Enzymes as Biomarker and Clinical Application

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Guest Lecture

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Non Degree Tranning

1. Chronic Inflammation : Mechanism and Regulation. Takeda Science Foundation, Osaka Japan (2017)
2. The Bioscience of Lipids. Robert Gordon University. Aberdeen Scotland UK (2014).
3. Laboratoire de Genetique Cellulaire et Moleculaire (LGCM), Universite de Poitiers Cedex, France. Under Professor A. Kitzis (2014).

Publication

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Conferences

Basic and Applied Science Conference (BASC) Virtual, April, 3-4. 2021. Oral Presentation

The 11th Annual ISCC Cancer Chemoprevention Conference, Virtual, November. 9-21. 2020

Are of Expertise : Metabolism. Enzyme Regulation, Oxidative Stress, Antioxidants & Herbal Medicine

ENZYMES

DEFINITION: they are the catalysts of biological system, colloidal, thermolabile & protein in nature.

BIOMEDICAL IMPORTANCE

- Without enzymes, life as we know it would not be possible.
- enzymes occupy central roles in health & diseases.
- Enzymes give information to physicians in diagnostic & prognosis.
- Deficiency may leads to inborn errors of metabolism.

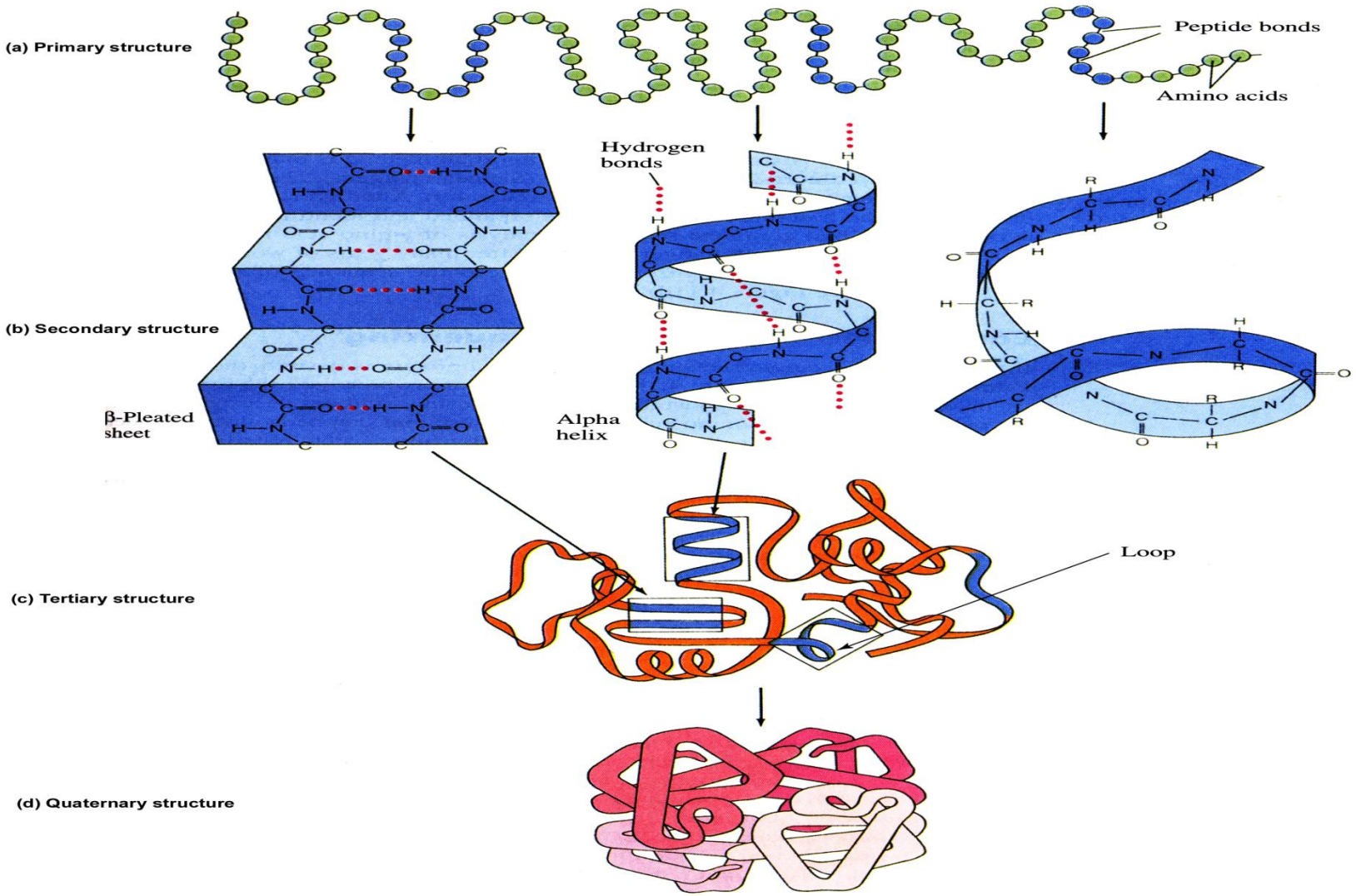
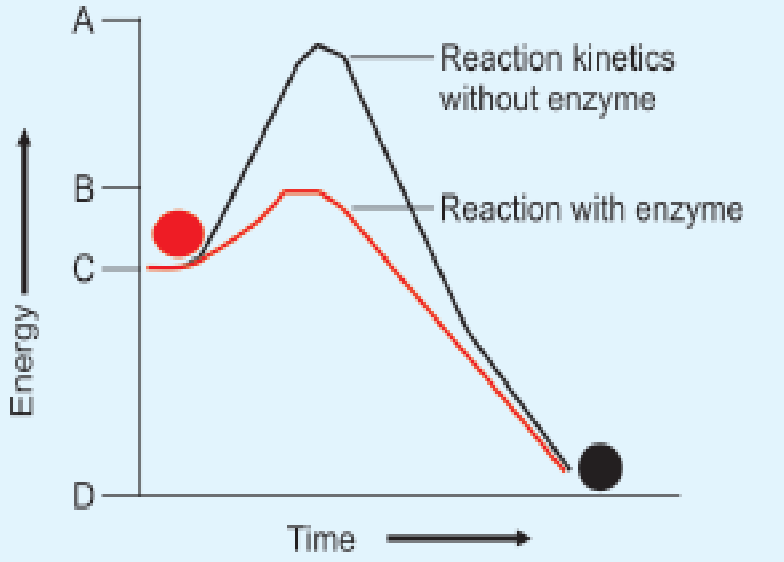
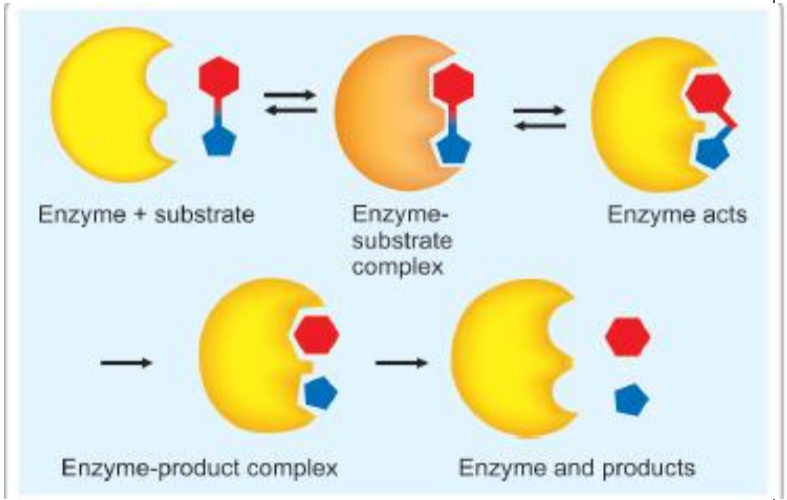


Figure 4.12
The relationships among the four levels of protein structure: (a) primary or covalent structure, (b) secondary structure, (c) tertiary structure, (d) quaternary structure.

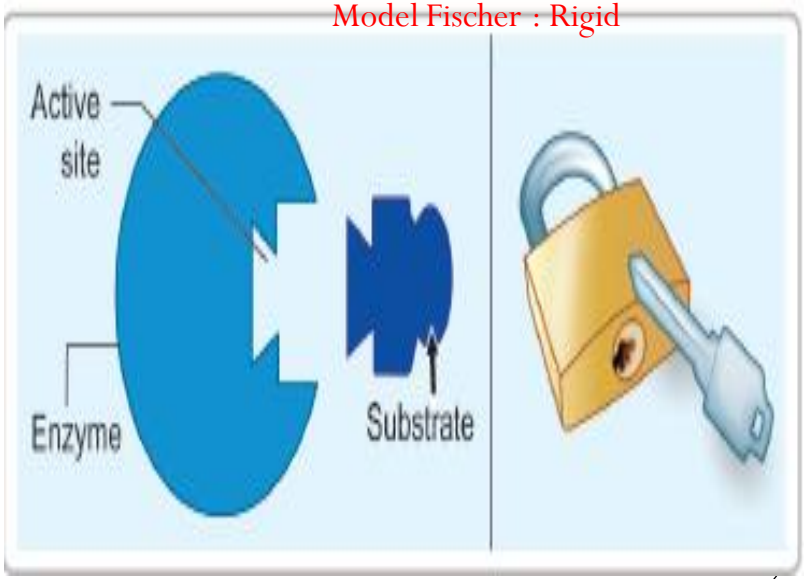
Enzyme is Biocatalysator



Red circle = substrate; black circle = product. C = energy level of 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



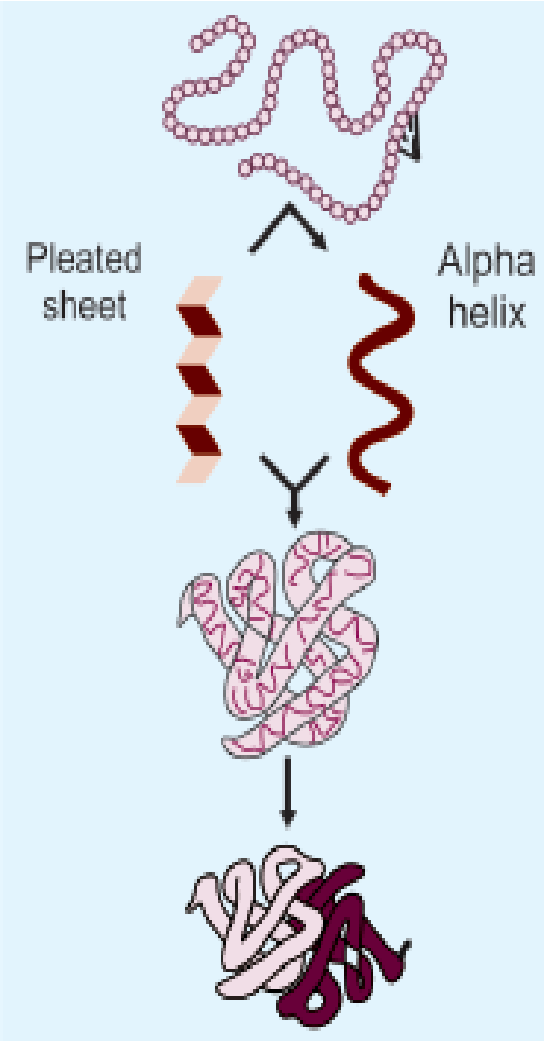
Model Fischer : Rigid



Koshland's Induced Fit Theory

The *substrate induces conformational changes in the enzyme*, such that precise orientation of catalytic groups is effected.

Model Activity of Enzyme



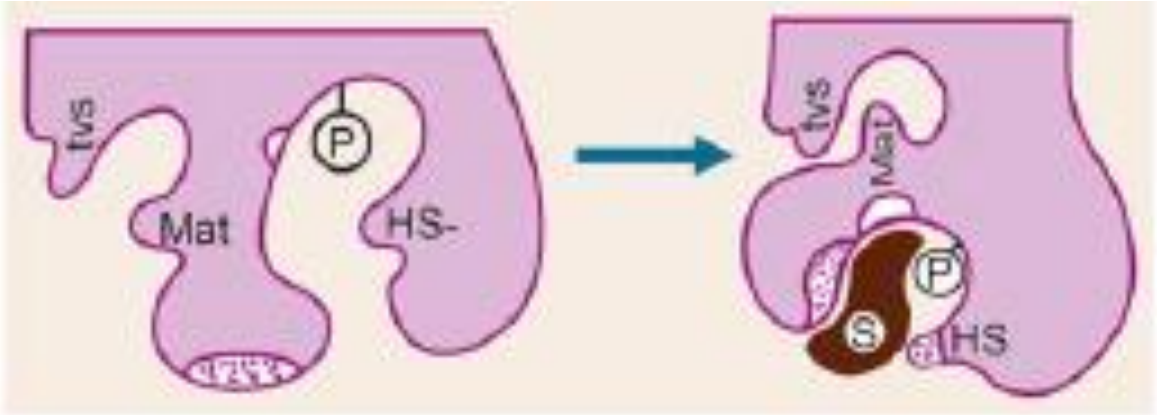
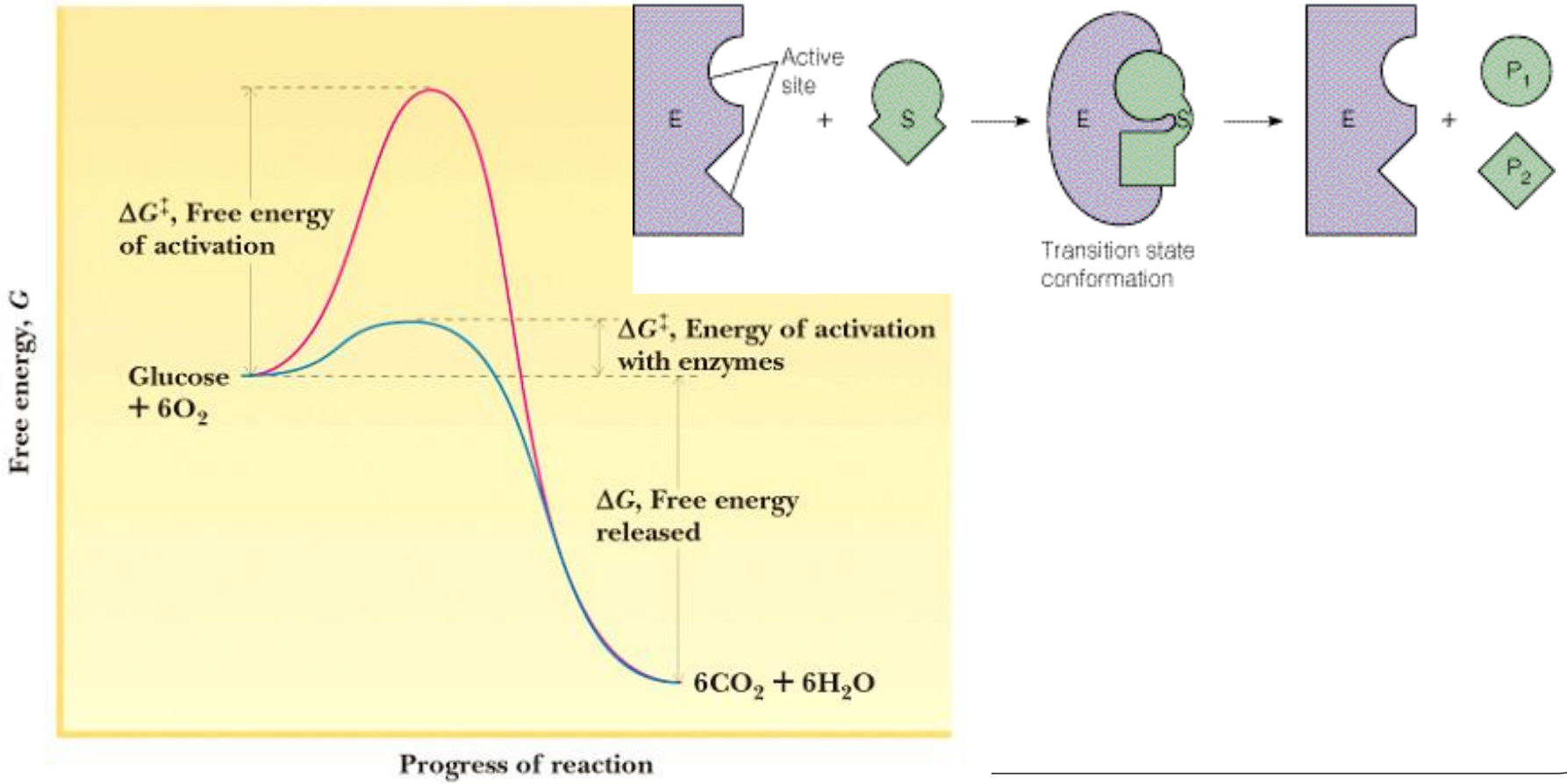


Fig. 9.2: Induced-fit model

TABLE 5.1: Examples of co-enzymes

Co-enzyme	*Group transferred*
Thiamine pyrophosphate (TPP)	Hydroxyethyl
Pyridoxal phosphate (PLP)	Amino group
Biotin	Carbon dioxide
Co-enzyme-A (Co-A)	Acyl groups
Tetrahydrofolate (FH4)	One carbon groups
Adenosine triphosphate (ATP)	Phosphate

(1) lowering the energy barrier (activation energy) for the product to form
(2) increases the favorable orientation of colliding reactant molecules for product formation to be successful (stabilize transition state intermediate)



Catalytic Power

- Enzymes can accelerate reactions as much as 10^{16} over uncatalyzed rates!
- Urease is a good example:
  - Catalyzed rate: 3×10^{4}/sec
  - Uncatalyzed rate: 3×10^{-10}/sec
  - Ratio is 1×10^{14}

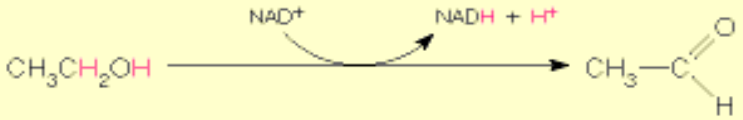
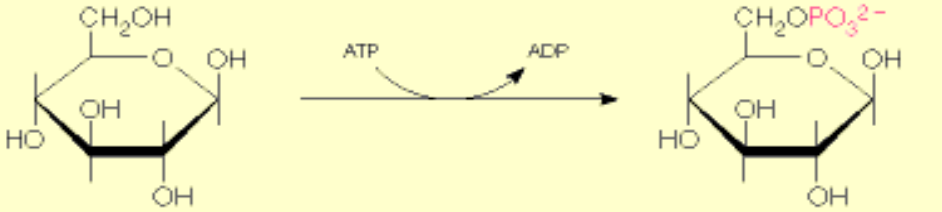
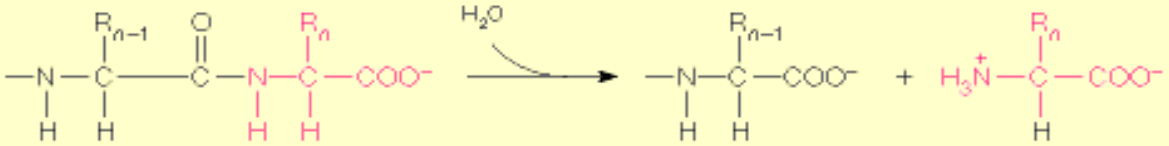
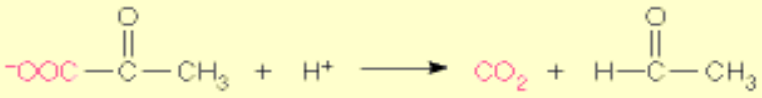
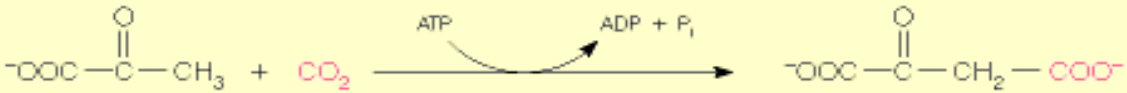
Specificity

- Enzymes selectively recognize proper substrates over other molecules
- Enzymes produce products in very high yields - often much greater than 95%
- Specificity is controlled by structure - the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield

Classes of enzymes (IUB System)

1. Oxidoreductases = catalyze oxidation-reduction reactions (NADH)
2. Transferases = catalyze transfer of functional groups from one molecule to another.
3. Hydrolases = catalyze hydrolytic cleavage
4. Lyases = catalyze removal of a group from or addition of a group to a double bond, or other cleavages involving electron rearrangement.
5. Isomerases = catalyze intramolecular rearrangement.
6. Ligases = catalyze reactions in which two molecules are joined.

Enzymes named for the substrates and type of reaction

Class	Example (reaction type)	Reaction Catalyzed
1. Oxidoreductases	Alcohol dehydrogenase (EC 1.1.1.1) (oxidation with NAD^+)	 **Ethanol** **Acetaldehyde**
2. Transferases	Hexokinase (EC 2.7.1.2) (phosphorylation)	 **D-Glucose** **D-Glucose-6-phosphate**
3. Hydrolases	Carboxypeptidase A (EC 3.4.17.1) (peptide bond cleavage)	 **C-terminus of polypeptide** **Shortened polypeptide** **C-terminal residue**
4. Lyases	Pyruvate decarboxylase (EC 4.1.1.1) (decarboxylation)	 **Pyruvate** **Acetaldehyde**
5. Isomerases	Maleate isomerase (EC 5.2.1.1) (*cis–trans* isomerization)	 **Maleate** **Fumarate**
6. Ligases	Pyruvate carboxylase (EC 6.4.1.1) (carboxylation)	 **Pyruvate** **Oxaloacetate**

Co-enzymes

- Non-protein molecules that help enzymes function
- Associate with active site of enzyme
- Enzyme + Co-enzyme = holoenzyme
- Enzyme alone = apoenzyme
- Organic co-enzymes – thiamin, riboflavin, niacin, biotin
- Inorganic co-factor – Mg^{++}, Fe^{++}, Zn^{++}, Mn^{++}

TABLE 5.2: Metallo-enzymes

Metal	*Enzymes containing metals*
Zinc	Carbonic anhydrase, carboxy peptidase, alcohol dehydrogenase
Magnesium	Hexokinase, phosphofructokinase, enolase, glucose-6-phosphatase
Manganese	Phosphoglucomutase, hexokinase, enolase, glycosyl transferases
Copper	Tyrosinase, cytochrome oxidase, lysyl oxidase, superoxide dismutase
Iron	Cytochrome oxidase, catalase, peroxidase, xanthine oxidase
Calcium	Lecithinase, lipase
Molybdenum	Xanthine oxidase

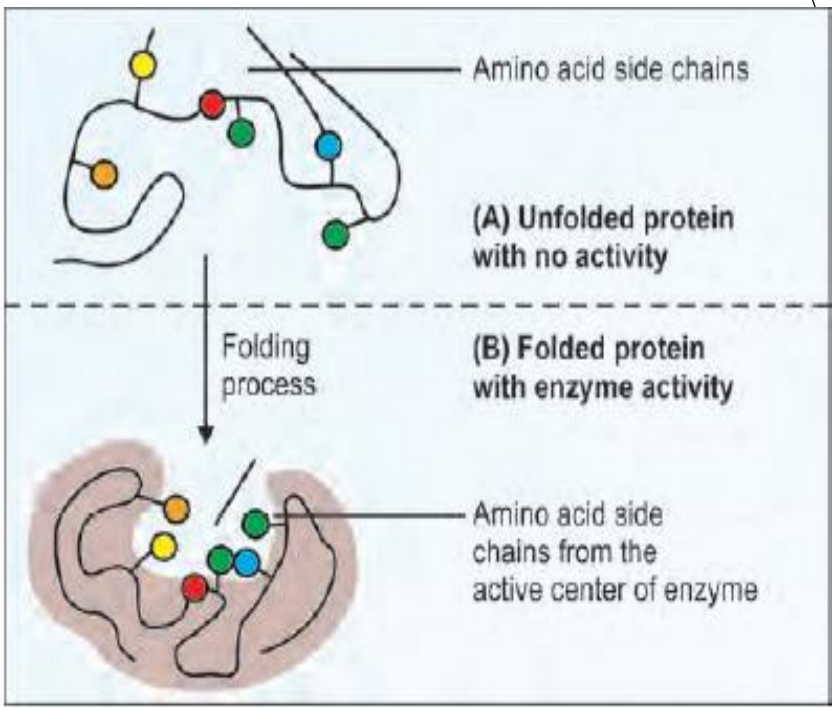


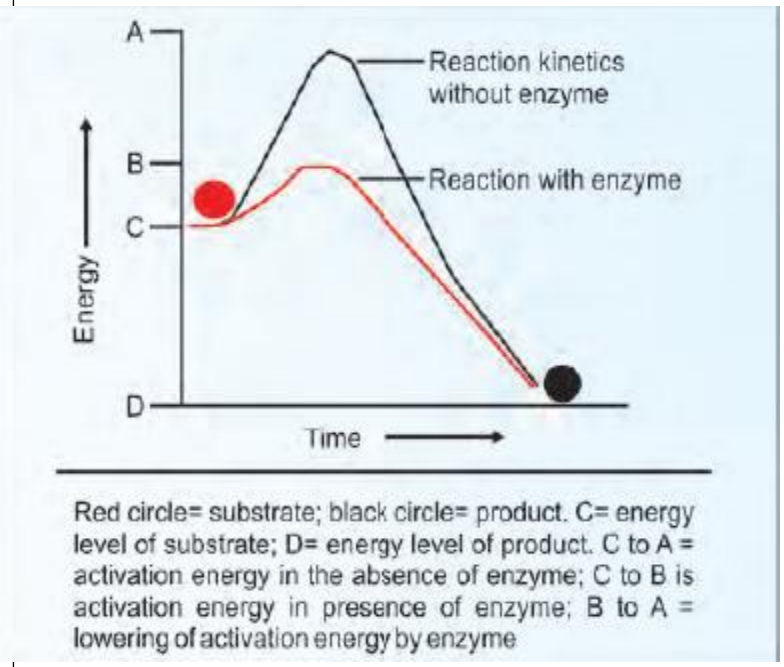
TABLE 5.4: Active center of enzymes

Name of enzyme	*Important amino acid at the catalytic site*
Chymotrypsin	His (57), Asp (102), Ser (195)
Trypsin	Serine, Histidine
Thrombin	Serine, Histidine
Phosphoglucomutase	Serine
Alkaline phosphatase	Serine
Acetylcholinesterase	Serine
Carbonic anhydrase	Cysteine
Hexokinase	Histidine
Carboxypeptidase	Histidine, Arginine, Tyrosine
Aldolase	Lysine

Box 5.7: 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.

Factor Effecting Enzyme Activity



Red circle= substrate; black circle= product. C= energy level of 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

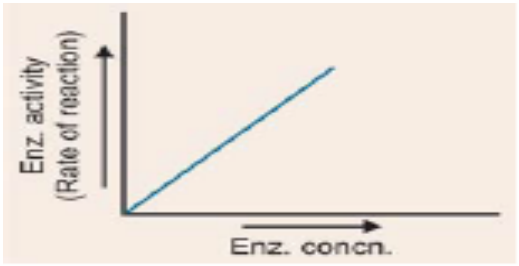


Fig. 9.8: Effect of enzyme concentration on enzymatic reaction

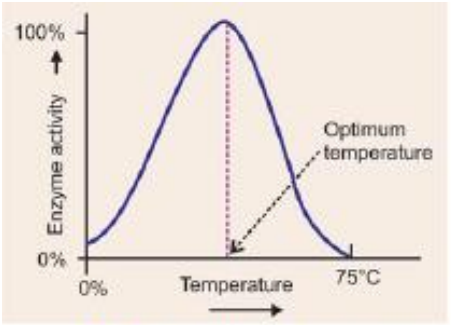


Fig. 9.6: Effect of temperature on enzymatic reaction

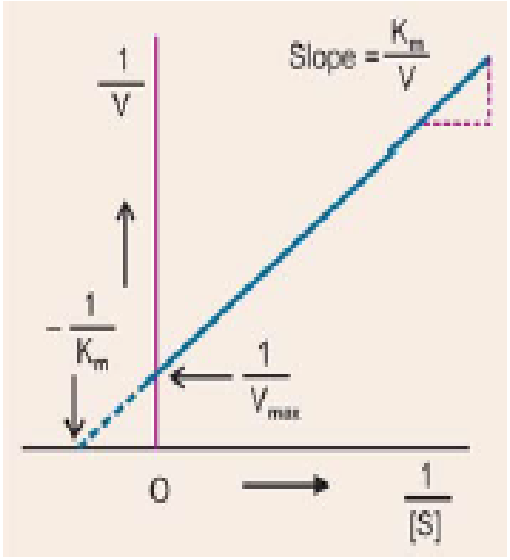


Fig. 9.4: Lineweaver-Burk plot

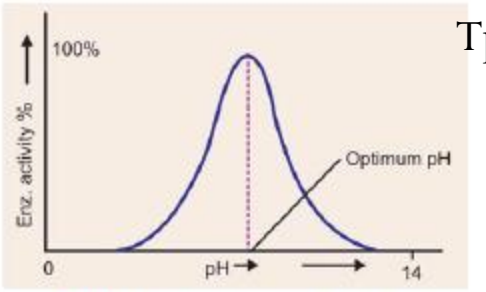
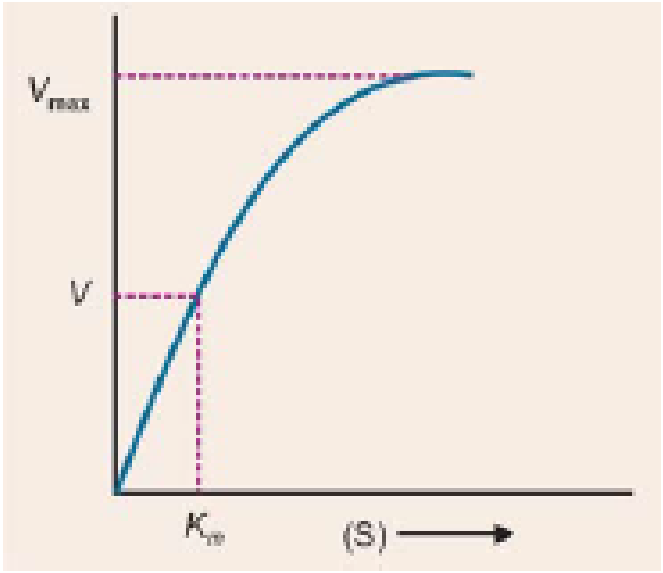


Fig. 9.7: Effect of pH on enzymatic 'reaction'



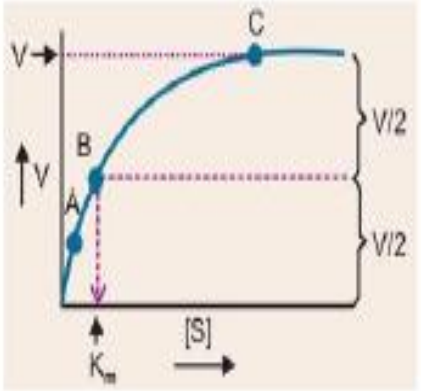
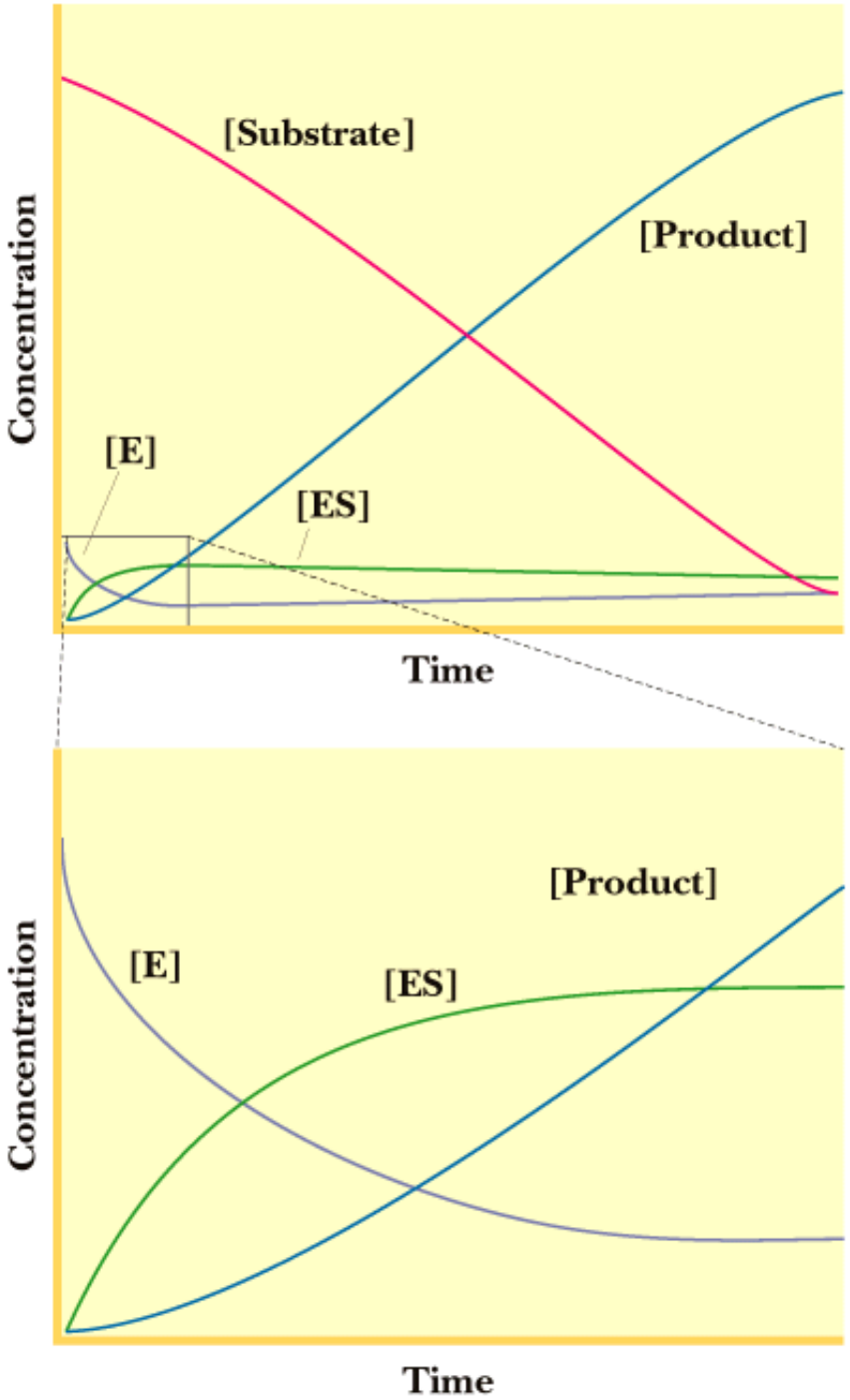
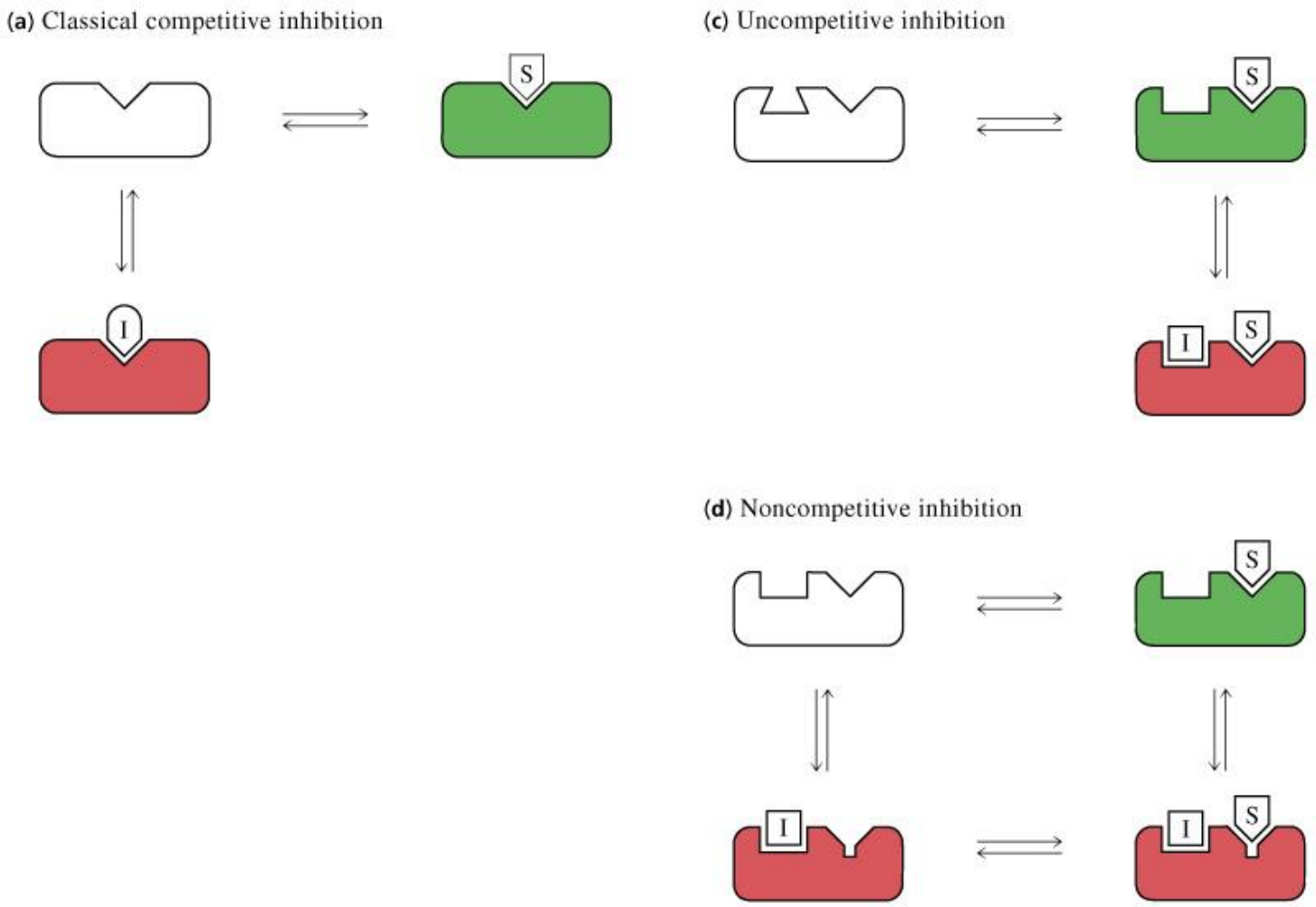


Fig. 9.9: Effect of substrate concentration on enzymatic reaction

Data from a single experiment performed with at a single [S].
(single point on Vo vs. [S] plot)



Types of Reversible Enzyme Inhibitors



Enzyme Inhibition

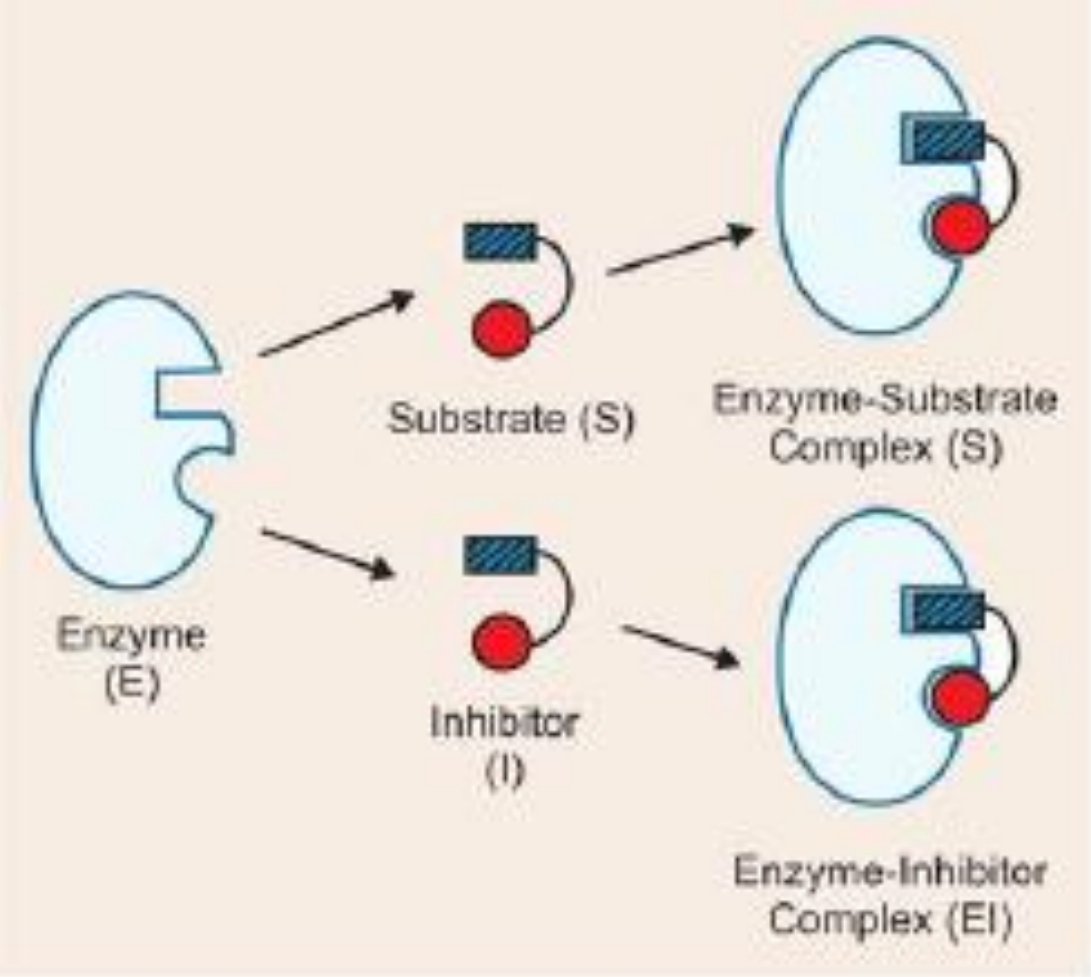


Fig. 9.10: Competitive inhibition

Following are few examples of competitive inhibitors.

Enzyme	Substrate	Competitive inhibitor
• *Lactate Dehydrogenase*	Lactate	Oxamate
• *Aconitase*	Cisaconitate	Transaconitate
• *Succinate Dehydrogenase*	Succinate	Malonate
• *HMG-CoA Reductase*	HMG-CoA	HMG
• *Dihydrofolate Reductase*	7,8 Dihydrofolate	Amethopterin

Diagrammatic presentation of competitive inhibition is given in Figure 9.10.

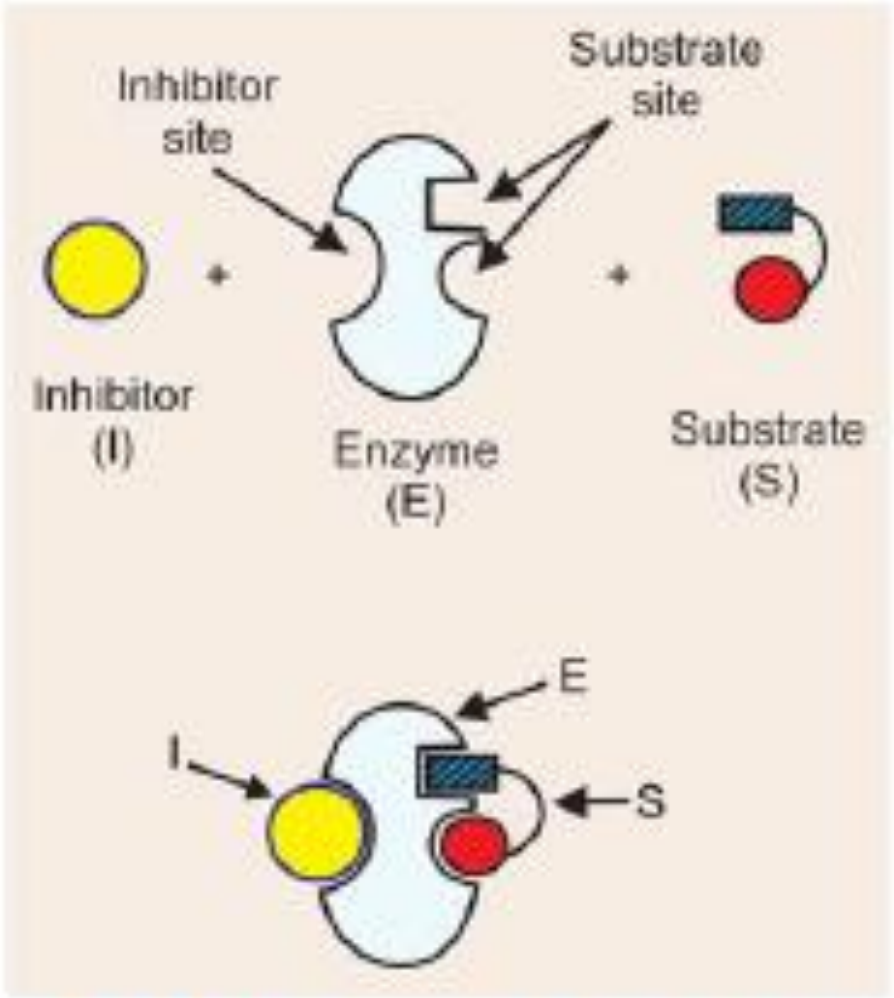


Fig. 9.11: Non-competitive inhibition

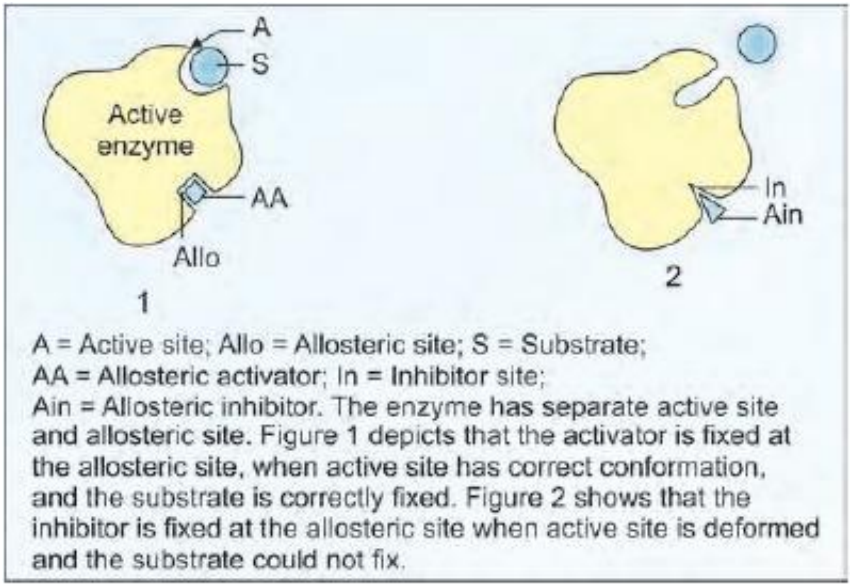


Fig. 5.25: Action of allosteric enzymes

TABLE 5.7: Examples of allosteric enzymes

Enzyme	*Allosteric inhibitor*	*Allosteric activator*
1. Phosphofructokinase	ATP, citrate	AMP, F-2,6-P
2. ALA synthase	Heme	
3. Aspartate trans-carbamoylase	CTP	ATP
4. HMG CoA-reductase	Cholesterol	
5. Pyruvate carboxylase	ADP	AcetylCoA
6. Acetyl CoA-carboxylase	AcylCoA	Citrate
7. Citrate synthase	ATP	
8. Carbamoyl phosphate synthetase I		NAG
9. Carbamoyl phosphate synthetase II	UTP	

Table 9.2: Differentiation of competitive and non-competitive inhibitions

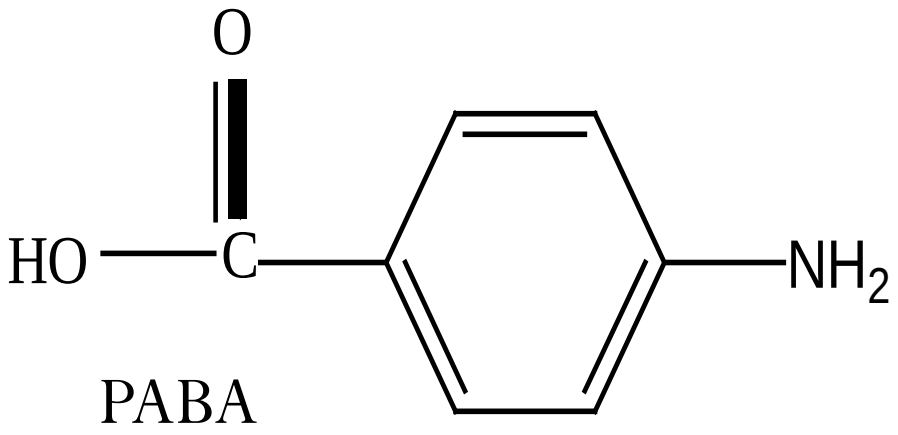
Competitive inhibition	*Non-competitive inhibition*
1. Reversible	1. Reversible or Irreversible
2. Inhibitor and substrate resemble each other in structure	2. Does not resemble
3. Inhibitor binds the active site	3. Inhibitor does not bind the active site
4. V_{max} is same	4. V_{max} lowered
5. K_m increased	5. K_m unaltered
6. Inhibitor cannot bind with ES complex	6. Inhibitor can bind with ES complex
7. Lowers the substrate affinity to enzyme	7. Does not change substrate affinity for the enzyme
8. Complex is E-I	8. Complex is E-S-I or E-I
9. Michaelis-Menten equation changed to $V = \frac{V_{max}[S]}{K_m \frac{1+(I)}{K_i} + S}$	9. Michaelis-Menten equation changed to: $V = \frac{V_{max}[S]}{K_m\left[\frac{1+(I)}{K_i}\right][K_m]+[S]}$
10. Lineweaver-Burk plot: $\frac{1}{V} = \frac{K_m}{V_{max}}[1+\frac{(I)}{K_i}] \times \frac{1}{[S]} + \frac{1}{V_{max}}$	10. Lineweaver-Burk plot: $\frac{1}{V} = \frac{K_m}{V_{max}}[1+\frac{(I)}{K_i}] \times \frac{1}{[S]} + \frac{1}{V_{max}} + [1+\frac{(I)}{K_i}]$
11. Eadie-Hofstee plot: No change in Y intercept (V_{max}) but possesses steeper slope and smaller X intercept	11. Eadie-Hofstee plot: No change in slope ($-K_m$) but Y intercept is lowered and X intercept declines in value

THERAPEUTIC USES OF ENZYMES

Name of the enzyme	*Availability*	*Mechanism of action*	*Indications*
A. Enzymes used systemically			
• *Streptokinase and* • *Urokinase*	Pure stabilised • Streptokinase available 750,000 to 15,00,000 IU vial • Urokinase—50,000 to 500,000 IU vial	Increases amounts of proteolytic enzyme *"plasmin"* by either • Increasing the circulating level of its precursor *"plasminogen"* or • Increasing the conversion of plasminogen to plasmin. Plasmin acts directly on "fibrin" breaking it down to achieve thrombolysis (Fig. 9.14)	• Acute myocardial infarction • Acute thrombosis of arteries • Deep vein thrombosis (DVT) • Pulmonary embolism
• *L-Asparaginase*	Available as *"Leunase"*, 10,000 KU of L-Asparaginase per vial	Certain tumour cells require L-Asparagine for growth L-Asparaginase hydrolyses L-Asparagine and growth of tumour cell suffer	• Acute leukaemia • Malignant lymphomas
• *Digestive enzymes Amylase, lipase and protease*	Available as tablets and syrup	Replacement therapy in pancreatic insufficiency	• Cystic fibrosis • Chronic pancreatitis • Following pancreatectomy
• *α-chymotrypsin*	5.775 mg sublingual tablets	Mucolytic and proteolytic activity	Used as adjunct therapy • In management of inflammatory oedema due to injury, Postsurgical infections and dental procedures
• *Serratopeptidase*	5 mg tablet	Fibrinolytic activity, high bradykinin decomposing activity, and potent caseinolytic activity	• Effective adjunct in inflammation after traumatic injury and after operation • Subconjunctival bleeding
B. Enzymes used locally			
• Hyaluronidase	Available as *"Hyalase"* 1500 IU per ml.	Brings about depolymerisation of ground substance and helps in absorption of fluids	• Promotes diffusion of fluids given subcutaneously (SC) • Intra-articular injection in joints to alleviate pain in osteoarthritis

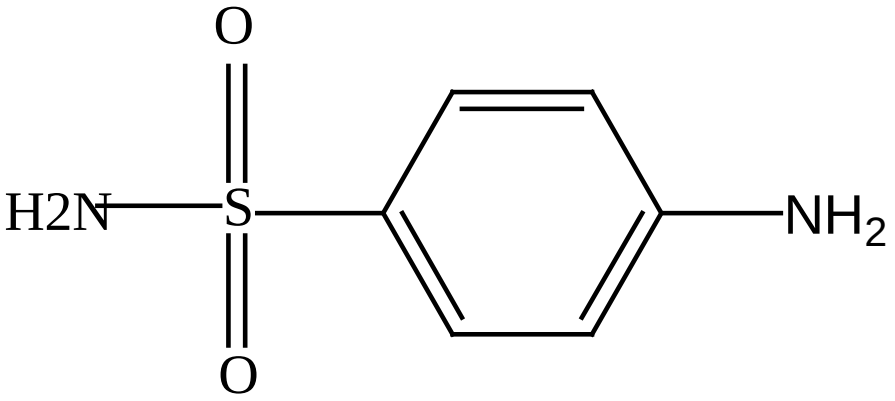
TABLE 5.5: Clinically useful competitive inhibitors

Drug	*Enzyme inhibited*	*Clinical use*
1. Allopurinol	Xanthine oxidase	Gout
2. Dicoumarol	Vitamin K-epoxide-reductase	Anticoagulant
3. Penicillin	Transpeptidase	Antibiotic
4. Sulfonamide	Pteroid synthetase	Antibiotic
5. Trimethoprim	FH2-reductase	Antibiotic
6. Pyrimethamine	Do	Malaria
7. Methotrexate	Do	Cancer
8. 6-mercapto-purine	Adenylosuccinate synthetase	Cancer
9. 5-fluorouracil	Thymidylate synthase	Cancer
10. Azaserine	Phosphoribosyl-amidotransferase	Cancer
11. Cytosine arabinoside	DNA polymerase	Cancer
12. Acyclovir	Do	Virus
13. Neostigmine	ACh-esterase	Myasthenia
14. Alpha-methyl dopa	Dopa-decarboxylase	Hypertension
15. Lovastatin	HMGCoA-reductase	Cholesterol lowering
16. Oseltamiver (Tamiflu)	Neuraminidase	Influenza



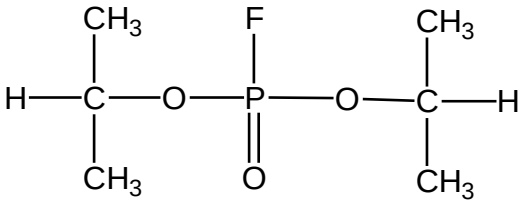
PABA

PABA precursor to folic acid in bacteria

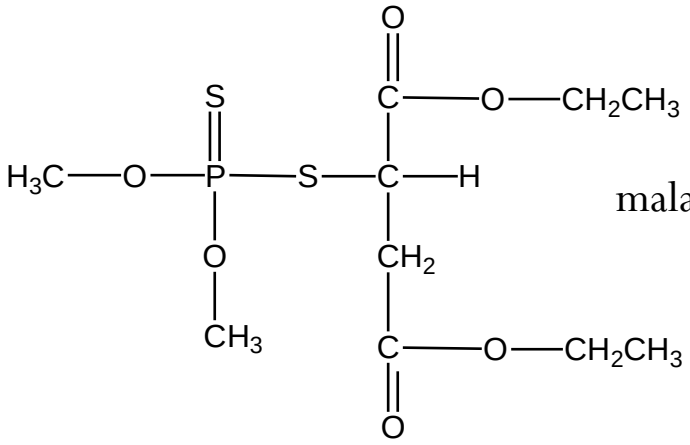


Sulfanilamide

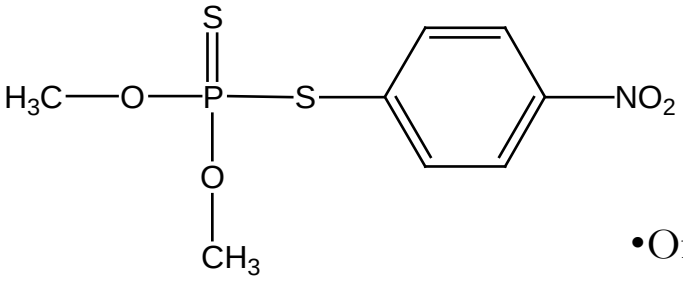
Irreversible Inhibitors



Diisopropyl fluorophosphate
(nerve gas)



malathion



parathion

- Organophosphates
- Inhibit serine hydrolases
- Acetylcholinesterase inhibitors

Inhibition of Enzyme Activity Neostigmine

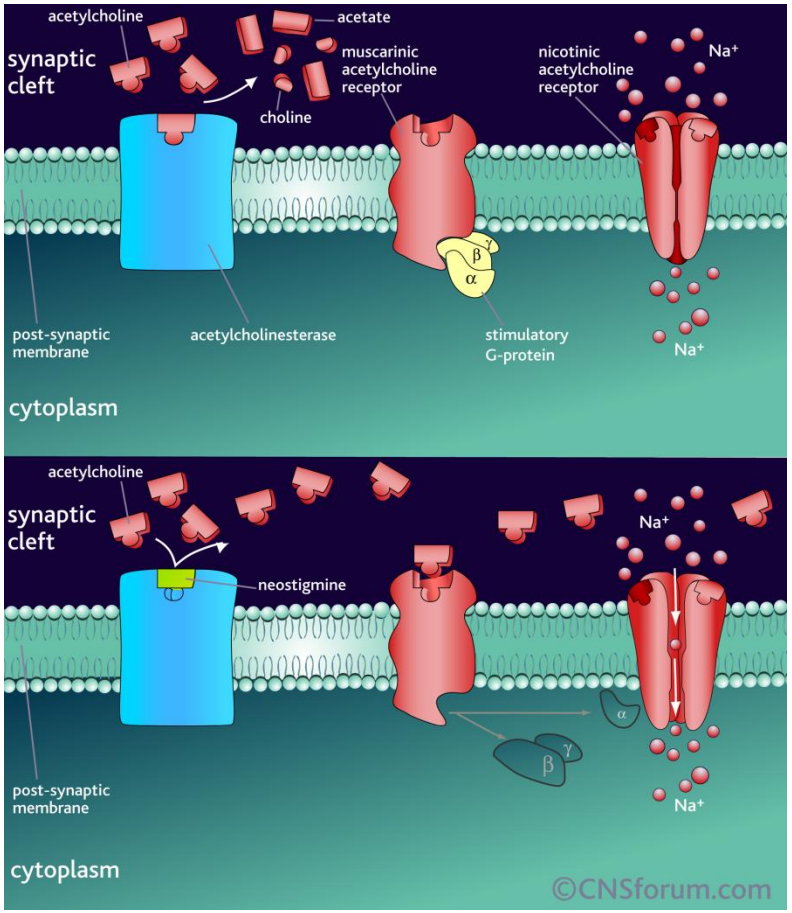
Mechanism of of action:

anti-cholinesterase

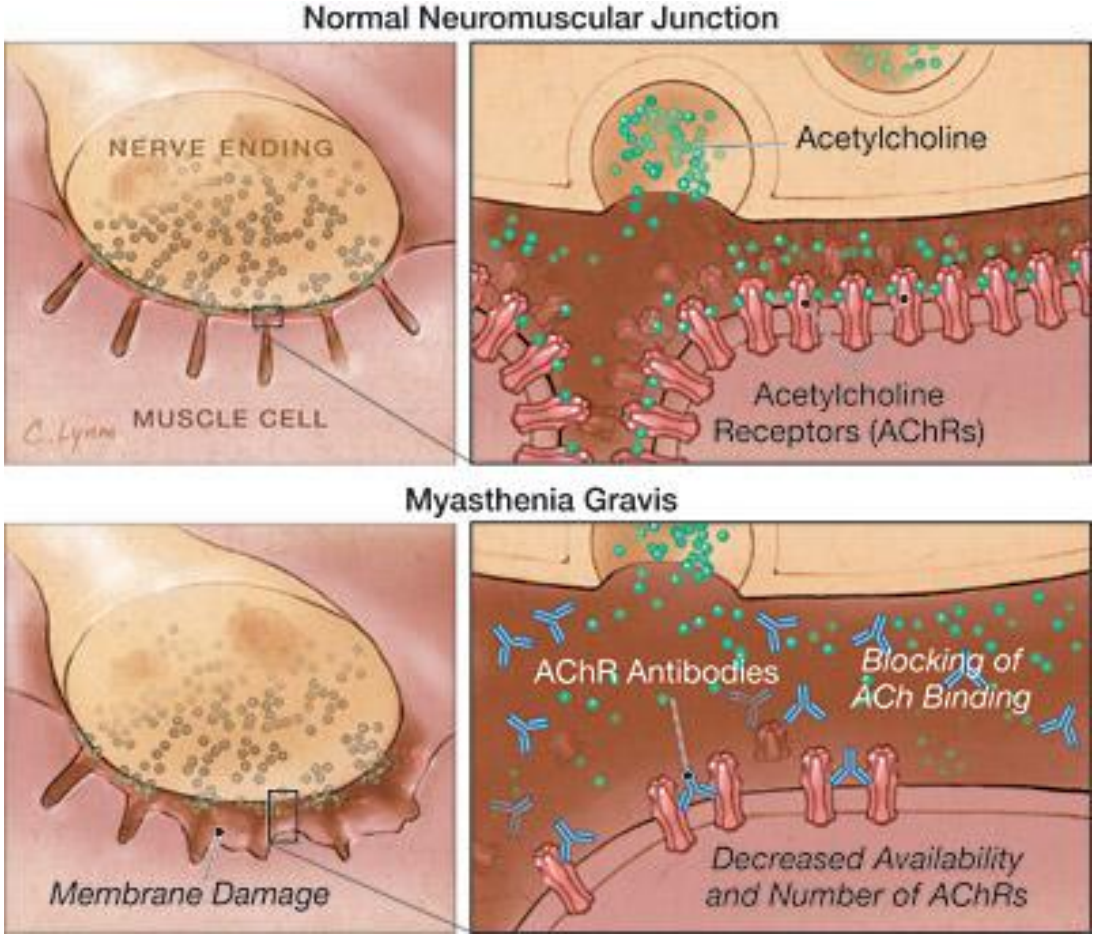
Inhibits activity of acetyl-cholinesterase

Effects: increase Acetylcholine (ACh) levels in the synapse.

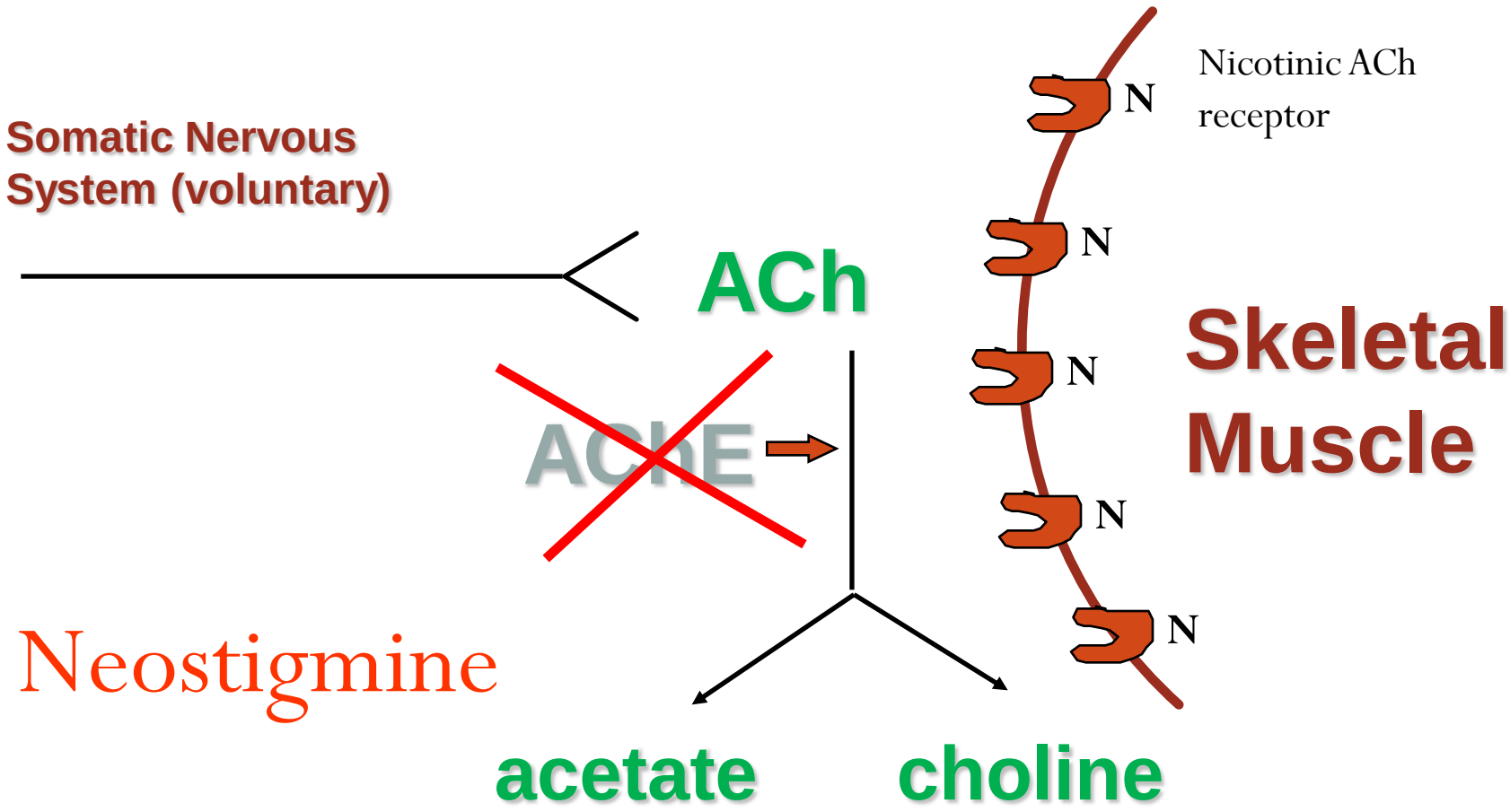
Clinical use: treatment of *myasthenia gravis*



Myasthenia Gravis: autoimmune disease



Action of Neostigmine



Inflammation and COX

Infection

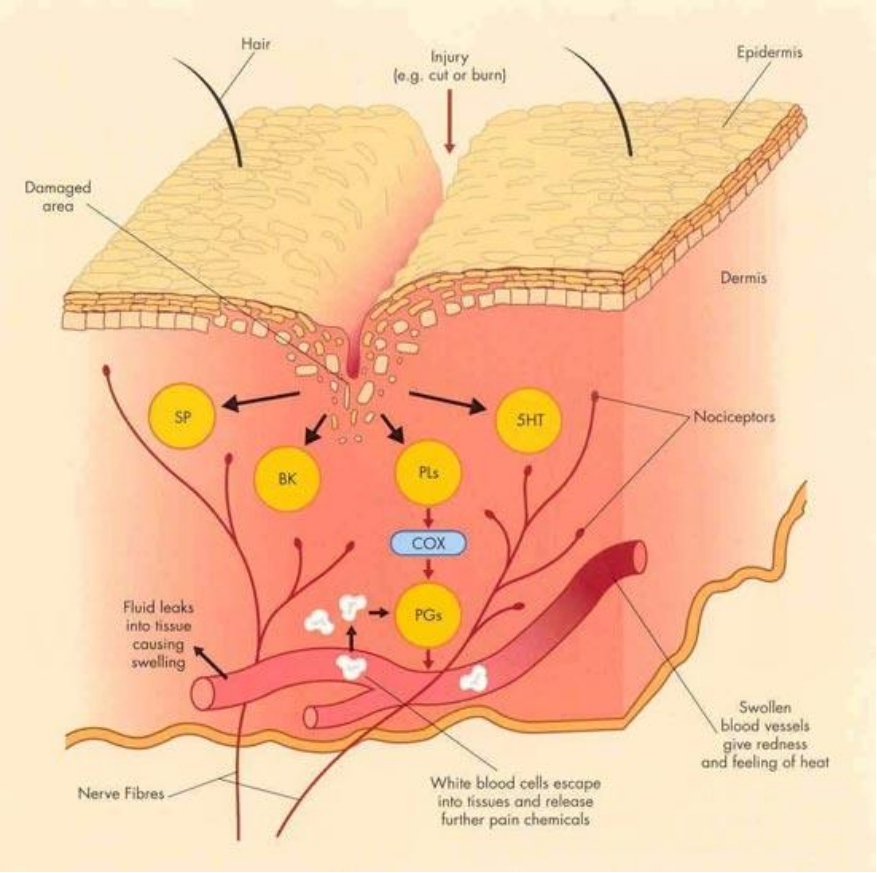
Arachidonic Acid

(released from cell membrane)

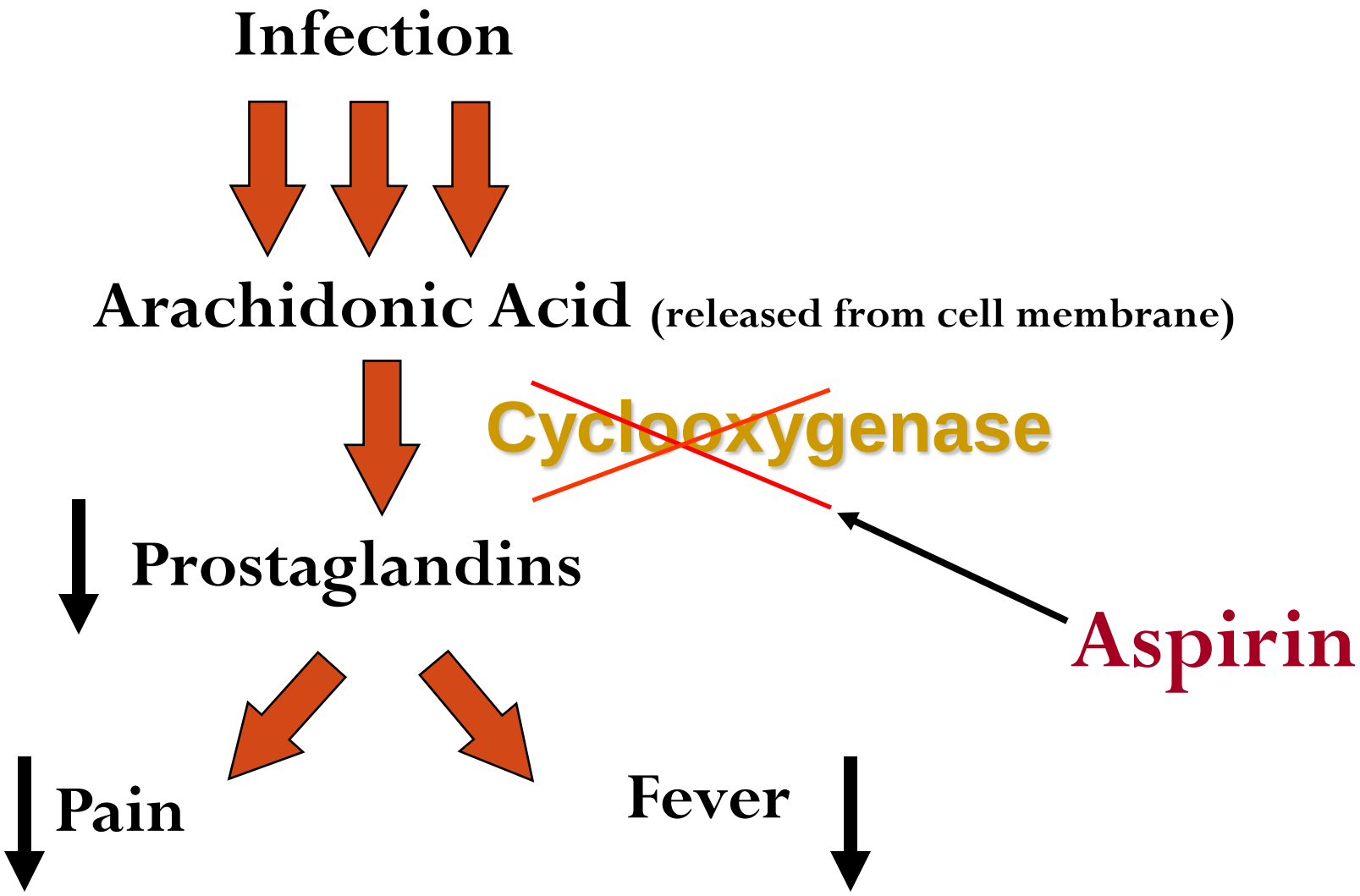
Cyclooxygenase (COX)

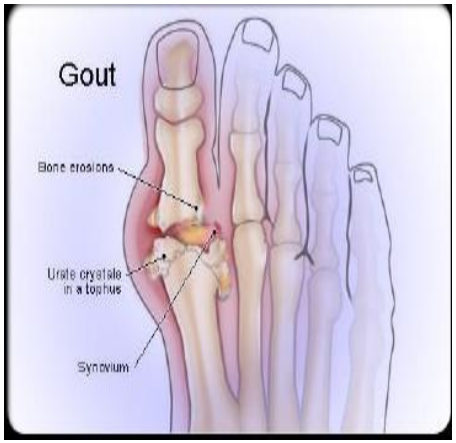
Prostaglandins

Pain **Fever**



Action of Aspirin

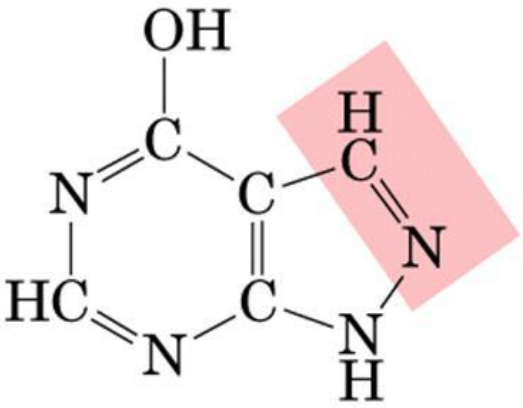




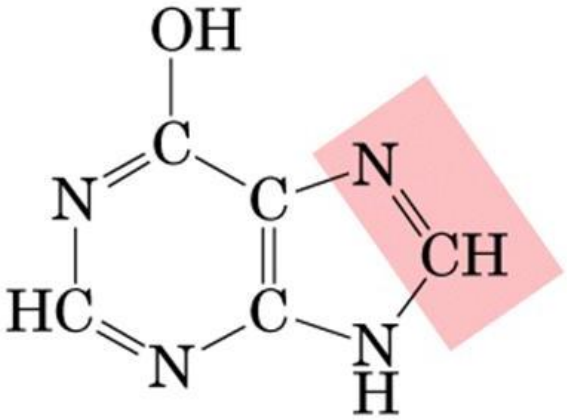
Gout

- Gout is a disease due to high levels of uric acid in body fluids
- 7.0 mg/dL and above (normal: 2.5-5 mg/dL)
- Uric acid accumulates because of:
  - Overproduction or
  - Underexcretion
- Gout used to be called the disease of the rich because the rich would always drink alcohol and eat plenty of meat - famous people who had gout were Benjamin Franklin and Isaac Newton

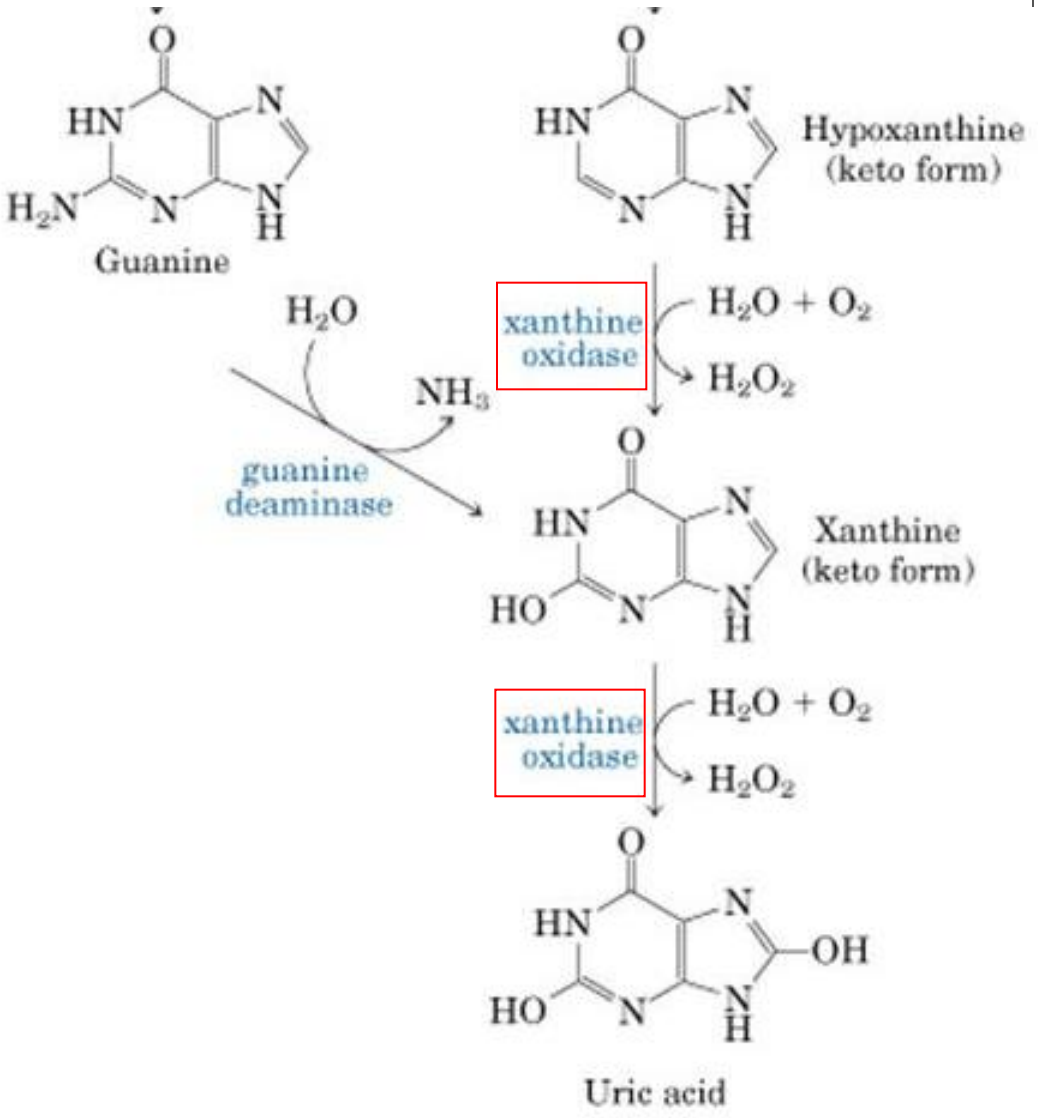
Allopurinol and Hypoxanthine



Allopurinol



Hypoxanthine
(enol form)



Regulation of Enzyme Activity

Enzyme quantity – regulation of gene expression (Response time = minutes to hours)

a) Transcription

b) Translation

c) Enzyme turnover

Enzyme activity (rapid response time = fraction of seconds)

a) Allosteric regulation

b) Covalent modification

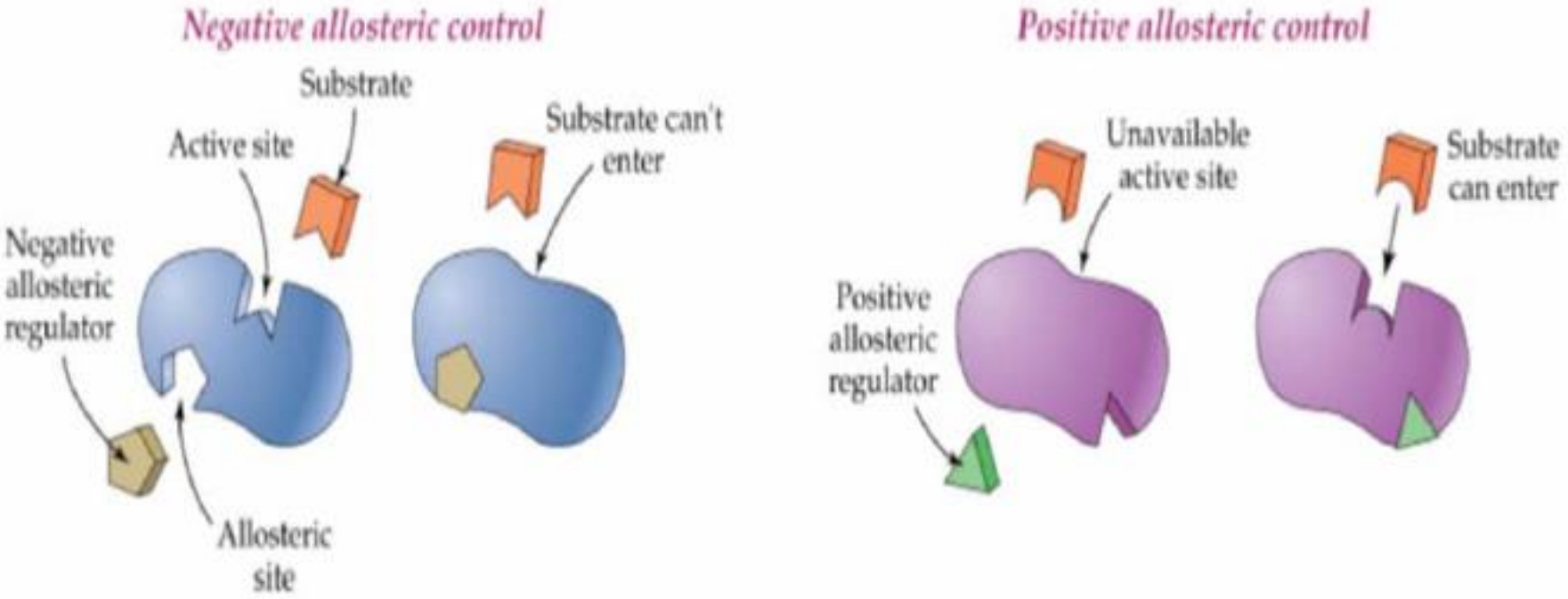
c) Association-disassociation'

d) Proteolytic cleavage of proenzyme

Allosteric Regulation

- End products are often inhibitors
- Allosteric modulators bind to site other than the active site
- Allosteric enzymes usually have 4° structure
- Vo vs [S] plots give sigmoidal curve for at least one substrate
- Can remove allosteric site without effecting enzymatic action

Allosteric control: either an activator or inhibitor acts on a portion of the enzyme other than the active site to regulate enzyme function.



ENZYMES WITH ALLOSTERIC EFFECTORS

Enzyme	Metabolic pathway	Inhibitor	Allosteric Activator
Hexokinase	Glycolysis	Glucose6-Po4	-
Phosphfructo kinase	Glycolysis	ATP	AMP,ADP
Pyruvate carboxylase	Glucooneogeonesis	–	Acetyl CoA
Acetyl CoA carboxylase	Fatty acid synthesis	Palmitate	Isocitrate

Phosphofructokinase(PFK)

Fructose-6-P + ATP -----> Fructose-1,6-bisphosphate + ADP

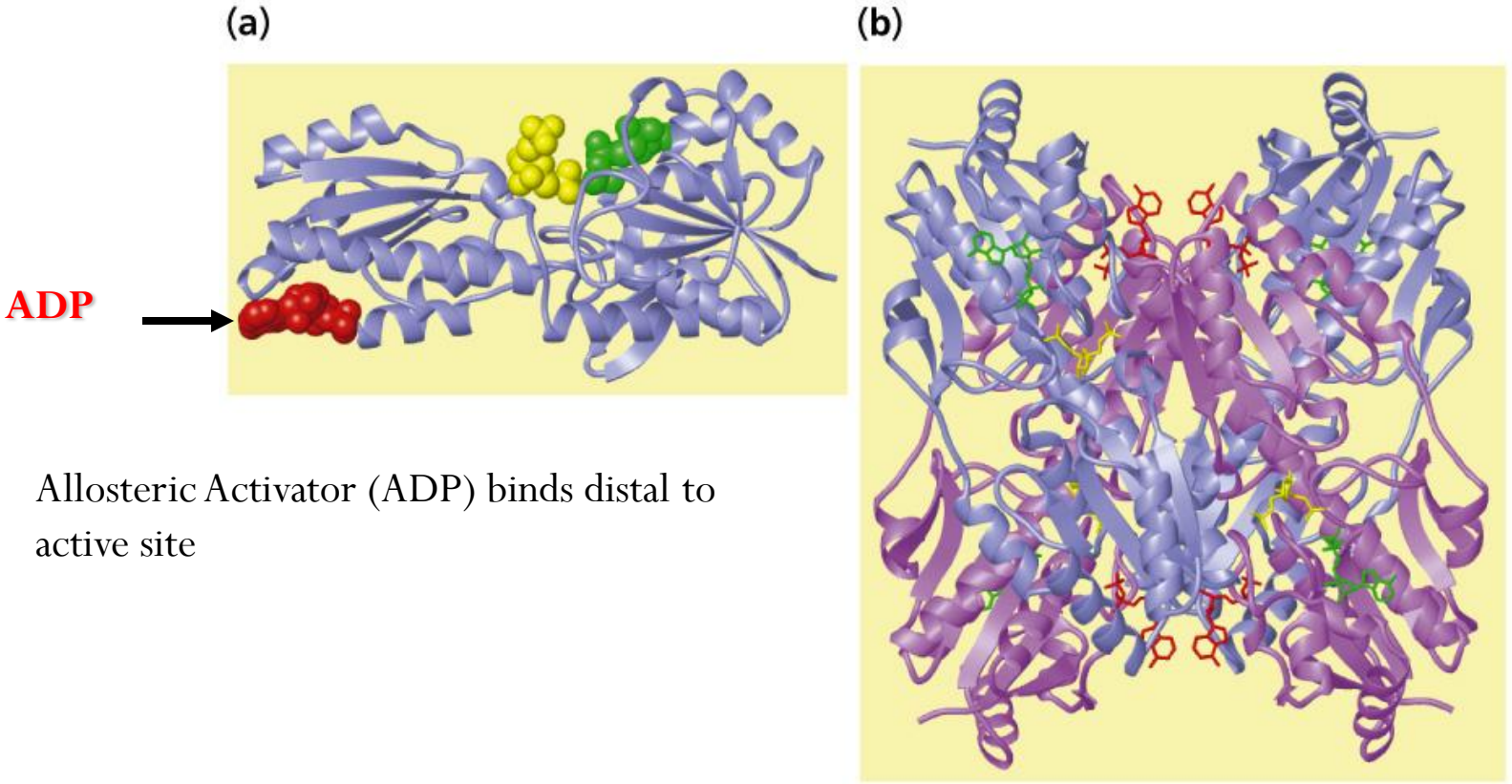
- **PFK catalyzes 1st committed step in glycolysis (10 steps total)**

(Glucose + 2ADP + 2 NAD^+ + 2Pi → 2pyruvate + 2ATP + 2NADH)

- **Phosphoenolpyruvate is an allosteric inhibitor of PFK**
- **ADP is an allosteric activator of PFK**

Allosteric modulators bind to site other than the active site and allosteric enzymes have 4° structure

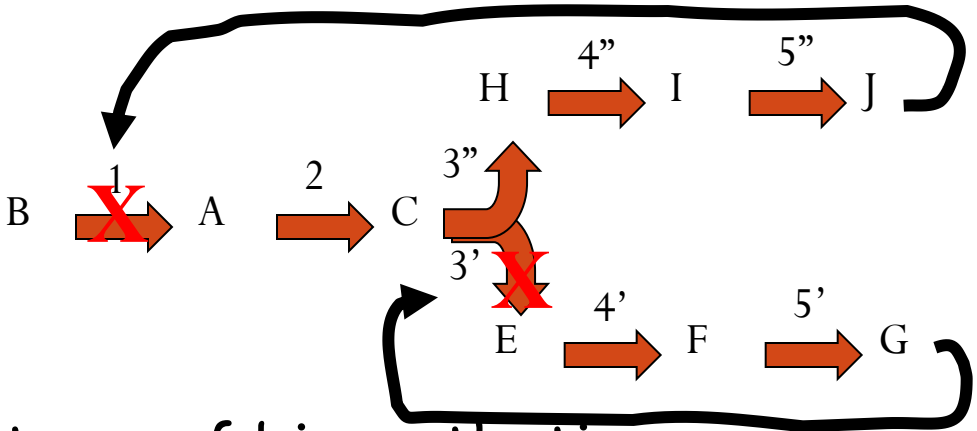
Fructose-6-P + ATP -----> Fructose-1,6-bisphosphate + ADP



Allosteric Activator (ADP) binds distal to active site

Regulation of Enzyme Activity (biochemical regulation)

- 1st committed step of a biosynthetic pathway or enzymes at pathway branch points often regulated by feedback inhibition.

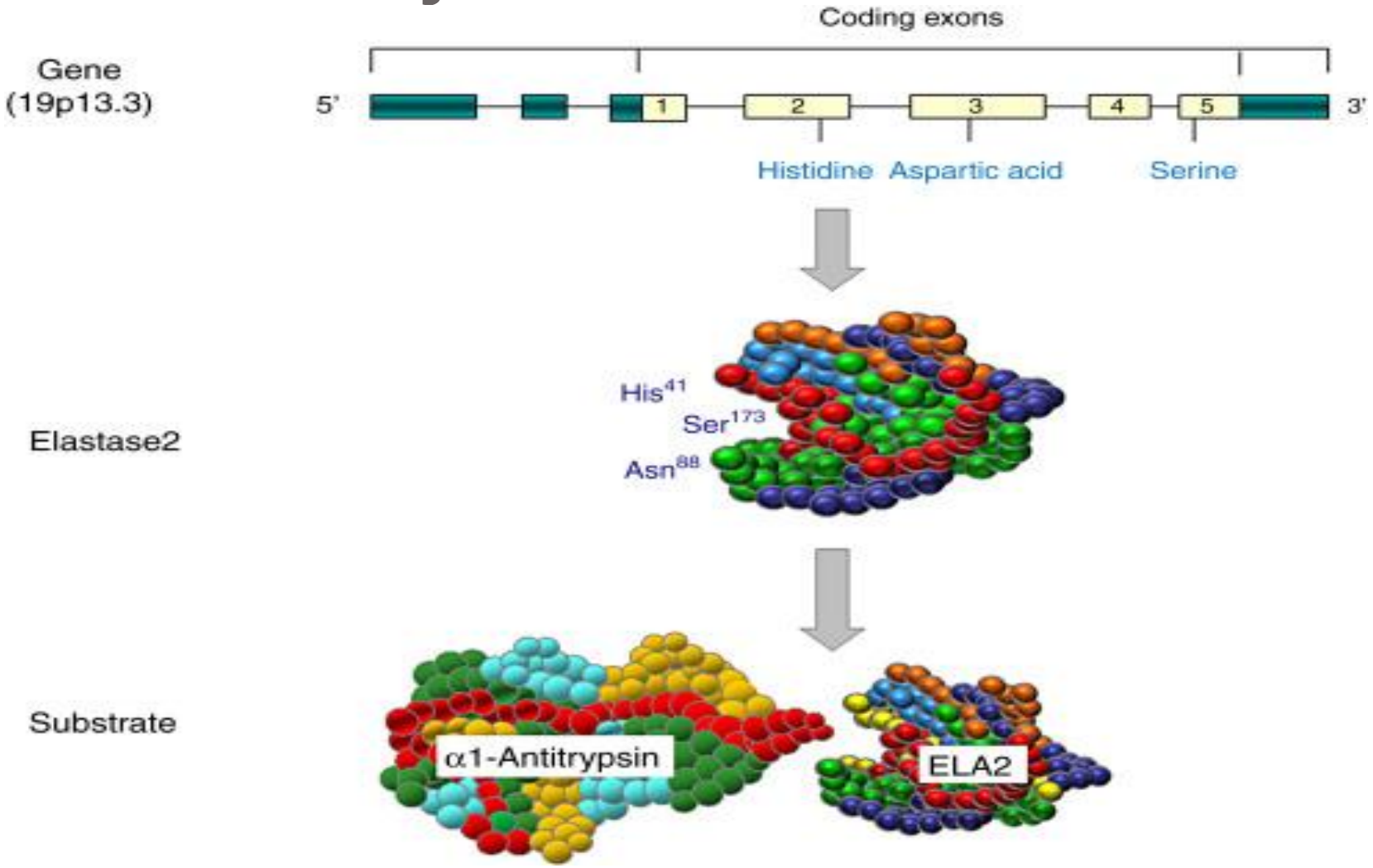


- Efficient use of biosynthetic precursors and energy

REGULATION BY COVALENT MODIFICATION

- Many enzymes exists in the active and inactive form which are intercovertible depending upon the needs of the body
- Covalent modification is the change in the activity of the enzyme by either adding a group to enzyme protein by covalent bond or removing a group by cleaving a covalent bond.
- Includes,
    1. Phosphorylation and dephosphorylation
    2. Adenylation and deAdeylation
    3. ADP ribosylation,Uridylation ,Methylation

Model Kerja Enzim Elastase



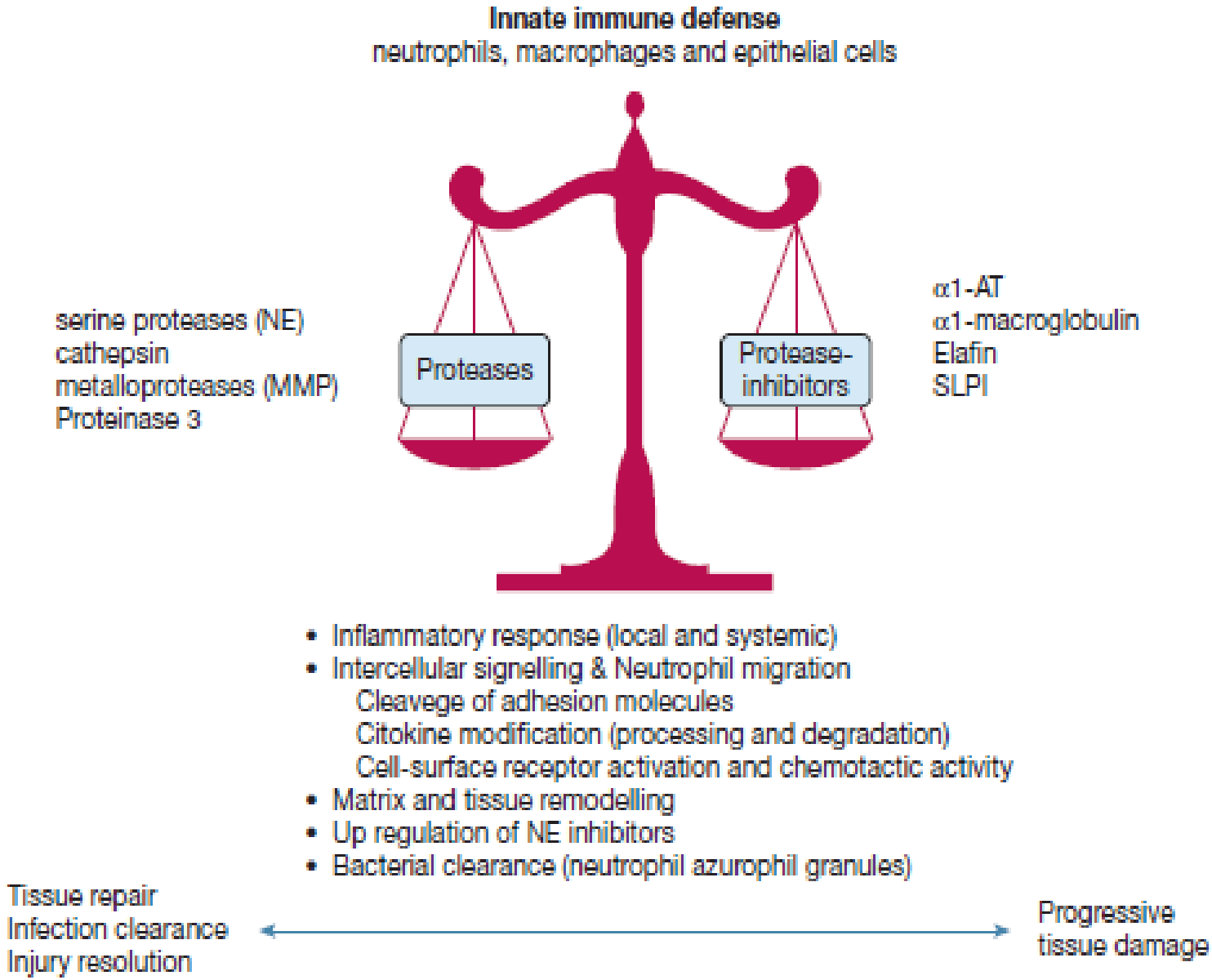


Figure 1 – *Innate immune defense. α1-AT = alpha1 antitrypsin; MMP = matrix metalloproteinase; NE = neutrophil elastase; SLPI = secretory leukocyte protease inhibitor.*

ACTIVATION OF LATENT ENZYMES

- Some enzymes exists in latent forms and latent forms as such are inactive for Eg enzymes may be synthesized as proenzymes or zymogens which undergo irreversible covalent activation by the breakdown of one or more peptide bonds
- E.g. chymotripsinogen, trypsinogen, and plasminogen are respectively converted to chymotrypsin, trypsin and plasmin

COARSE CONTROL

- Modification in the concentration of the enzyme this is done by regulating the rate of enzyme synthesis that is Induction and repression of enzyme synthesis
- Induction – Increased synthesis of the enzyme
- Repression – Decreased synthesis of the enzyme
- This is coordinated at the level of gene and is also mediated mainly through the hormones

CLINICAL APPLICATION OF ENZYMES

- Enzymes are present in all the tissues
- It is having different applications clinically

1. Diagnosing a disease
2. To diagnose a congenital disorder
3. Measurement of compounds
4. As therapeutic agents
5. Enzymes linked to insoluble materials are used as chemical reactors.
6. Most of the drugs are acting by inhibiting the enzymes

ENZYMES IN DIAGNOSIS

Functional	Non- functional
Specific to plasma	Enters plasma from tissues
Have definite function	No function in plasma
Present in high concentration	Normally the level will be low

These non- functional enzymes are more important for clinician to diagnose a disease

ENTRY OF TISSUE ENZYMES INTO PLASMA

They enter

- when disease process cause changes in cell membrane permeability
- When cell dies
- Since there is gross difference between intracellular and extra cellular concentration

ENZYME LEVELS IN PLASMA

- The levels are maintained by the following factors

1. Rate of release of enzymes into plasma
2. Stability of enzymes in plasma
3. The clearance rate of enzymes by reticulo - endothelial system (Half –life)

Plasma enzymes

1. Plasma specific enzymes:

   cholinesterase,

   plasma superoxid dismutase,

   lecithin-cholesterol acyltransferase,

   Serin proteases – inactive zymogens of coagulation factors and factors of fibrinolysis (faktor II - prothrombin, factor VII, IX, XIII) and complement system components, non-specific immune system (components C1 – C9).

Plasma enzymes (cont.)

Enzyma name	**abbreviation**	**Causes leading to increased levels**
Alanine aminotransferase	ALT	liver and biliary tract disease pancreatic disease decompensated heart defects
Aspartate aminotransferase	AST	liver diseases myokardium damage disease of skeletal muscle and myocardium
akcaline phosphatase	ALP	liver and biliary tract disease bone diseases
Creatin kinase	CK	disease of skeletal muscle and myocardium
Lactate dehydrogenase	LD_{1-5}	Myocardium disease (LD_1, LD_2) and muscle disease hepatopathy
γ-glutamy ltransferasa	GMT	liver and biliary tract disease and pancreatic disease

Enzyme	Therapeutic Application
Asparginase	Acute Lymphoblastic Leukemia
Streptokinase	To lyse Intravascular Clot
Urokinase	Do
Streptodornase	DNAse; applied locally
Hyaluronidase	Enhances Local Anaesthetics
Pancreatin(Trypsin and Lipase)	Pancreatic Insufficiency; oral administration
Papain	Anti-Inflammatory
Alpha 1 – Antitrypsin	AAT Deficieny; Emphysema


thank you