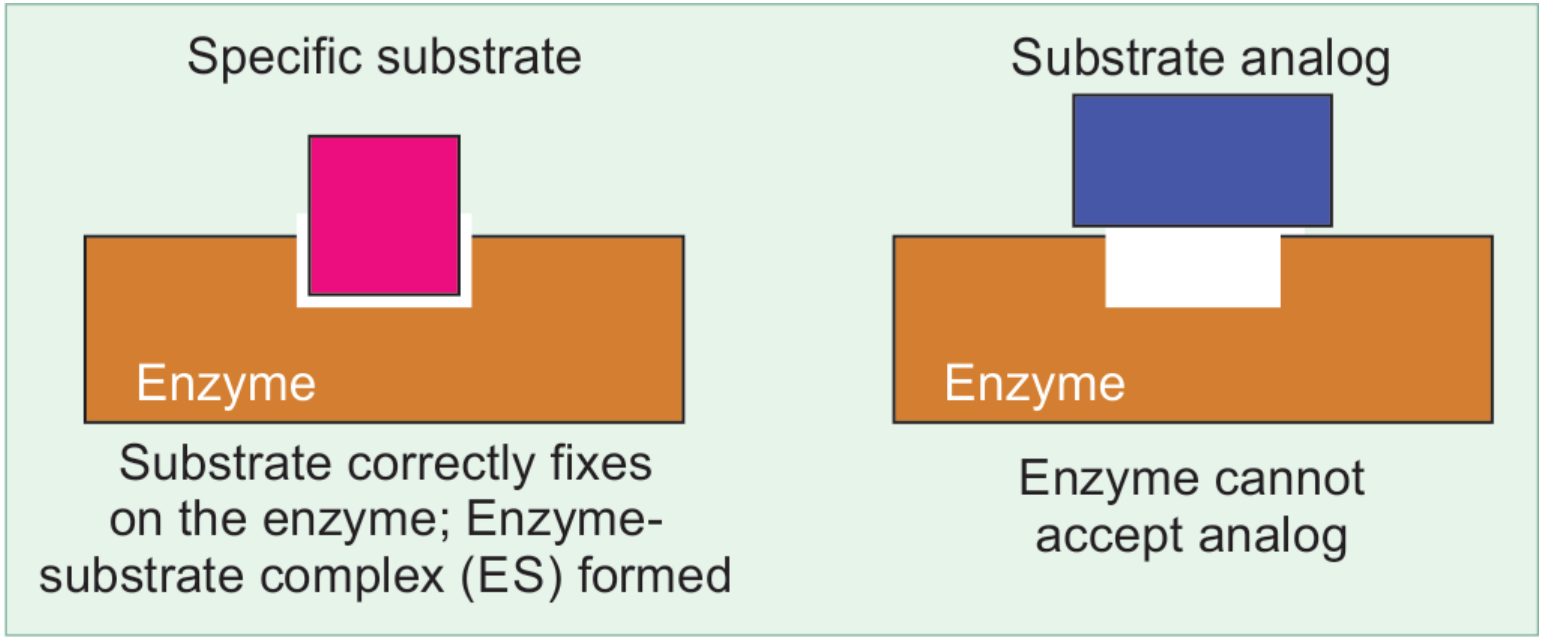
- In 1913, Michaelis and Menten put forward the **Enzyme–Substrate complex theory**.
- Accordingly, the enzyme (E) combines with the substrate (S), to form an enzyme-substrate (ES) complex, which immediately breaks down

  to the enzyme and the product (P)
- E + S E–S Complex E + P



Enzyme substrate complex

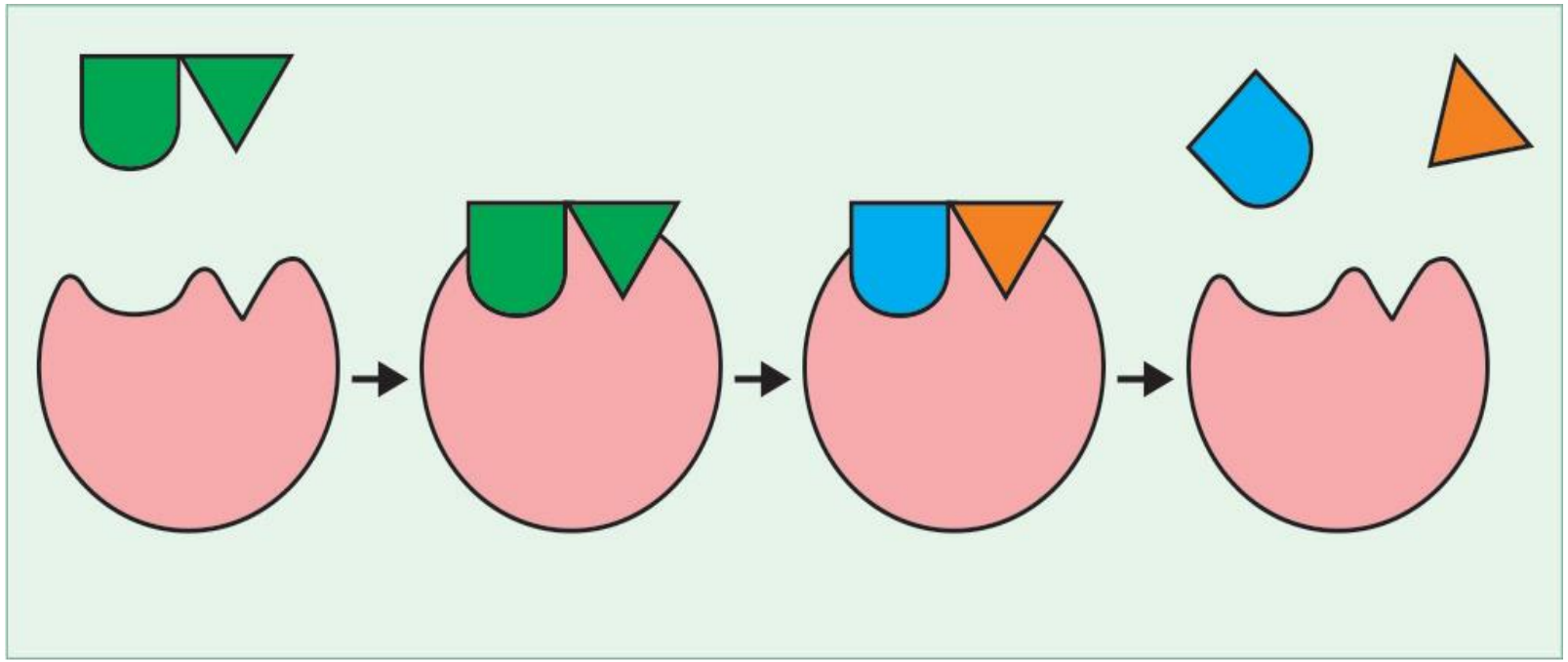
Fischer's Template Theory



Fischer's Template Theory

This theory states that the three dimensional structure of the active site of the enzyme is complementary to the substrate. Thus **enzyme and substrate fit each other**. Substrate fits on the enzyme, similar to **lock and key**. The lock can be opened only by its specific key. However, Fischer envisaged a rigid structure for enzymes, which could not explain the flexibility shown by enzymes.

Koshland’s induced fit theory



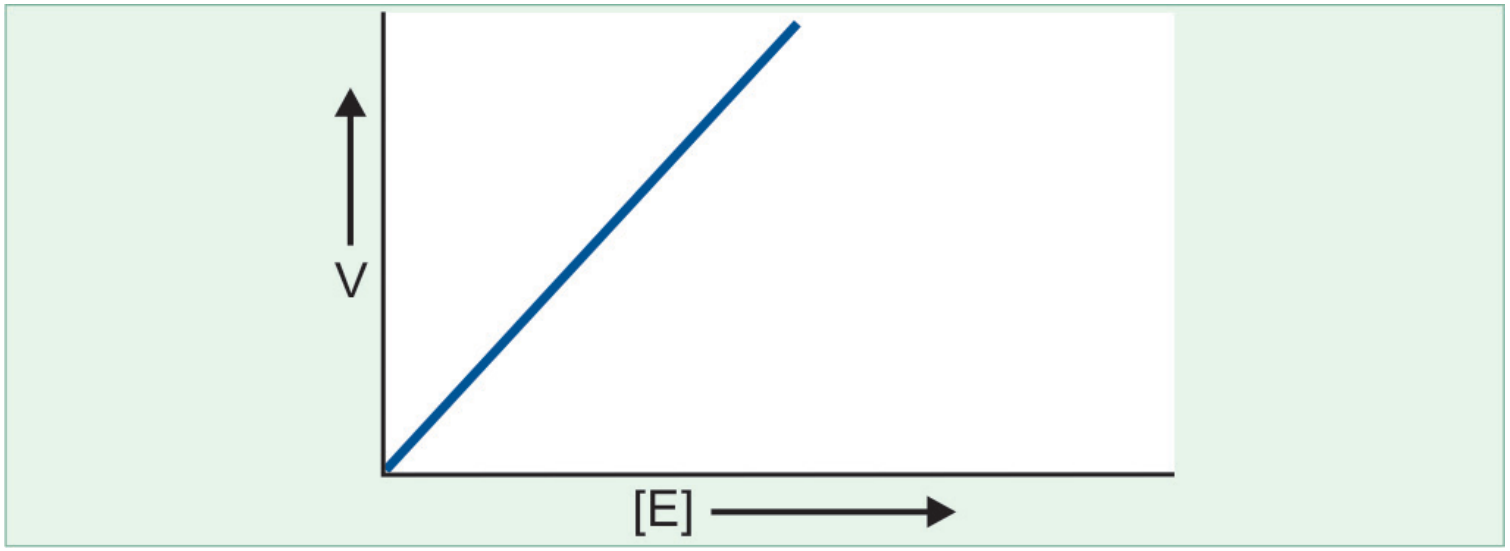
Koshland’s induced fit theory.

(A) Enzyme has shallow grooves; substrate alignment is not correct.
(B) Fixing of substrate induces structural changes in enzyme.
(C) Now substrate correctly fits into the active site of enzyme.
(D) Substrate is cleaved into two products

Factors Affecting Enzyme Activity

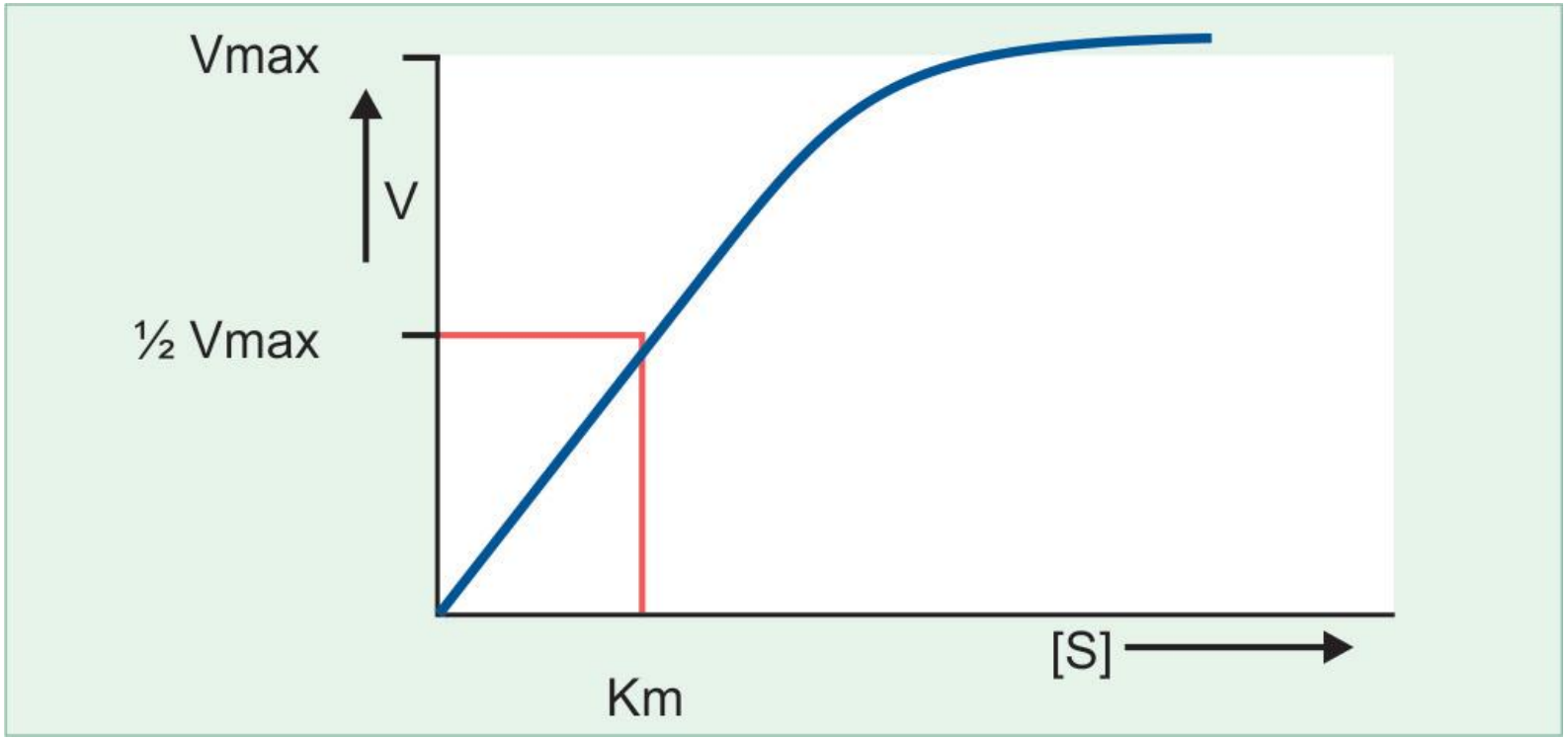
1. Enzyme concentration
2. Substrate concentration
3. Product concentration
4. Temperature
5. Hydrogen ion concentration (pH)
6. Presence of activators
7. Presence of inhibitors
8. Presence of repressor or derepressor
9. Covalent modification

Effect of enzyme concentration



Rate of a reaction or velocity (V) is directly proportional to the enzyme concentration, when sufficient substrate is present. Velocity of reaction increases proportionately with the concentration of enzyme, provided substrate concentration is unlimited.

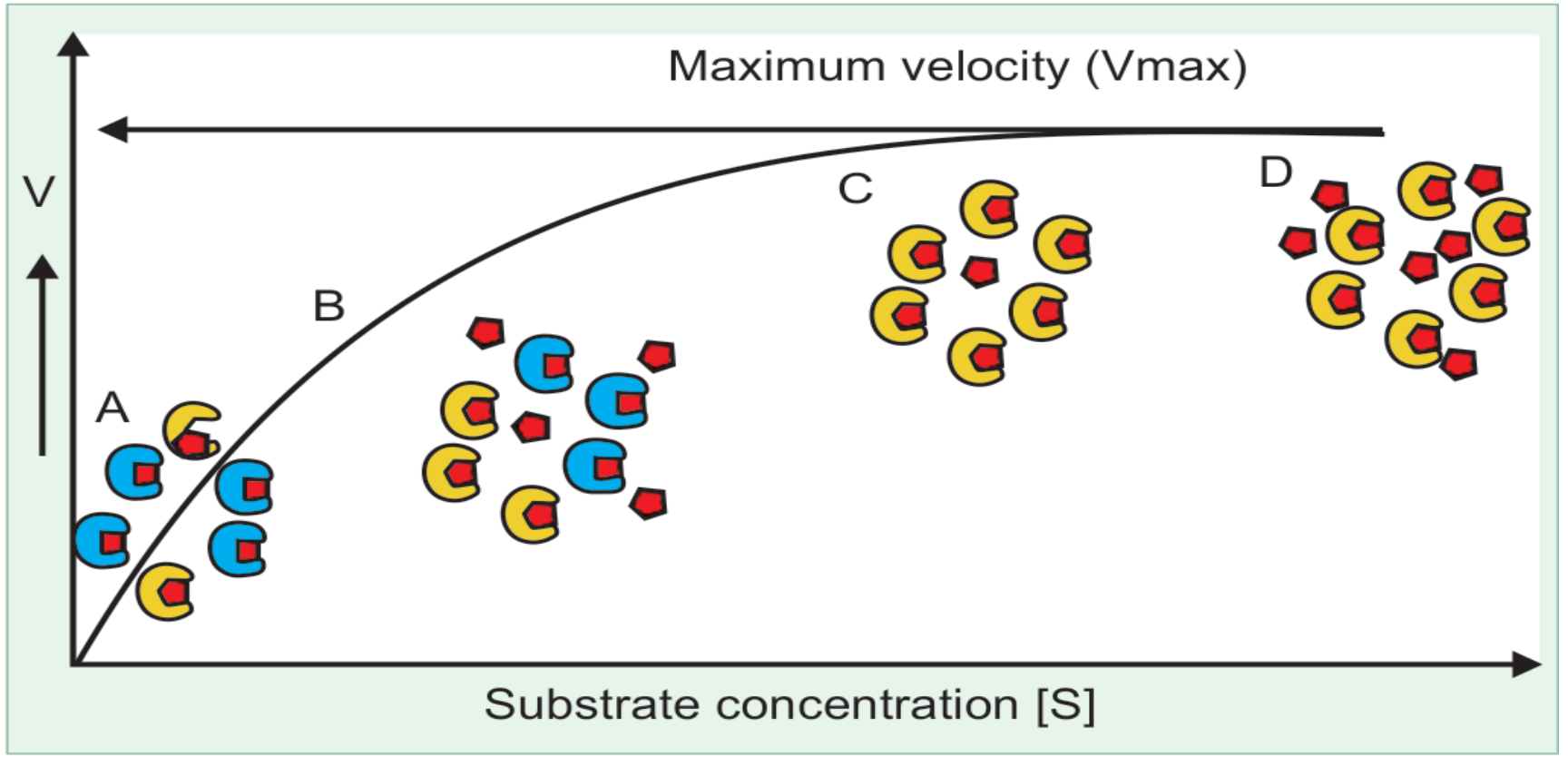
Effect of substrate concentration



Effect of substrate concentration (substrate saturation curve).

As substrate concentration is increased, the velocity is also correspondingly increased in the initial phases; but the curve flattens afterwards.

Effect of substrate concentration on enzyme activity.



Enzyme molecular are shown as half-circles. Substrate molecules are red dots.

(A) Substrate molecules are low; so only a few enzyme molecules are working and velocity is less.

(B) At half-maximal velocity (Km). 50% enzyme molecules are bound with substrate.

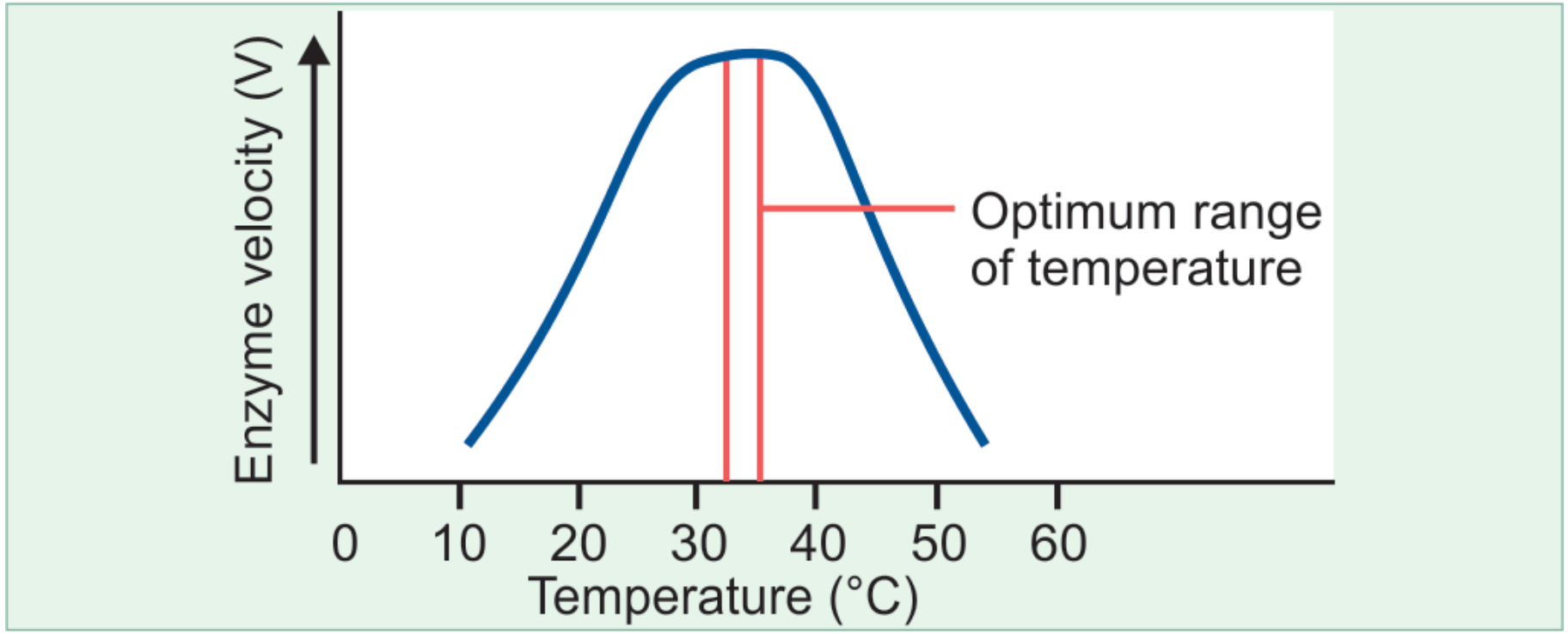
(C) As a lot of substrate molecules are available, all enzyme molecules are bound.

(D) Further increases in the substrate will not increase the velocity further.

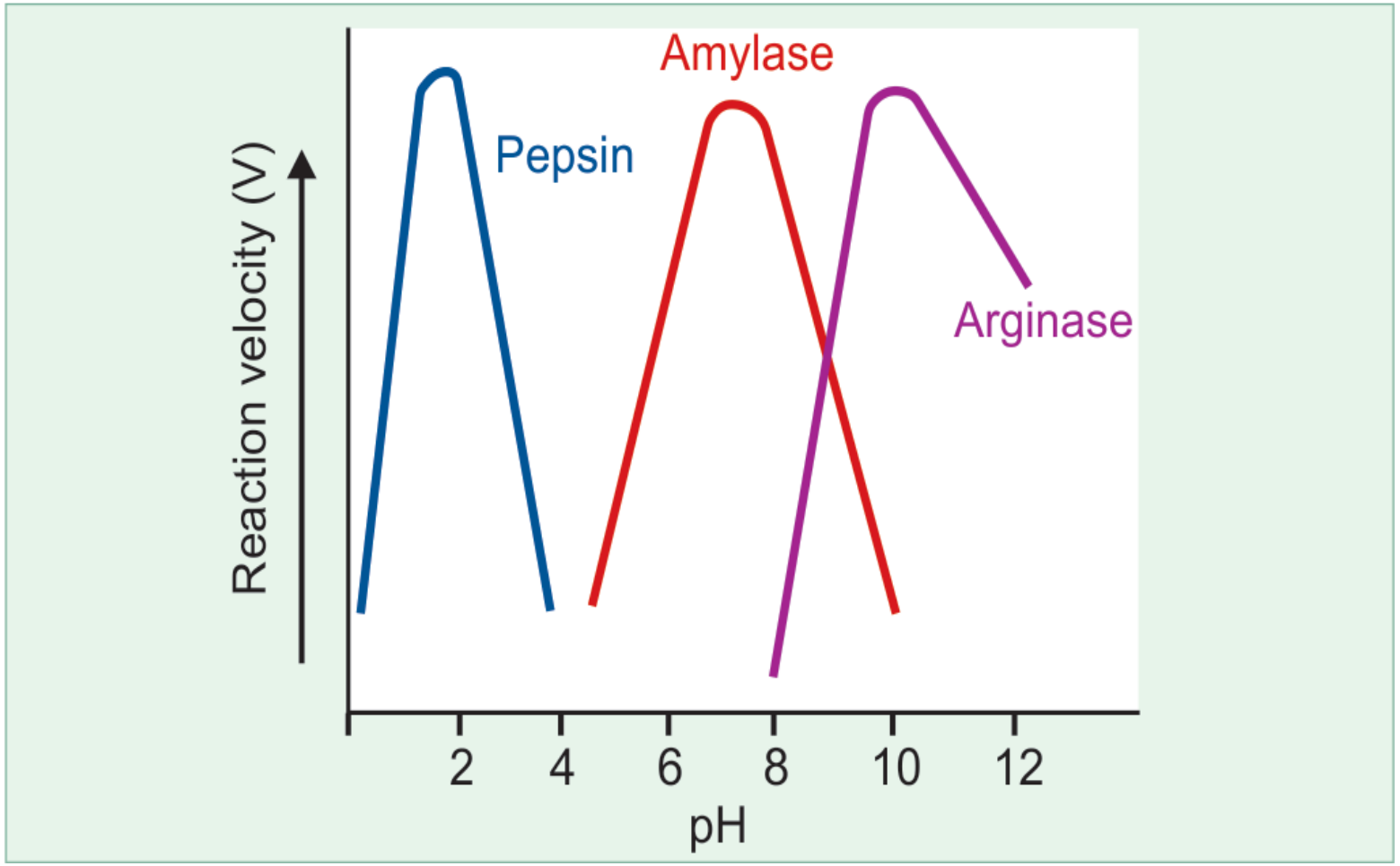
Salient Features of Km

1. **Km value is substrate concentration** (expressed in moles/ L) **at half-maximal velocity.**
2. It denotes that **50% of enzyme molecules are bound with substrate molecules** at that particular substrate concentration.
3. **Km is independent of enzyme concentration.** If enzyme concentration is doubled, the Vmax will be double. But the Km will remain exactly same. In other words, irrespective of enzyme concentration, 50% molecules are bound to substrate at that particular substrate concentration.
4. Km is the signature of the enzyme. Km value is thus a constant for an enzyme. It is the **characteristic feature of a particular enzyme** for a specific substrate.
5. **Km denotes the affinity of enzyme for substrate.** The lesser the numerical value of Km, the affinity of the enzyme for the substrate is more

Effect of temperature on velocity.



Effect of pH on enzyme velocity.



Enzyme inhibition

Competitive :

- Inhibitor molecules are competing with the normal substrate molecules for binding to the active site of the enzyme, because the inhibitor is a structural analog of the substrate

Non competitive

- There is no competition between substrate and inhibitor. The inhibitor usually binds to a different domain on the enzyme, other than the substrate binding site.

Comparison of Two Types of Enzyme Inhibition

	Competitive inhibition	*Non-competitive inhibition*
Acting on	Active site	May or may not
Structure of inhibitor	Substrate analog	Unrelated molecule
Inhibition is	Reversible	Generally irreversible
Excess substrate	Inhibition relieved	No effect
Km	Increased	No change
Vmax	No change	Decreased
Significance	Drug action	Toxicological

Clinical importance of competitive inhibition

1. **Sulfonamides**: They are commonly employed antibacterial agents. Bacteria synthesize folic acid by combining PABA with pteroyl glutamic acid. Sulfa drugs, being structural analog of PABA, will inhibit the folic acid synthesis in bacteria, and they die.

2. Methotrexate is a structural analogue of folic acid, and so can competitively inhibit folate reductase enzyme. Therefore, methotrexate is used as an anticancer drug.

3. Dicoumarol: It is structurally similar to vitamin K and can act as an anticoagulant by competitively inhibiting the vitamin K activity.

4. **Isonicotinic acid hydrazide (INH)** is an antituberculous drug. It is structurally similar to pyridoxal, and prolonged use of INH may cause pyridoxal deficiency and peripheral neuropathy.

Noncompetitive Inhibition (Irreversible)

Examples are:

(a) **Cyanide** inhibits cytochrome oxidase.

(b) **Fluoride** will remove magnesium and manganese ions and so will inhibit the enzyme, enolase, and consequently the glycolysis.

(c) **Iodoacetate** would inhibit enzymes having -SH group in their active centers.

(d) **BAL** (British Anti Lewisite; dimercaprol) is used as an antidote for heavy metal poisoning.

Isoenzymes

They are **physically distinct forms of the same enzyme activity**. Different molecular forms of the same enzyme synthesized from various tissues are called isoenzymes.

Hence study of isoenzymes is very useful to understand diseases of different organs.

If the subunits are all the same, the protein is a **homomultimer** represented by a single gene.

If the subunits are different, the protein is said to be a **heteromultimer**, produced by different genes.

Isoenzymes may be Formed in Different Ways

They may be products of different genes (more than one locus) in which case they are known as **true isoenzymes**.
The genes may be located on different chromosomes, e.g. salivary and pancreatic amylase.

In certain cases, all the different forms are present in the same individual, e.g. **lactate dehydrogenase** (LDH) has 5 isoenzymes and all are seen in all persons in the population.

Isoenzymes of LDH

Lactate dehydrogenase (LDH) enzyme is a tetramer with 4 subunits. But the subunit may be either H (heart) or M (muscle) polypeptide chains.

So 5 combinations of H and M chains are possible. These combinations are H4, H3M1, H2M2, H1M3 and M4 varieties. All these 5 forms are seen in all persons.

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Clinical Enzymology

Plasma contains many **functional enzymes,** which are actively secreted into plasma. For example, enzymes of blood coagulation.

On the other hand, there are a few **non functional enzymes** in plasma, which are coming out from cells of various tissues due to normal wear and tear. Their normal levels in blood are very low; but are drastically increased during cell death (necrosis) or disease. Therefore, assays of these enzymes are very useful in **diagnosis of Diseases.**

Enzyme Patterns (Enzyme Profiles) in Diseases

I. Hepatic diseases

1. Alanine aminotransferase (ALT): Marked increase in parenchymal liver diseases
2. Aspartate aminotransferase (AST): Elevated in parenchymal liver disease
3. Alkaline phosphatase (ALP): Marked increase in obstructive liver disease
4. Gamma glutamyl transferase (GGT): Increase in obstructive and alcoholic liver

II. Myocardial infarction

1. Cardiac troponins (CTnT and CTnI). (These are not enzymes, but are specific and sensitive and elevated very early in MI).
2. Creatine kinase (CK-MB): CK-MB isoenzyme is specific

- III. Muscle diseases

1. Creatine kinase (CK-MM): Marked increase in muscle diseases.
2. Aspartate aminotransferase (AST): Increase in muscle disease; not specific
3. Aldolase (ALD): Earliest enzyme to rise, but not specific

- IV. Bone diseases

1. Alkaline phosphatase (ALP) Marked elevation in rickets and Paget's disease

- V. Prostate cancer

1. Prostate specific antigen (PSA): Marker for prostate cancer. Mild increase in benign prostate enlargement
2. Acid phosphatase (ACP): Marker for prostate cancer. Metastatic bone disease especially from a primary form prostate. Inhibited by L tartrate.

VI. Pancreatic disease

1. Amylase: Marker for acute pancreatitis and inflammation of salivary glands
2. Lipase: Marker of pancreatitis, more specific than amylase

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Enzymes as Therapeutic Agents

- **Streptokinase** (from Streptococcus) or **Urokinase** (from urine) can lyse intravascular clots and are therefore used in myocardial infarction.

- **Pepsin** and **trypsin** are given to patients with defective digestion.

- **Asparaginase** is used as an anticancer drug.


Thank You