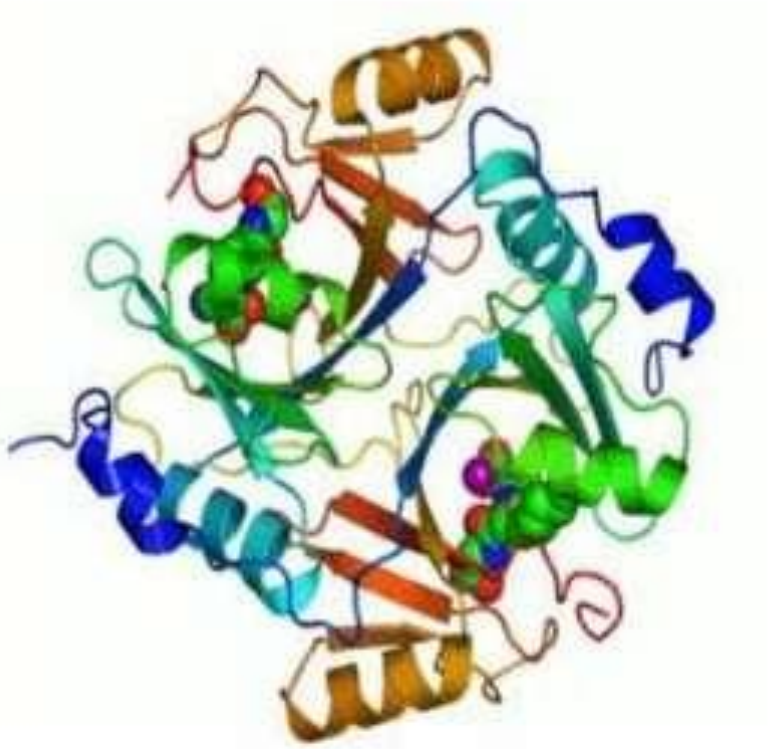


ENZYME'S ACTIVITY

Mr. OMAR ALAJIL (أ.عمر العجيل)
M.Sc Food Technology

IMPORTANCE OF ENZYME'S ACTIVITY

- **In human body**
  the enzymes involved in all essential biological reaction for life such as DNA replication and transcription, protein synthesis, metabolism and signal transduction

- **In industry**
  enzymes play role in industrial products and processes, for example, within the detergent, textile and starch industries.

- **In food processing and storage**
  for producing some foods like cheese, and also some enzyme cause deteriorative actions and enzymic browning

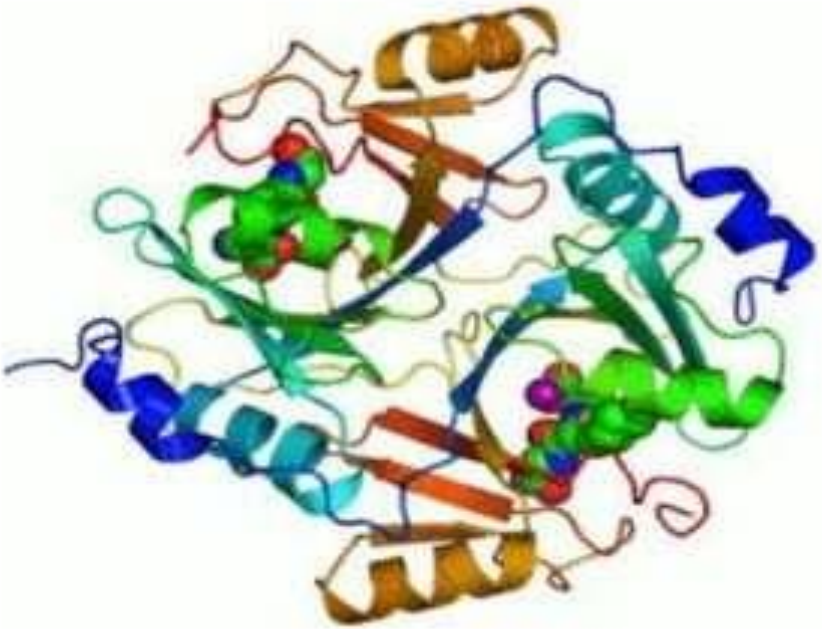
TABLE OF CONTENT

What is enzymes

- Enzymes are natural catalysts (biological catalysts) in the form of proteins.
- They are produced by living organisms to increase the rate of an immense and diverse set of chemical reactions required for life.
- They are involved in all processes essential for life such as DNA replication and transcription, protein synthesis, metabolism and signal transduction, etc.
- And their ability to perform very specific chemical transformations has made them increasingly useful in industrial processes.

- What the meaning of catalyst

- A Catalyst is defined as "a substance that increases the rate of a chemical reaction without being itself changed in the process."



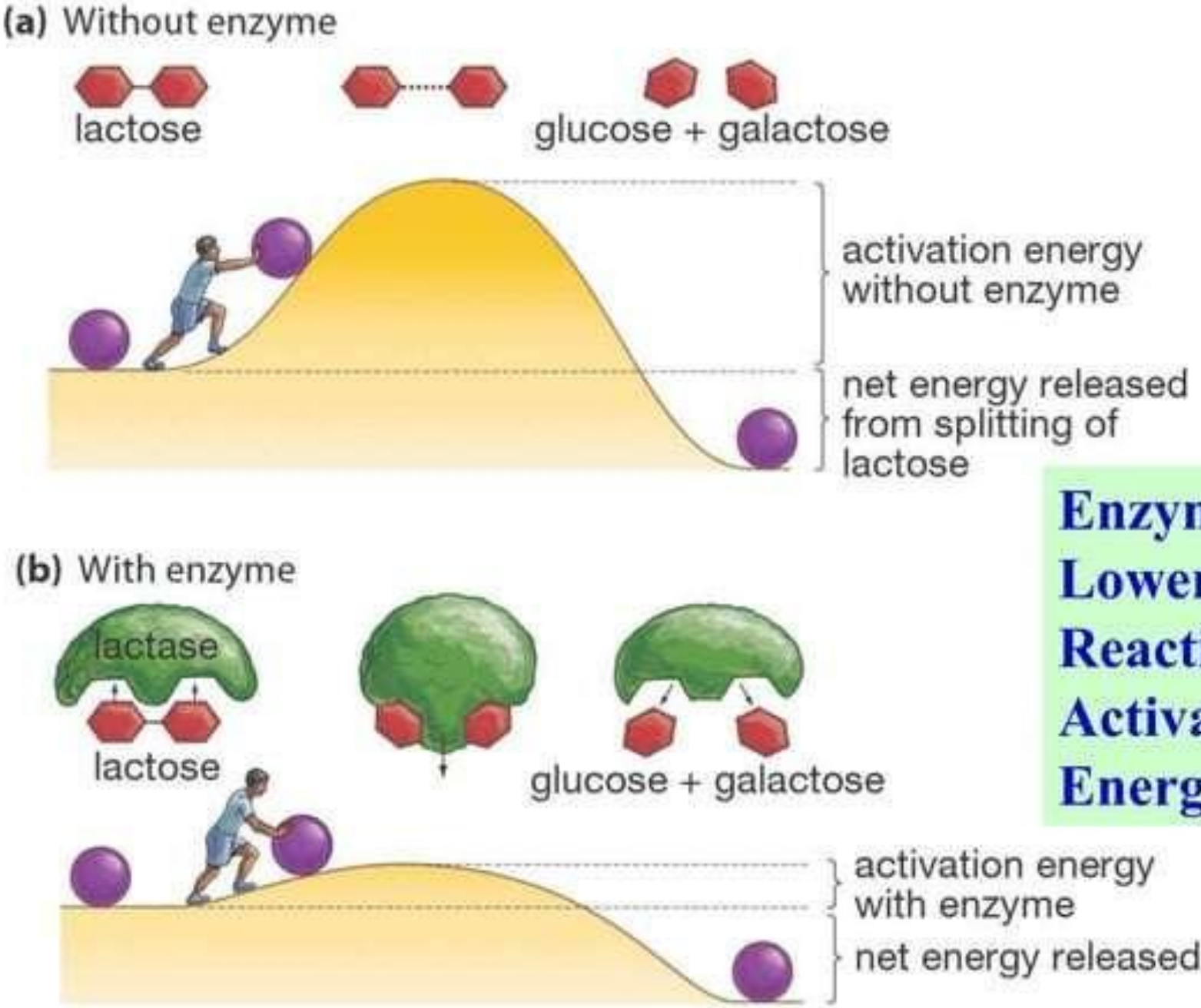
PROPERTIES OF ENZYMES

- **Catalytic efficiency – high efficiency, 10^3 to 10^{17} faster than the corresponding uncatalyzed reactions**

- **Specificity - high specificity, interacting with one or a few specific substrates and catalyzing only one type of chemical reaction.**

- **Mild reaction conditions- 37°C, physiological pH, ambient atmospheric pressure**

How can an enzyme increase reaction rates?



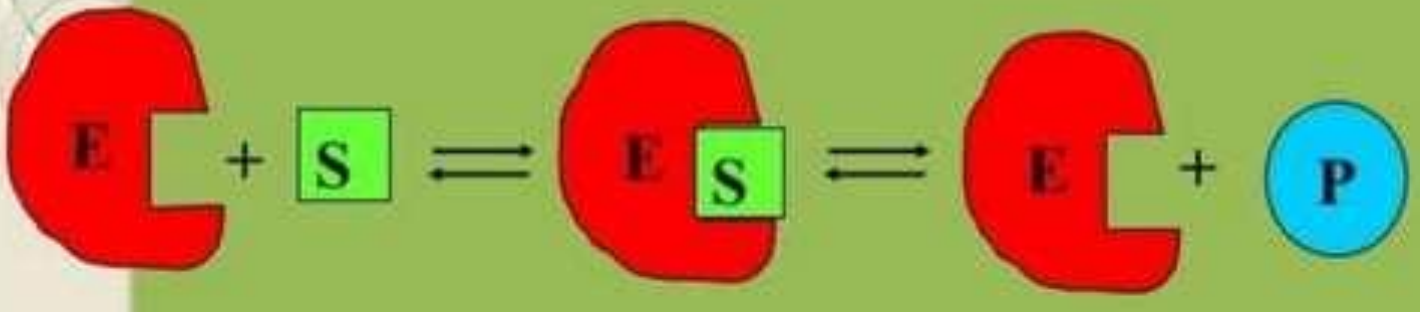
Enzymes Lower a Reaction's Activation Energy

How can an enzyme reduce the activation energy?

- In enzymatic reactions, binding groups and catalytic centers ("active sites") in enzyme molecules bind substrate molecules to form intermediate complexes with lower energy contents than those of the transition states of the uncatalyzed reactions.

- These complexes undergo certain atomic and electronic rearrangements, after which the products are released

- Thus,the enzymes work by providing alternative reaction pathways with lower activation energies than those of the uncatalyzed reaction



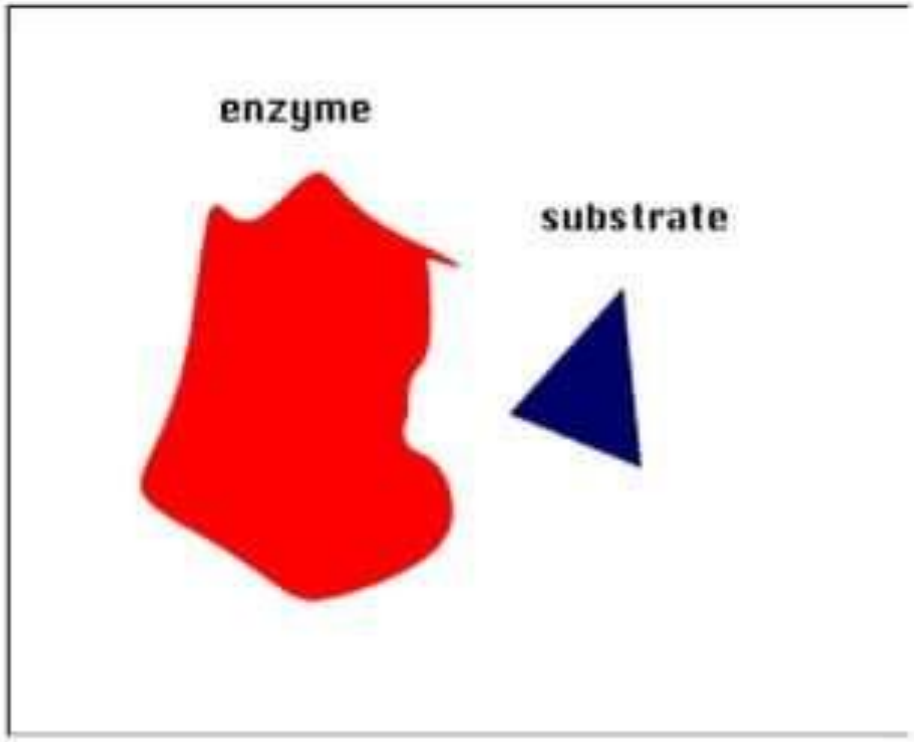
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

How can an enzyme reduce the activation energy?

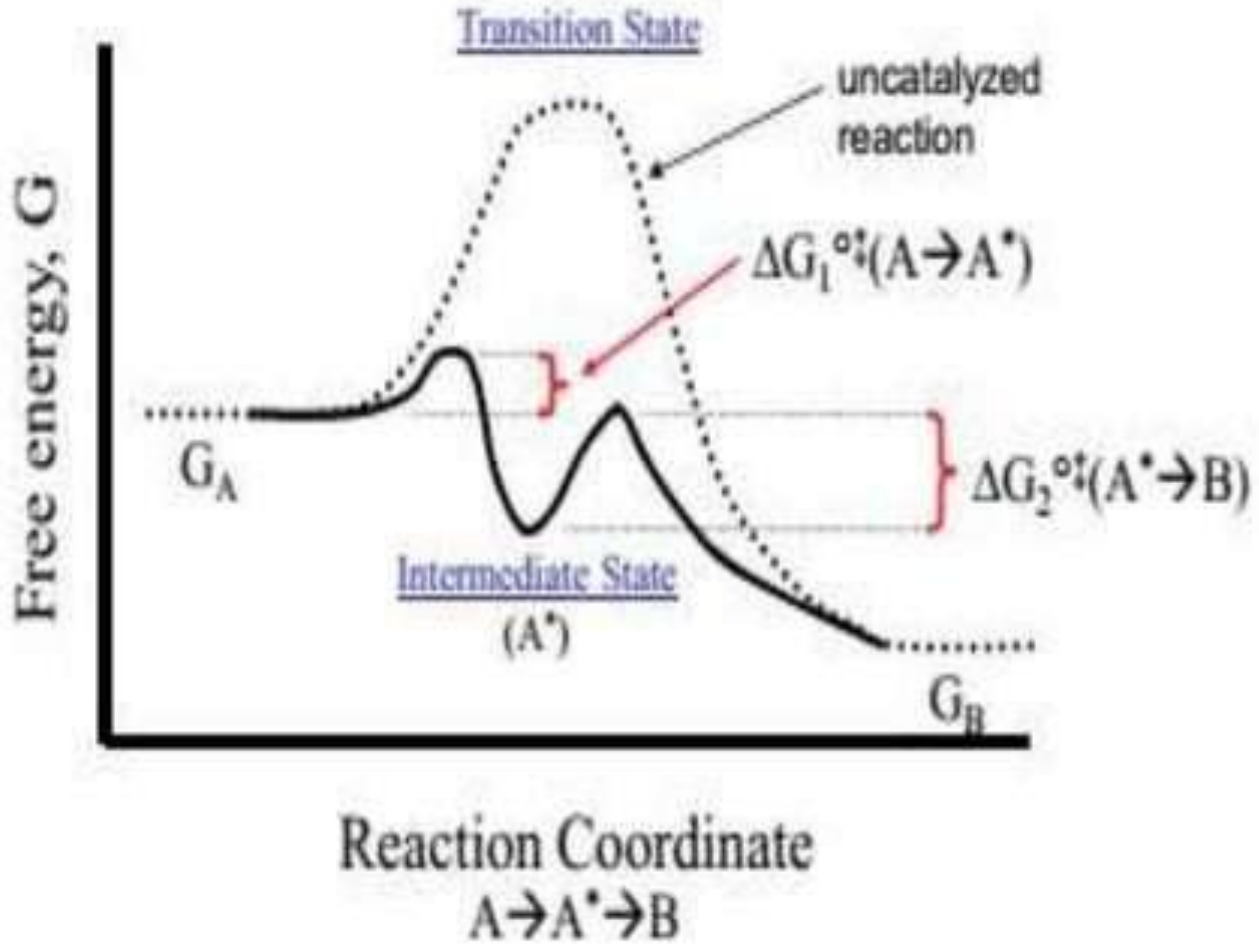
(1) Binding to the substrate in active site

(2) Orientation and positioning of substrate(s)

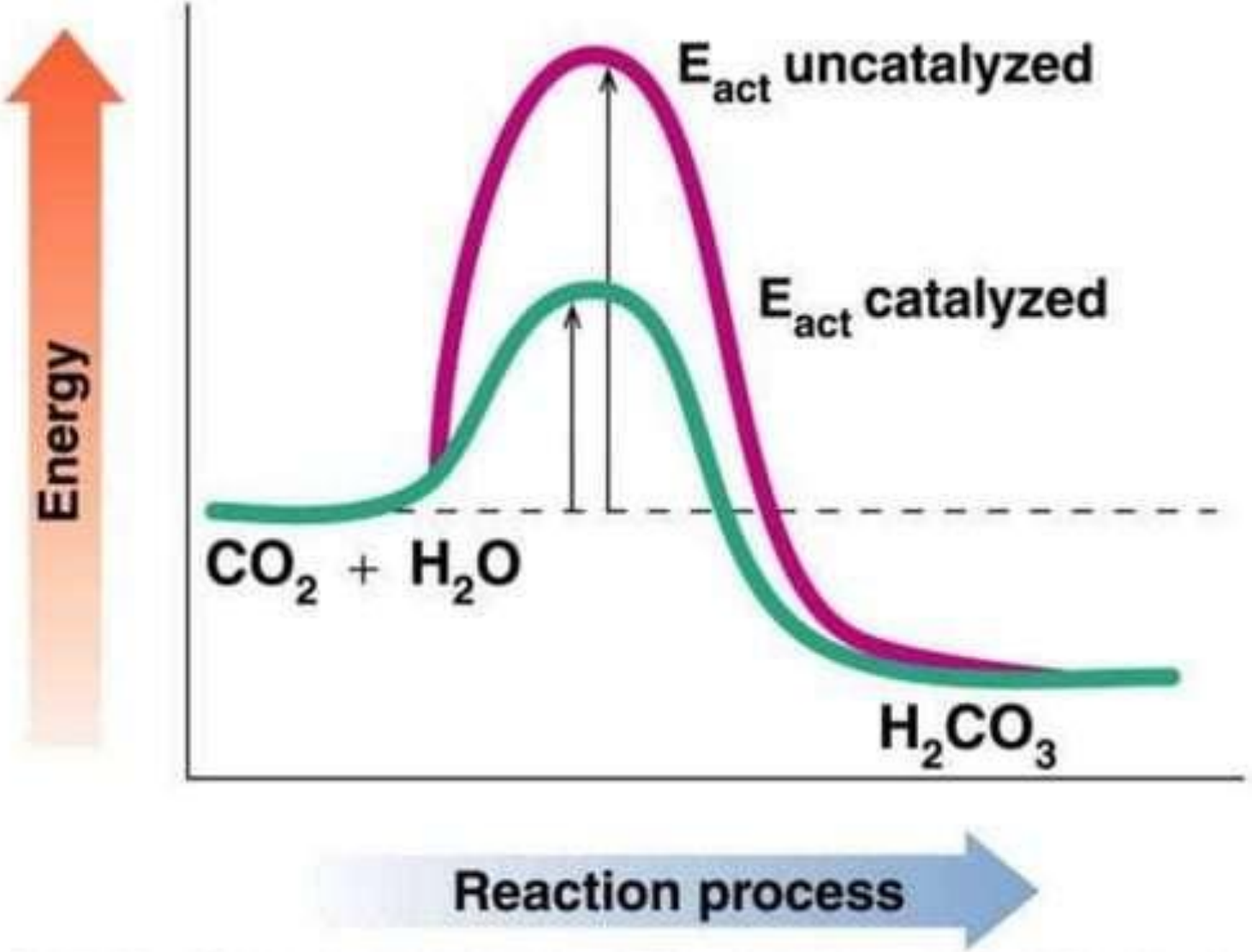
(3) Bonds in the substrate



ENZYME ROLE IN REACTION ENERGY REDUCTION



Enzymes as Biological Catalysts



EXAMPLE

- Carbonic anhydrase, which catalyzes the hydration of carbon dioxide to speed up its transfer in aqueous environments like the **blood**, is one of the fastest enzymes known.
- Each molecule of the enzyme can hydrate 100,000 molecules of carbon dioxide per second.
- This is ten million times faster than the non-enzyme catalyzed reaction.

ENZYME CLASSIFICATION

ENZYMES – INTERNATIONAL CLASSIFICATION

- There are six main types/groups of enzymes classified based on their chemical reaction mechanism

1. Oxidoreductases
   - Catalyze oxidations or reductions of substrates
   - Some important food reaction examples:
     - Lipid oxidation – lipoxygenase (adds an oxygen on fatty acids)
     - Browning – polyphenol oxidase (oxidizes phenols in food)
2. Transferases
   - Catalyze a shift of a chemical group from a donor to acceptor substrate e.g. Kinases regulate metabolism by transferring phosphate from ATP to other molecules.
   - Not so important in foods

Enzymes – General properties

3. Hydrolases

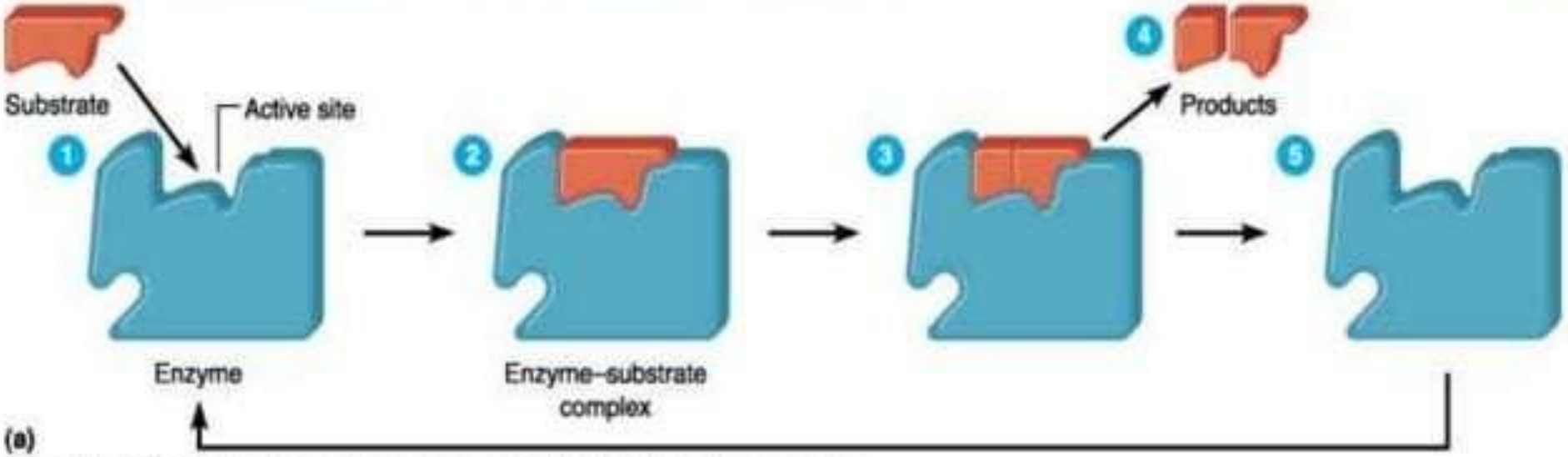
- Catalyze the hydrolysis (with help of water) of substrates (i.e. breaking of bonds)
- By far the **most important** enzymes with respect to **food quality** and use in food processing
- Some important food reaction examples:
  - Texture, protein modification – proteases (cleave the peptide bond)
  - Texture, carbohydrate modification – e.g. amylases (cleave glycosidic bonds) and pectinases (act on several groups/bonds)
  - Hydrolytic rancidity, fat crystallization modification – lipases (cleave ester bonds)

ENZYMES – GENERAL PROPERTIES

4. Lyases
   - Catalyze the removal or addition of chemical groups to substrates
   - Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds.
   - Not so important in foods
5. Isomerases
   - Catalyze intramolecular rearrangements
   - Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others.
   - An important food reaction example:
     - Sweetness (Glu → Fru) – glucose isomerase (converts aldose to ketose)
6. Ligases
   - Catalyze combinations of substrates with the use of energy from ATP
   - Not so important in foods

MECHANISM OF ACTIVITY

ENZYMATIC REACTION STEPS

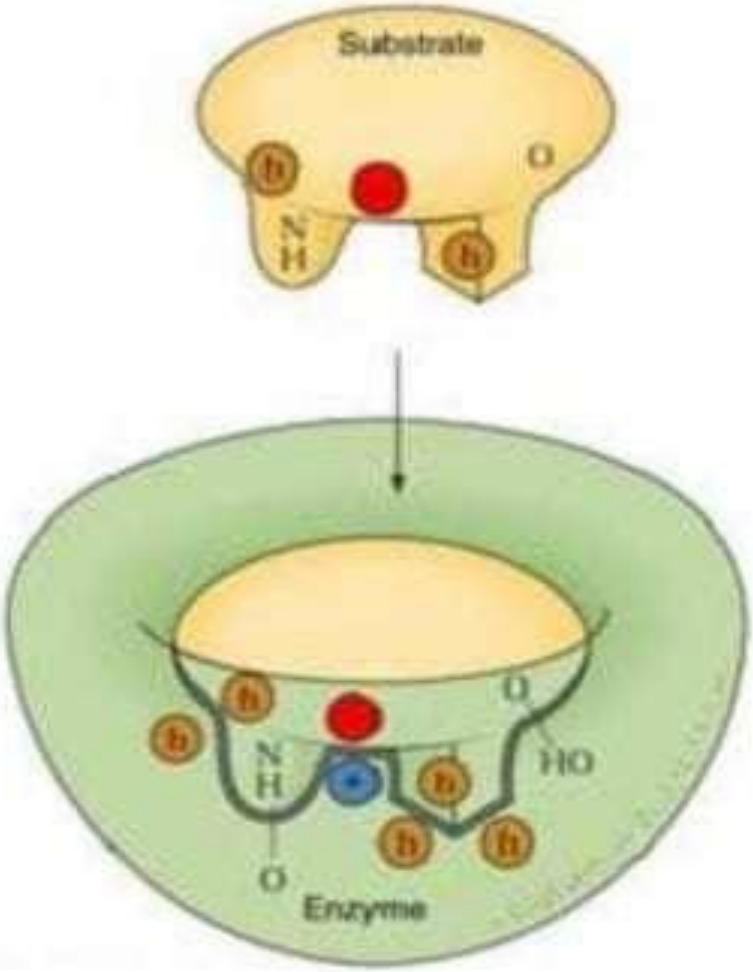


1. Substrate approaches active site
2. Enzyme-substrate complex forms
3. Substrate transformed into products
4. Products released
5. Enzyme recycled

Active Site

- The area of an enzyme that binds to the substrate Structure has a unique geometric shape that is designed to fit the molecular shape of the substrate
  - Each enzyme is substrate specific
  - Thus the active site that is complementary to the geometric shape of a substrate molecule
- There is tow models of active sites
  1. **Lock and Key model**
  2. **Induced Fit mode**

Lock and Key Model



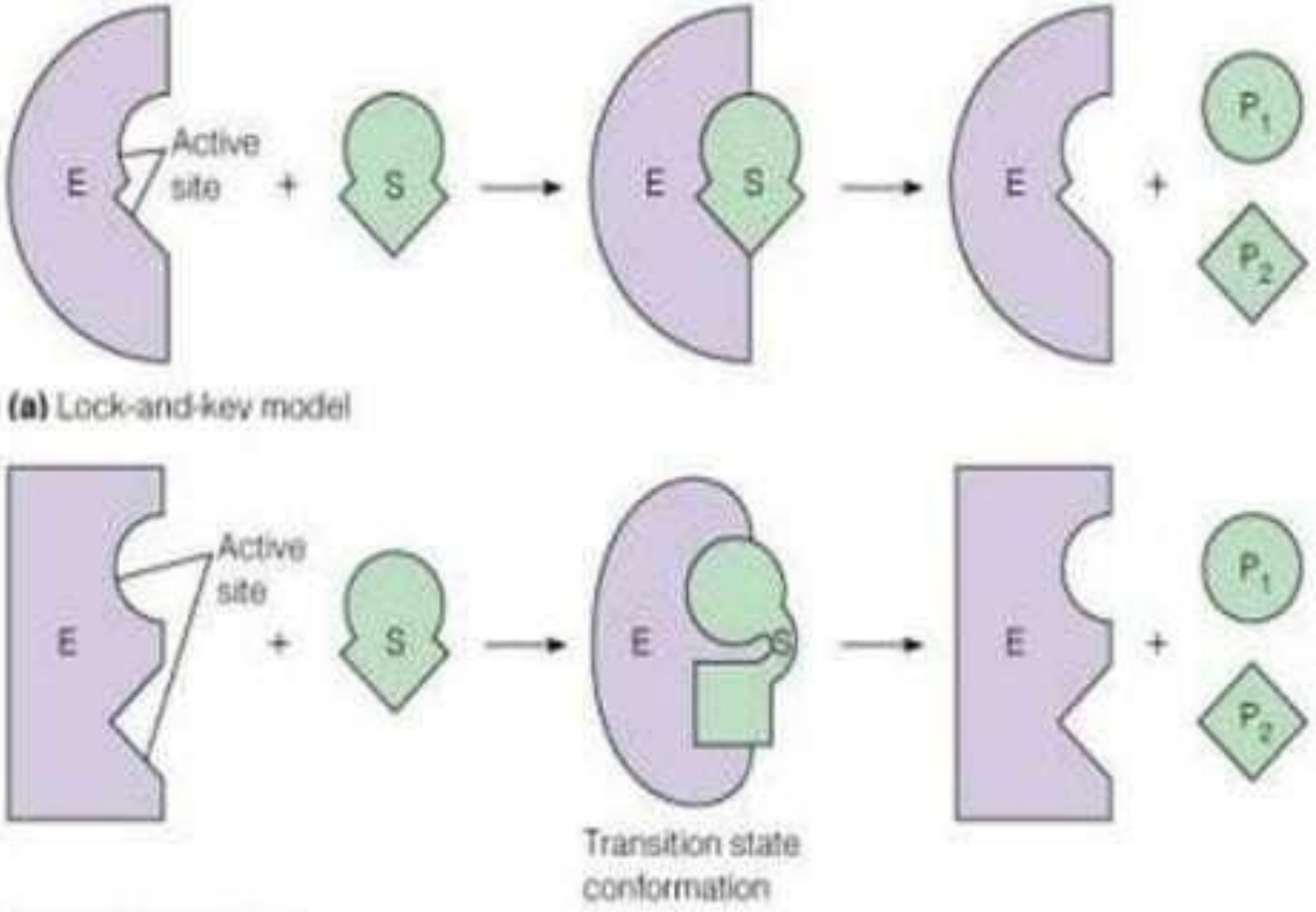
Lock and Key Model

- 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

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
  - transition-state like configuration

Enzyme-Substrate Interaction



Measuring enzyme activity

MEASURING ENZYMATIC ACTIVITY

- Enzymes are never expressed in terms of their concentration (as mg or µg etc.), but are expressed only as activities.
- Enzyme activity = moles of substrate converted to product per unit time.
  - The rate of appearance of product or the rate of disappearance of substrate
  - Test the absorbance: spectrophotometer
- Measuring Enzymatic Rates ideally done with a system where the **product or substrate** absorb a particular wavelength of light this depends on enzyme reaction can be monitored with a spectrophotometer by measuring either
- **the appearance of product**

  or
- **disappearance of substrate**

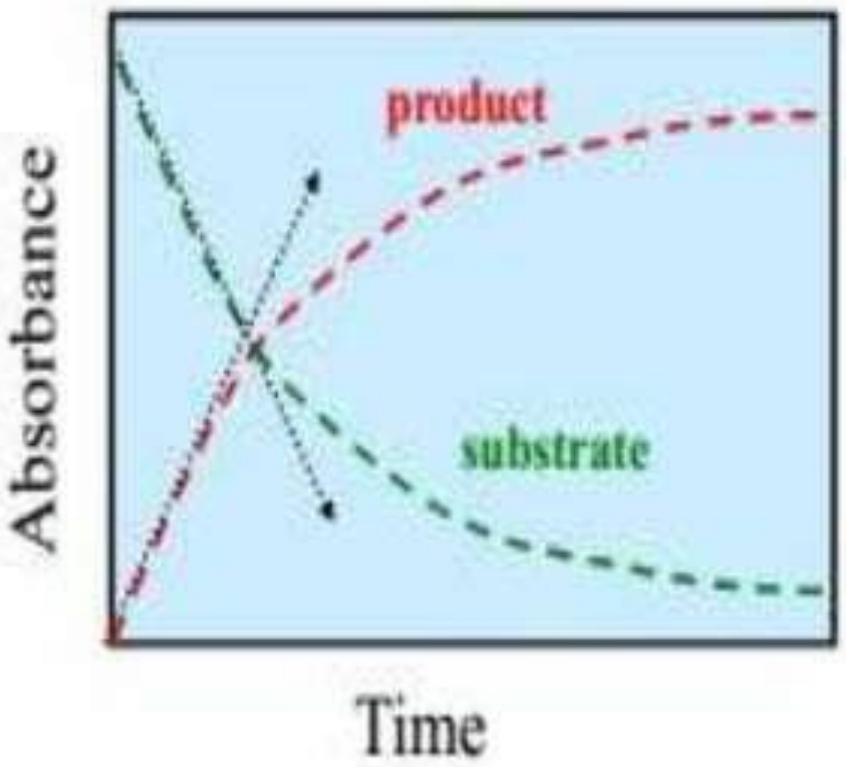
Beer Lambert's law

$Abs = \varepsilon lc$

ε = extinction Coefficient

l = path length (cm) ~1

c = Concentration (M)



measurement of the initial slope → rate (conc.)/(time)

MICHAELIS-MENTEN ENZYME KINETICS

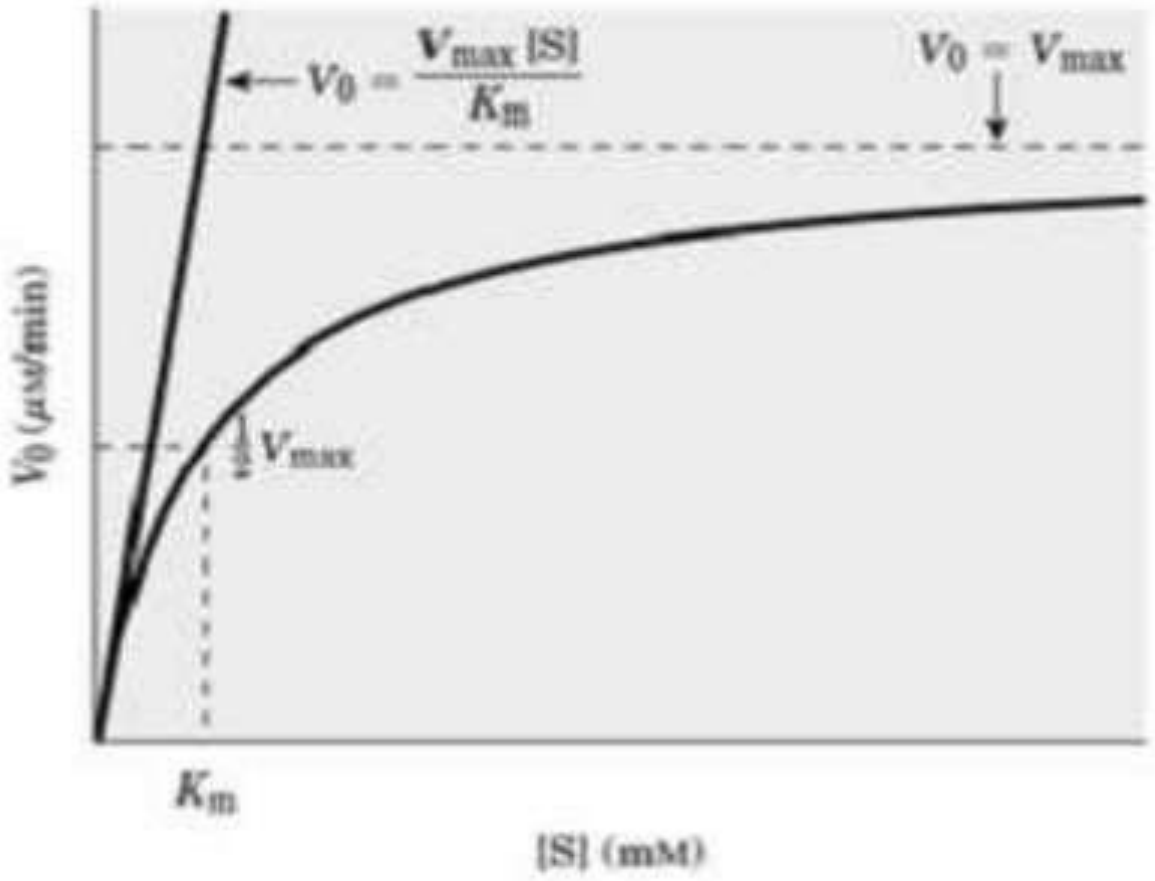
Reaction Scheme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Michaelis-Menten Equation $$v = \frac{V_m[S]}{K_m + [S]}$$

where $V_m = k_2[E_t]$ and $Km = \frac{k_{-1} + k_2}{k_1}$

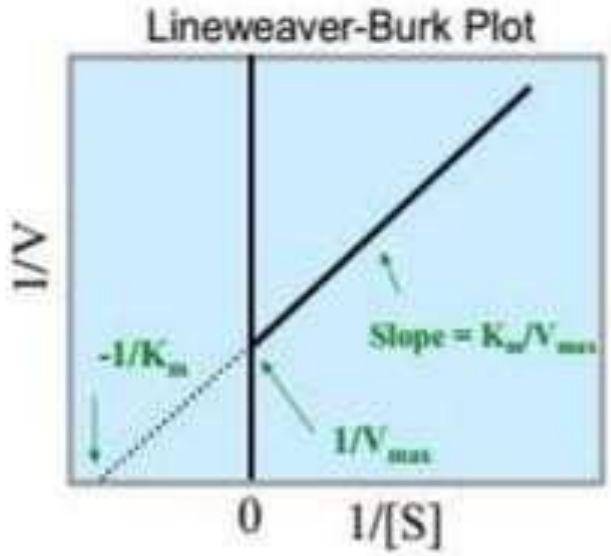
$$v = \frac{d[P]}{dt} = k_{cat}[ES]$$



low [S], v is proportional to [S] - first order
high [S], v is independent of [S] - zero order

- In order to change this equation to a form we can use in our analysis of enzymatic rate constants, we invert both sides of the equation:

$$V = \frac{V_{max}[S]}{K_m + [S]} \longrightarrow \frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]}$$



$$\frac{1}{V} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \left(\frac{1}{V_{max}}\right)$$

$$V_{max} = k_{cat}[E]$$

TURNOVER NUMBER (KCAT)

- The kcat is a direct measure of the conversion of substrate to product
- The number of substrate molecules turned over per enzyme molecule per second, hence "turnover number".
- The overall rate of a reaction is limited by its slowest step

K_m — High K_m means strength of binding is low

Relates to how strongly an enzyme binds its substrate

k_{cat} — High k_{cat} means high speed of catalysis

Relates to how rapid a catalyst the enzyme is

V_{max} — High V_{max} means high rate of catalysis

Related to k_{cat} and [E] by: $V_{max}=k_{cat}[E]$

Factors affecting the activity

FACTORS AFFECTING ENZYME ACTIVITY

1) Enzyme concentration
2) substrate concentration
3) Temperature
4) pH
5) Water activity
6) Inhibitors

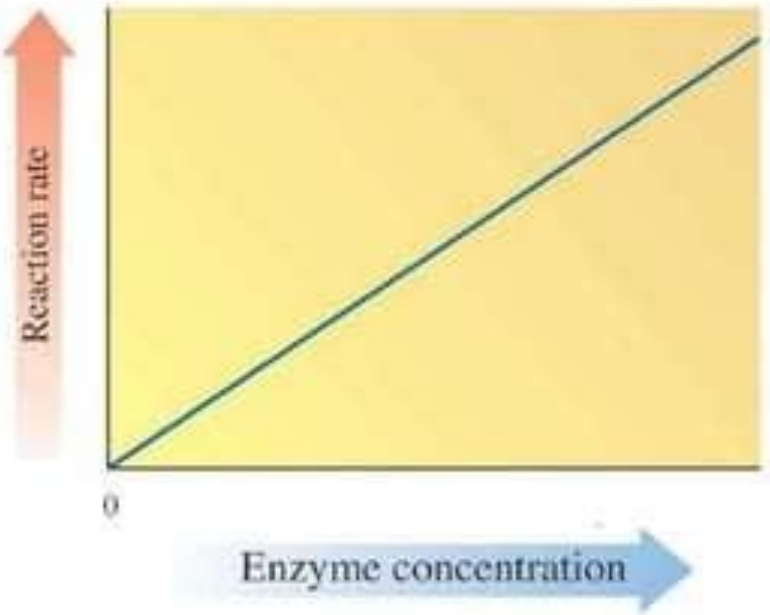
1-The effect of Enzyme concentration

- at low enzyme concentration there is **great competition** for the active sites and the rate of reaction is **low**.
- As the enzyme concentration **increases**, there are **more active** sites and the reaction can proceed at a **faster** rate.
- Eventually, increasing the enzyme concentration *beyond a certain point* has no effect because the substrate concentration becomes the *limiting* factor.

Enzyme Concentration

An increase in *enzyme concentration*

- increases the rate of reaction (at constant substrate concentration)
- binds more substrate with enzyme



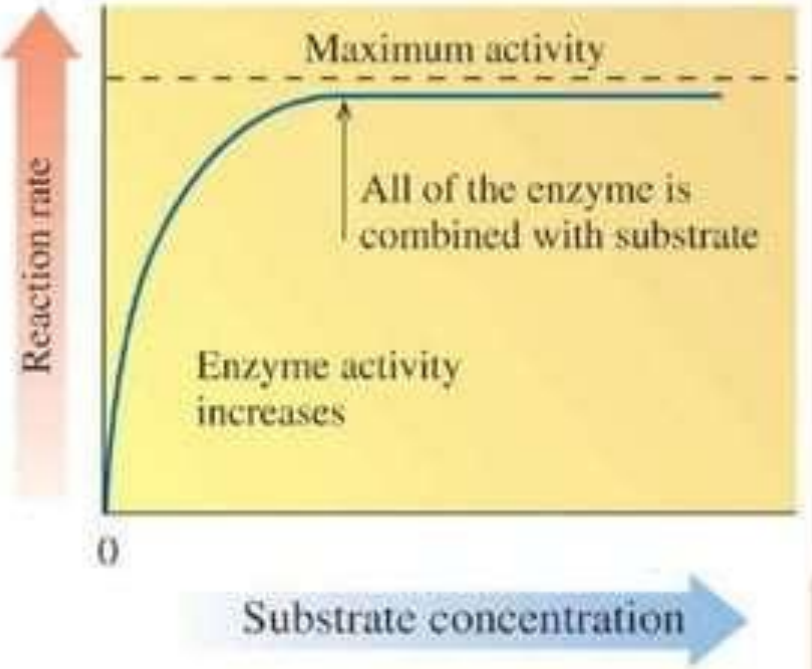
2-The effect of Substrate concentration

- at a low substrate concentration there are many active sites that are not occupied. This means that the reaction rate is low.
- When more substrate molecules are added, more enzyme-substrate complexes can be formed.
- As there are more active sites, and the rate of reaction increases.
- Eventually, increasing the substrate concentration yet further will have no effect. The active sites will be saturated so no more enzyme-substrate complexes can be formed.

Substrate Concentration

An increase in *substrate concentration*

- increases the *rate of reaction* (at constant enzyme concentration)
- eventually saturates an enzyme with substrate to give maximum activity



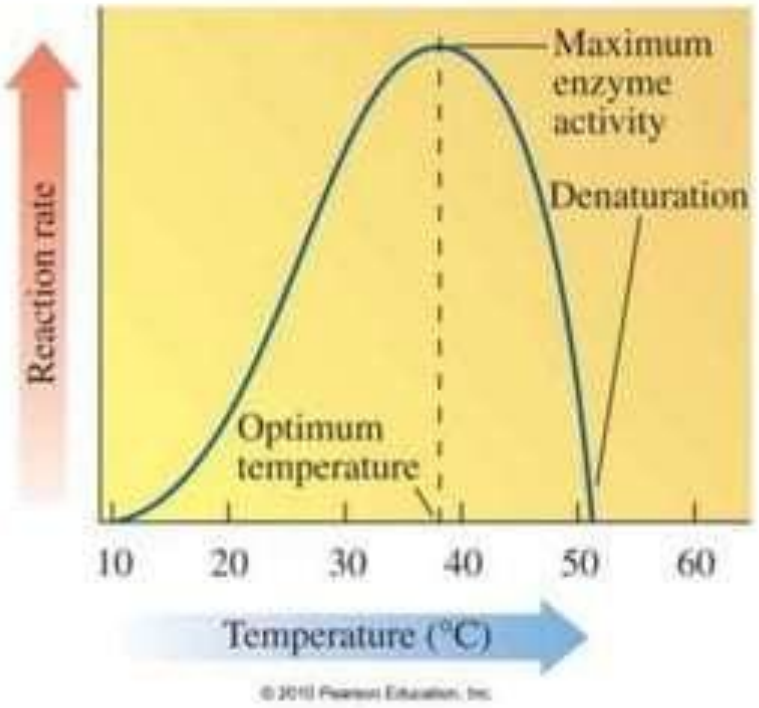
3- The effects of change in temperature

- Temperature: enzymes work best at an optimum temperature.
- An increase in temperature provides more kinetic energy to the molecules involved. The numbers of collisions between enzyme and substrate will **increase** so the rate will too.
- Too high temperature above the optimum temperature cause the enzymes to be *denatured*.
- Bonds holding the structure together will be broken and the active site loses its shape and will no longer work.

Temperature and Enzyme Action

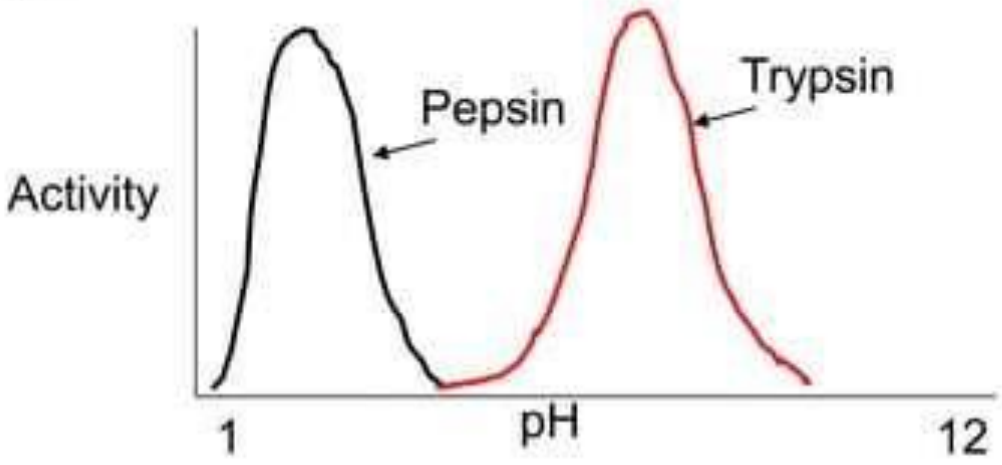
Enzymes

- are most active at an optimum temperature (usually 37 °C in humans)
- show little activity at low temperatures
- lose activity at high temperatures as denaturation occurs
- Different enzymes have different temperature optima's (the point when max activity is)



4- PH AND ENZYME ACTIVITY

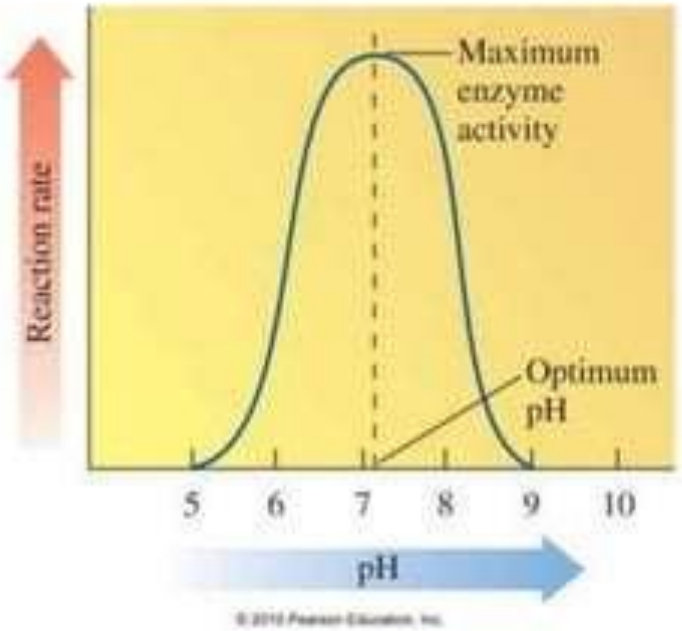
- All enzymes have a certain narrow range of pH where they perform best
  - Most active between 4.5 - 8
  - Some active at very low (e.g. pepsin) or high pH
- Extremes of pH can affect the enzyme by *denaturing* it (remember it is a protein) or *affecting* the *charge* of critical amino acids in its active site (or charge on the substrate)
- For this reason pH control of foods with undesirable enzymes is important



pH and Enzyme Action

Enzymes

- are most active at *optimum pH*
- contain R groups of amino acids with proper charges at optimum pH
- lose activity in low or high pH as tertiary structure is disrupted



Optimum pH Values

Enzymes in

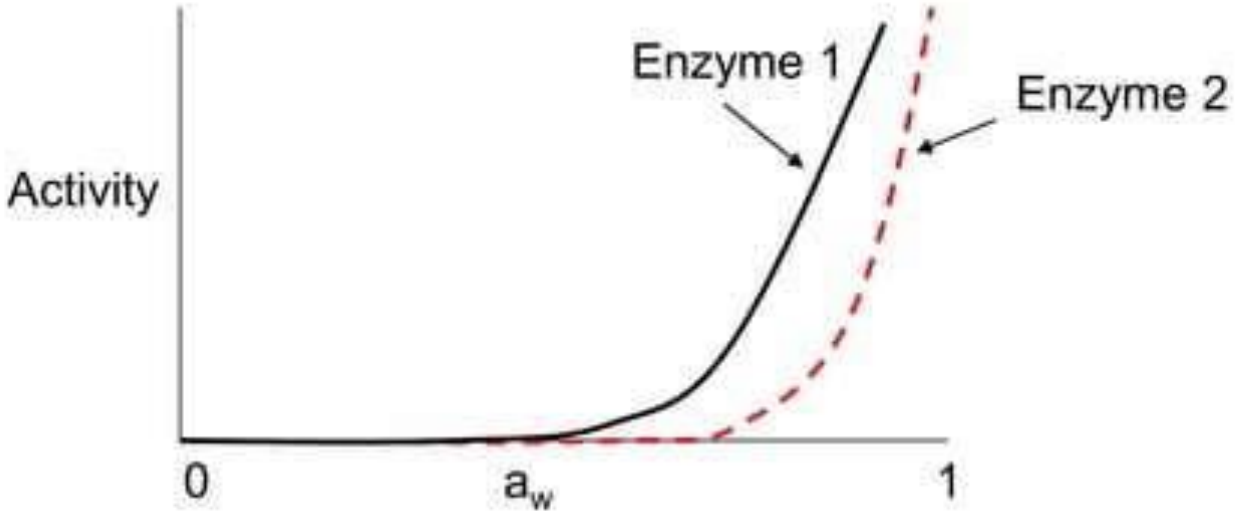
- the body have an *optimum pH* of about 7.4
- certain organs have enzymes that operate at lower and higher optimum pH values

TABLE 20.5 Optimum pH for Selected Enzymes

Enzyme	Location	Substrate	Optimum pH
Pepsin	Stomach	Peptide bonds	1.5–2.0
Sucrase	Small intestine	Sucrose	6.2
Amylase	Pancreas	Amylose	6.7–7.0
Urease	Liver	Urea	7.0
Trypsin	Small intestine	Peptide bonds	7.7–8.0
Lipase	Pancreas	Lipid (ester bonds)	8.0
Arginase	Liver	Arginine	9.7

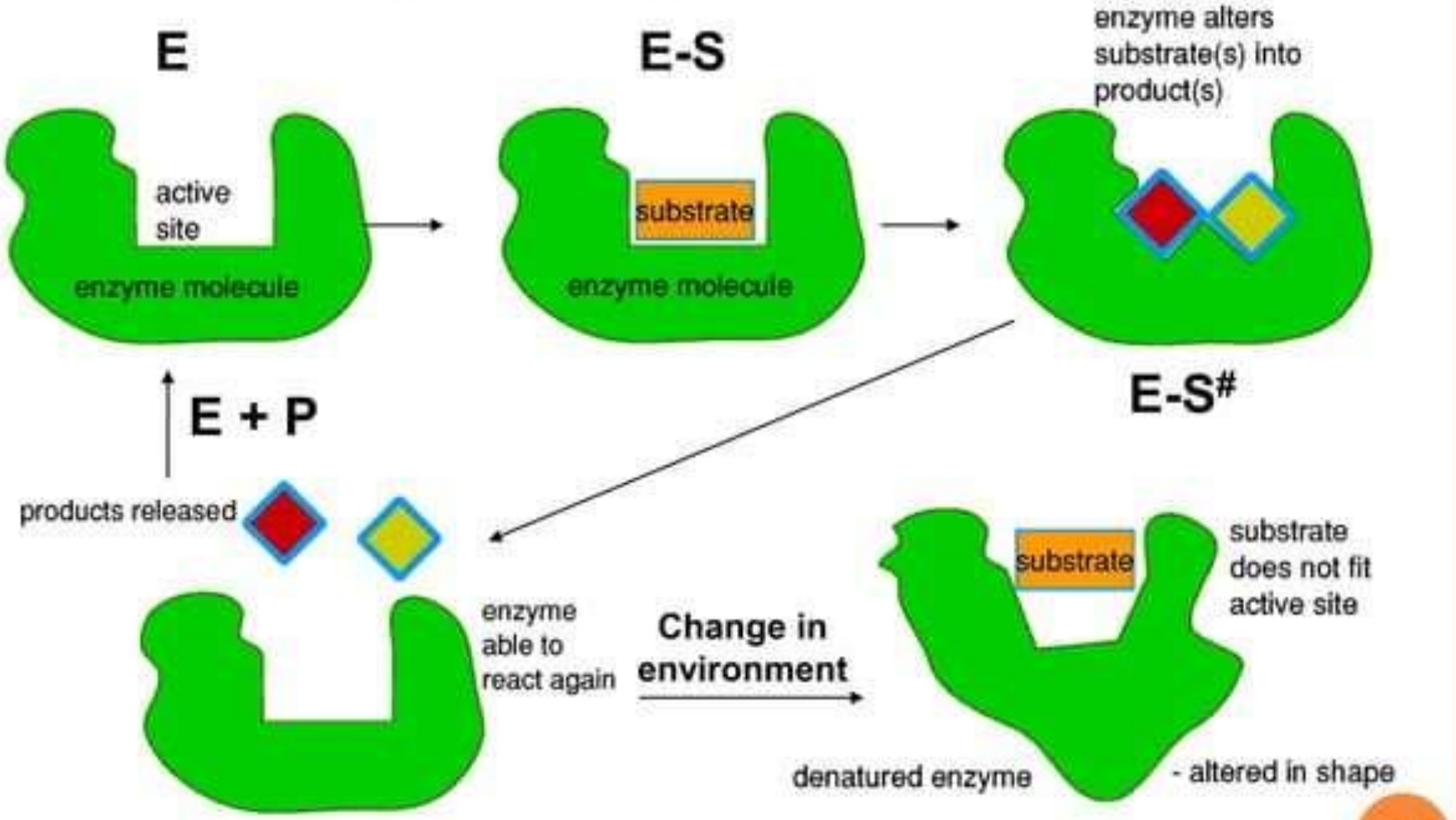
4. Water activity

- Water can influence an enzyme in many ways
  - It can be critical for the S→P reaction (e.g. hydrolysis)
  - It can be critical to solubilize the substrate and product
  - It can be critical for the flexibility of the enzyme structure
- Water activity can be varied in foods to slow down enzymatic activity



NO INHIBITOR

$$E + S \rightarrow ES \rightarrow E + P$$

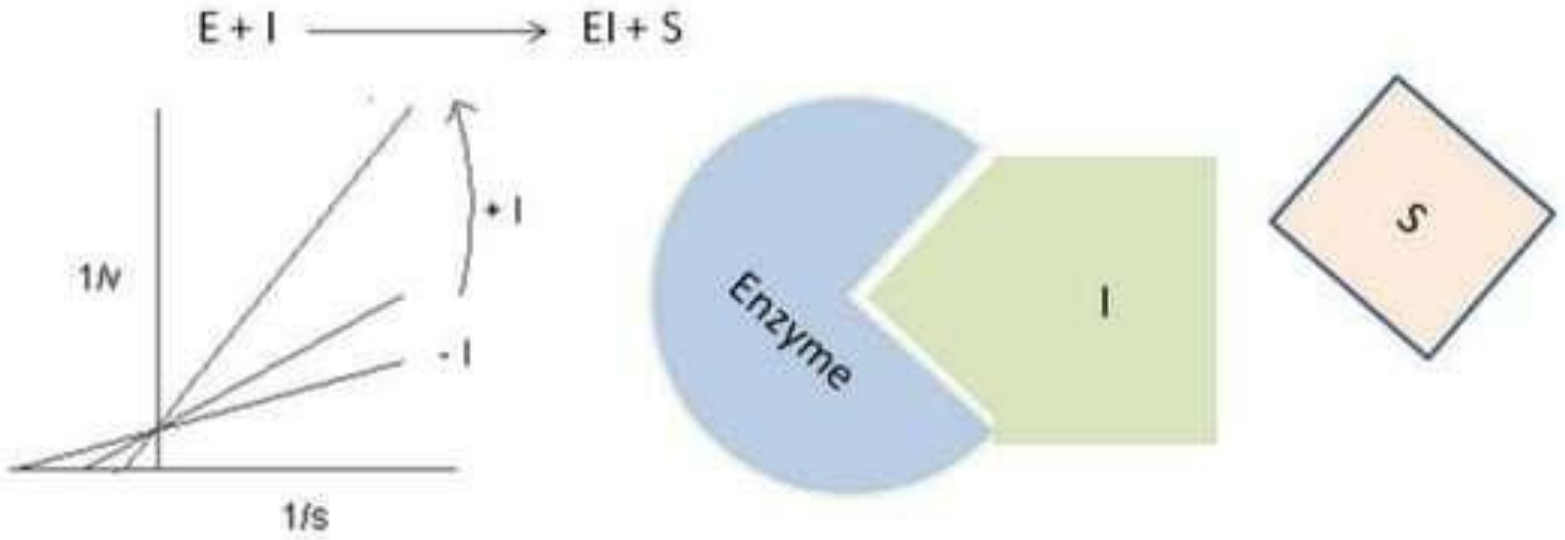


Enzymes – General properties

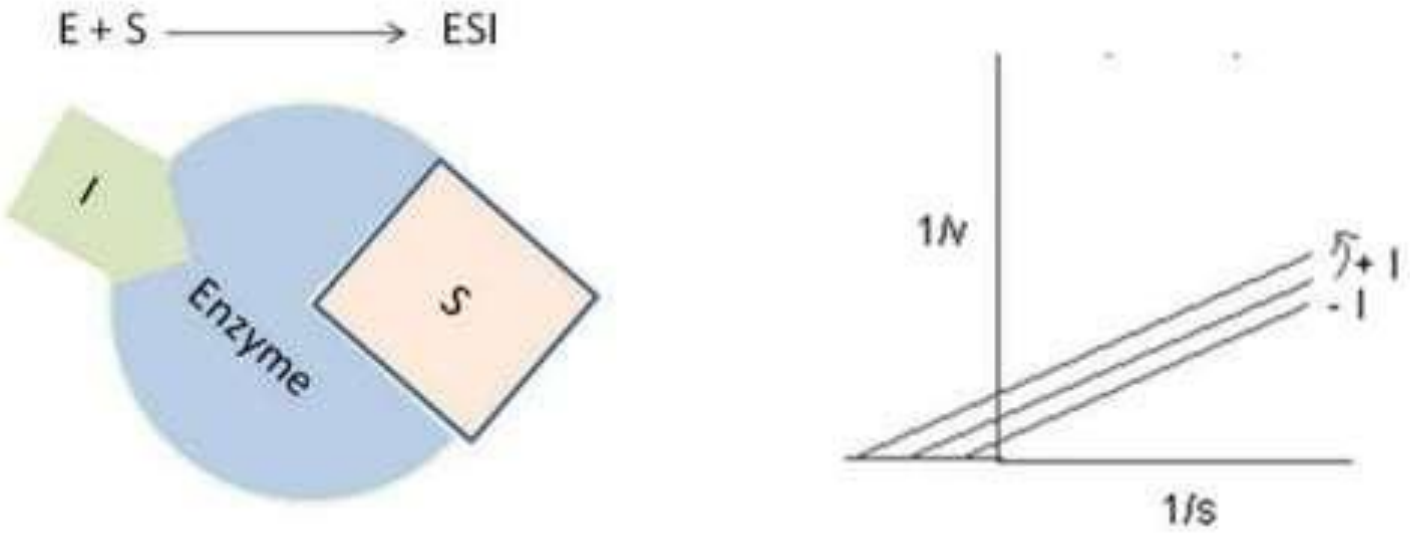
5. Inhibitors

- Chemical compounds that inhibit or slow down the activity of enzymes
  1. Competitive inhibitors
     - Compete with the substrate for the active site
     - Enzyme can only bind to either S (substrate) or I (inhibitor) at one time
  2. Non-competitive inhibitors
     - Bind to enzyme at another site than active site
     - Enzyme can bind to both S and I at the same time
  3. Un-competitive inhibitors
     - Can only bind to the E-S complex (the intermediate state)
     - Enzyme binds first to S and then can bind to I
- These can be reversible or irreversible

Competitive

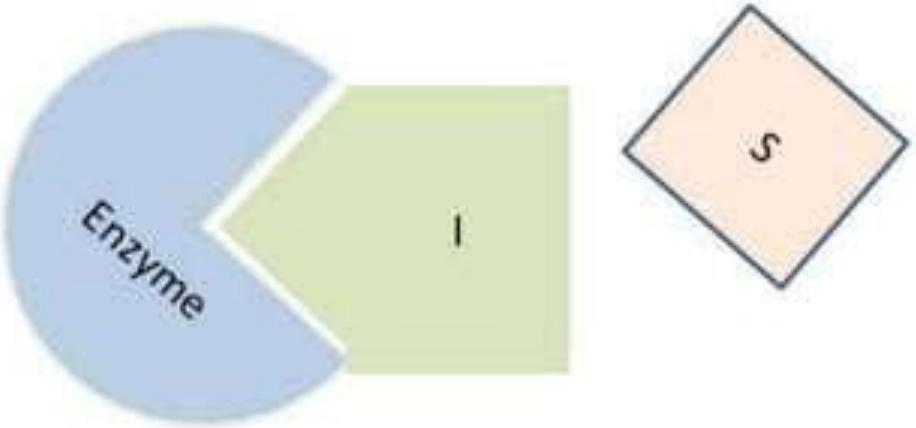


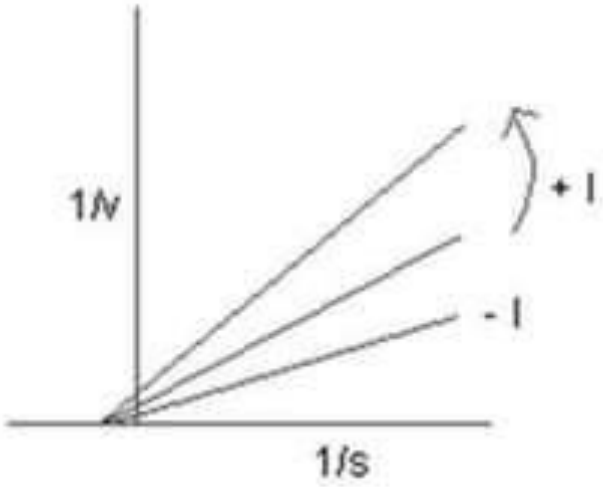
Uncompetitive

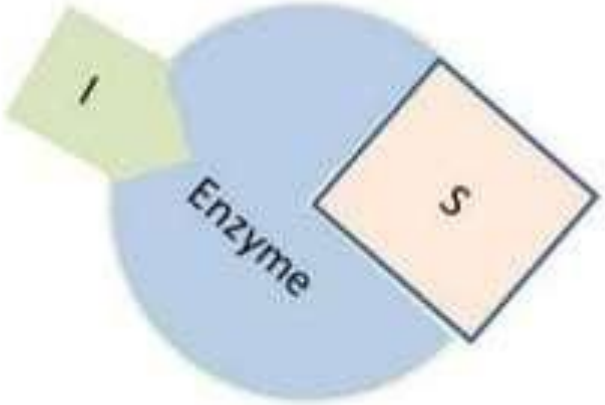


Noncompetitive

$$E + I \longrightarrow EI + S$$







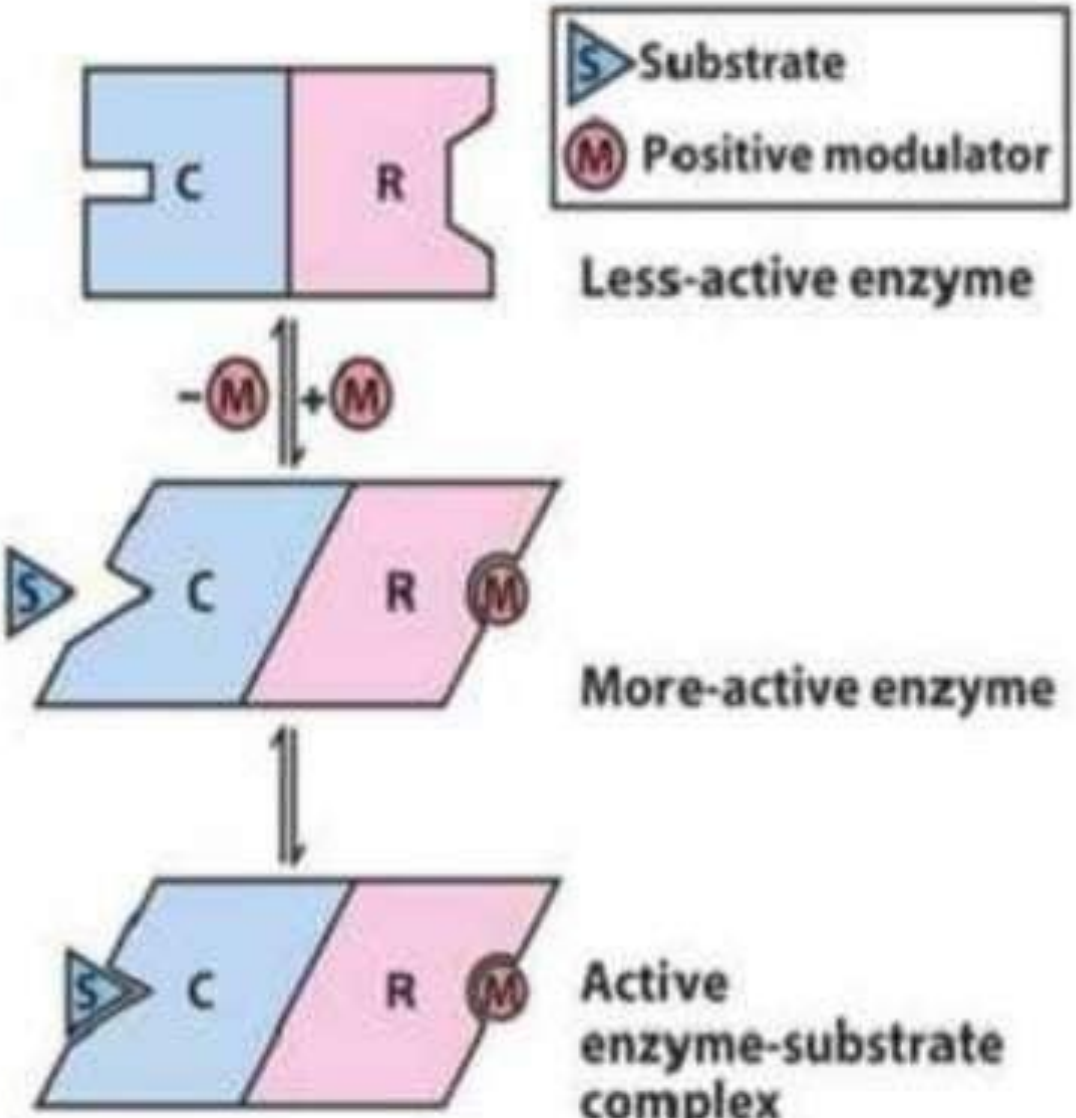
$$E + S \longrightarrow ESI$$

SOME EXAMPLE OF INHIBITORS

- antibiotics affecting bacterial metabolism by inhibit enzymes
- Nerve Gases cause irreversible enzyme inhibition
  - Insecticides – choline esterase inhibitors
  - Many heavy metal poisons work by irreversibly inhibiting enzymes, especially cysteine residues

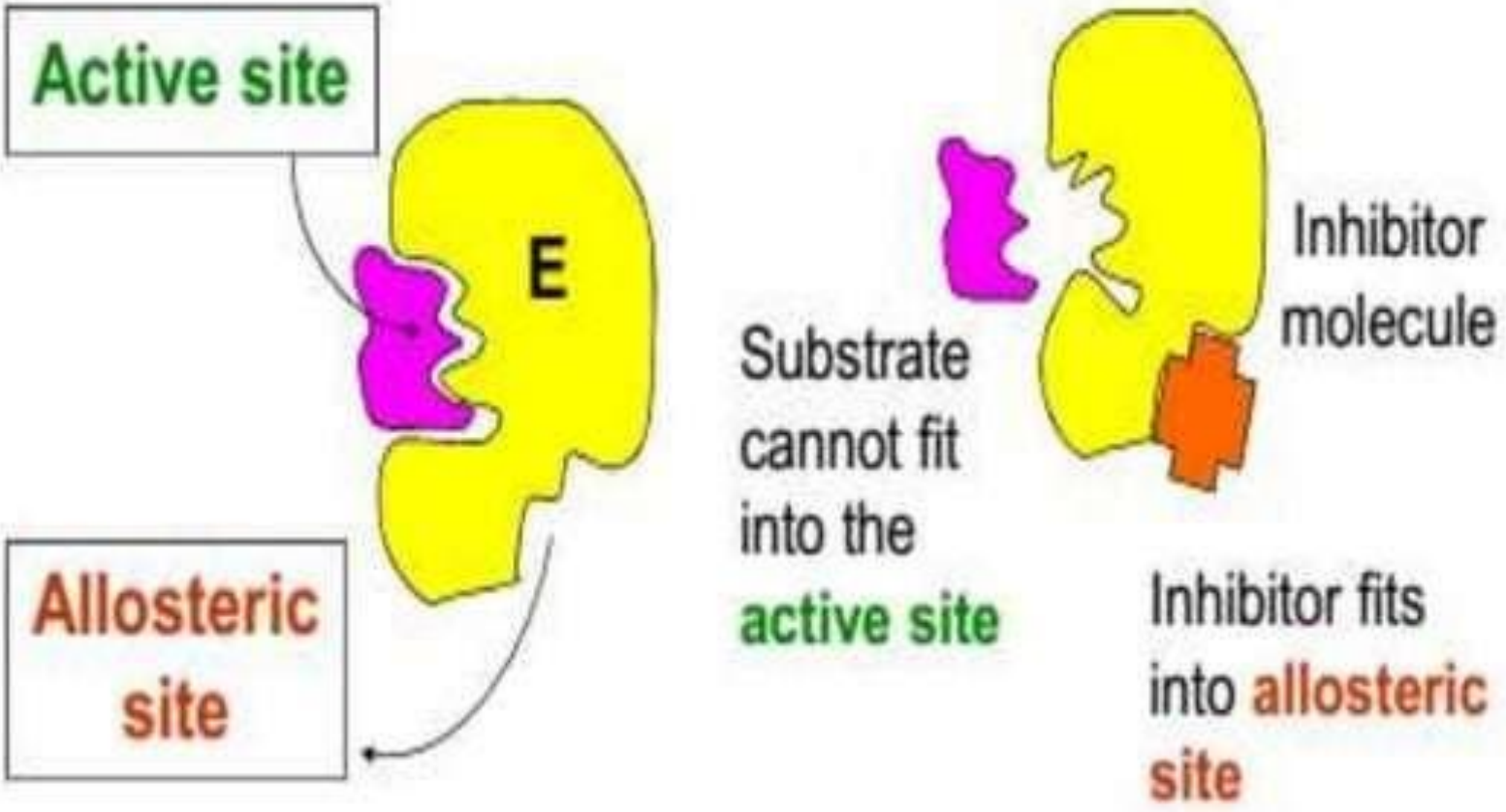
ALLOSTERIC REGULATION

- When a small molecule can act as an effector or **regulator** to activate or inactivate an action of a protein- the protein is said to be under **allosteric** control.


S Substrate
M Positive modulator
C
R
Less-active enzyme
-M
+M
S
C
R
M
More-active enzyme
S
C
R
M
Active enzyme-substrate complex

ALLOSTERIC INHIBITION

ALLOSTERIC MEANS "OTHER SITE"



Feedback Inhibition

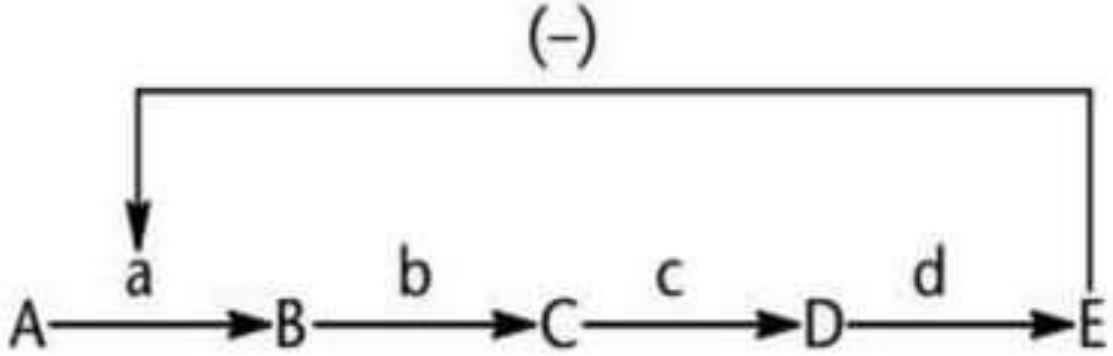
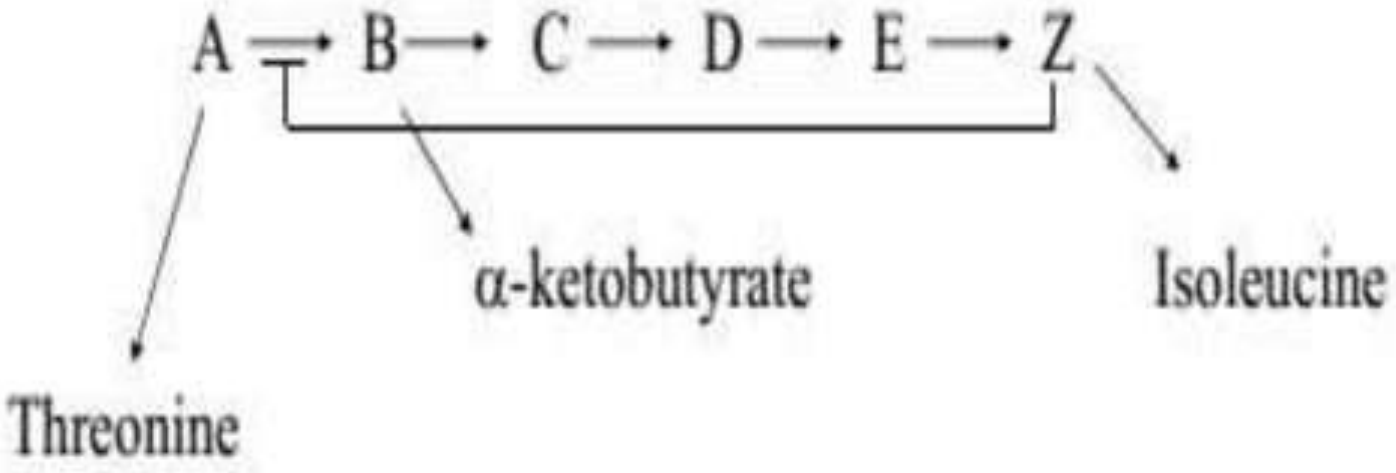


Figure 1 Feedback inhibition. As concentrations of product E builds up, they act to inhibit/regulate the catalytic activity of enzyme 'a'.

- By inhibiting enzyme 'a', the unnecessary utilization of substrate 'A' as well as the accumulation of the final product 'E' is prevented.
- Frequently, feedback inhibition occurs through a steric mechanism whereby the product occupies a portion of the active site of the enzyme, thus preventing substrate binding.
- In this way, the regulation is the product of simple mass action relationships.
- That is, if the product accumulates, the enzyme itself senses this fact and exhibits reduced activity.

Feedback Inhibition



Industrial Enzyme Applications

History of Enzyme Use in Food Production

- Enzymes extracted from edible plants and the tissues of food animals, as well as those produced by microorganisms (bacteria, yeasts, and fungi), have been used for centuries in food manufacturing.
- Rennet is an example of a natural enzyme mixture from the stomach of calves or other domestic animals that has been used in cheese making for centuries.
- Rennet contains a protease enzyme that coagulates milk, causing it to separate into solids (curds) and liquids (whey).
- Alternatively, for centuries enzymes produced by yeast have been used to ferment grape juice in order to make wine.

Major Enzyme Applications in Food Industry

- In food industry, enzyme has been used to produce and to increase the quality and the diversity of food.
- Some examples of products that use enzyme are cheese, yoghurt, bread syrup etc.
- Ancient traditional arts such as brewing, cheese making, meat tenderization with papaya leaves and condiment preparation (e.g., soy sauce and fish sauce) rely on proteolysis, albeit the methods were developed prior to our knowledge of enzymes.
- Early food processes involving proteolysis were normally the inadvertent consequence of endogenous or microbial enzyme activity in the foodstuff.

Modern Production of Food Enzymes

- Today, **microorganisms are the most important source of commercial enzymes.**
- Although microorganisms do not contain the same enzymes as plants or animals, a microorganism can usually be found that produces a related enzyme that will catalyse the desired reaction.
- Enzyme manufacturers have optimized microorganisms for the production of enzymes through natural selection and classical breeding techniques.

- To date, scientists have deposited around **35,000** known structures of enzyme molecules in the Enzyme Structures Database (http://www.ebi.ac.uk/thornton-srv/databases/enzymes/).

- but just approximately **200** microbial original types are used commercially

- However, only about **20** enzymes are produced on truly industrial scale

No.	Enzymes	Microorganisms	Usage
1	Amylase	*Aspergillus niger*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus subtilis*, *Trichoderma longibrachiatum* *, *Aspergillus oryzae* *	Corn silage, corn, corn gluten feed, soybean meal, wheat, wheat middlings, barley, grain sorghum, oat, pea, tapioca, millet, rice
2	Pullulanase	*Bacillus acidopullulyticus*	
3	α-Galactosidase	*Aspergillus niger*	Soybean meal
4	Cellulase	*Trichoderma longibrachiatum*	Corn, barley, wheat, wheat bran, rye, grain sorghum
5	β-Glucanase	*Aspergillus niger* , *Bacillus subtilis* , *Trichoderma longibrachiatum*, *Penicillium funiculosum* *	Wheat, barley, canola meal, wheat byproduct, oat groats, rye, triticale, grain sorghum
6	Glucose Oxidase	*Penicillium notatum*	Glucose
7	Lipase	*Aspergillus niger*	Plant and animal sources of fats and oils
8	Maltase	*Bacillus subtilis*	Maltose
9	Mannanase	*Bacillus lentus*	Corn, soybean meal, guar meal
10	Pectinase	*Aspergillus niger*	Corn, wheat
11	Phytase	*Aspergillus niger* , *Aspergillusoryzae*	Corn, soybean meal, sunflower meal, hominy, tapioca, plant byproducts
12	Protease	*Aspergillus niger*, *Aspergillus oryzae*, *Bacillus subtilisi* , *Trichoderma longibrachiatum* *	Plant and animal proteins
13	Xylanase	*Aspergillus oryzae*, *Humicolainsolens* , *Trichoderma longibrachiatum* , *Bacillus subtilis* , *Penicillium funiculosum* *	Corn, barley, rye, wheat, grain sorghum, triticale, oats

Food processing	Baking industry	α-amylases	Degrading starch in flours and controlling the volume and crumb structure of bread.
		β-xylanases	Improving dough handling and dough stability.
		Oxidoreductases	Giving increased gluten strength.
		Lipases	Improving stability of the gas cells in dough.
		Proteases	Reducing the protein in flour.
	Juice industry	Amylases, glucoamylases	Breaking down starch into glucose. Clarifying cloudy juice, especially for apple juice.
		Pectinases	Degrading pectins which are structural polysaccharides present in the cell wall. Increasing the overall juice production.
		Cellulases, hemicellulases*	Acting on soluble pectin hydrolysis and on cell wall components with pectinases Lowering viscosity and maintenance of texture.
		Laccase	Increasing the susceptibility of browning during storage.
		Naringinase and limoninase	Acting on compounds that cause bitterness in citrus juices
	Starch processing	α-amylases	Cleaving α-1,4-glycosidic bonds in the inner region of the starch. Causing a rapid decrease in substrate molecular weight and viscosity.
		Pullulanases	Attacking α-1,6- linkages, liberating straight-chain oligosaccharides of glucose residues linked by α-1,4-bonds.
		Neopullulanases, amylopullulanases	Acting on both α-1,6- and α-1,4-linkages.
		β-amylases	Cleaving α-1,4-linkages from non-reducing ends of amylose, amylopectin and glycogen molecules. Producing low-molecular weight carbohydrates, such as maltose and "β-limit dextrin".
		Glucoamylases	Attacking α-1,4-linkages and α-1,6-linkages from the non-reducing ends to release β-d-glucose
		Isoamylases	Hydrolyzing α-1,6-linkages in glycogen and amylopectin.
		Glucose isomerases	Catalyzing isomerization of glucose to fructose
		Glycosyltransferases	Transferring a segment of a 1,4-α-D-glucan chain to a primary hydroxy group in a similar glucan chain to create 1,6-linkages. Increasing the number of branched points to obtain modified starch with improved functional properties such as higher solubility, lower viscosity, and reduced retrogradation.

SOME MAJOR APPLICATIONS BY TYPES OF ENZYMES ARE

- **Rennet** in cheese industries
- **Lactases** in milk industries
- **Catalases** in food preservation
- **Lipases** **in** oil industries
- **Proteases** in milk industries
- **Amylases** in food and beverages (baking), brewing, starch, sugar industries

CONCLUSION

- Enzymes are proteins that catalyze biological reaction and make them faster by reducing activation energy.
- We can measure enzyme activity by either measuring appearance of products or disappearance of substrates.
- Enzyme activity is affected by concentration of enzyme and substrate, temperature, pH, and water activity, inhibitors.
- Enzymes have a lot of application in industry and in food processing and preservation.

THANK YOU