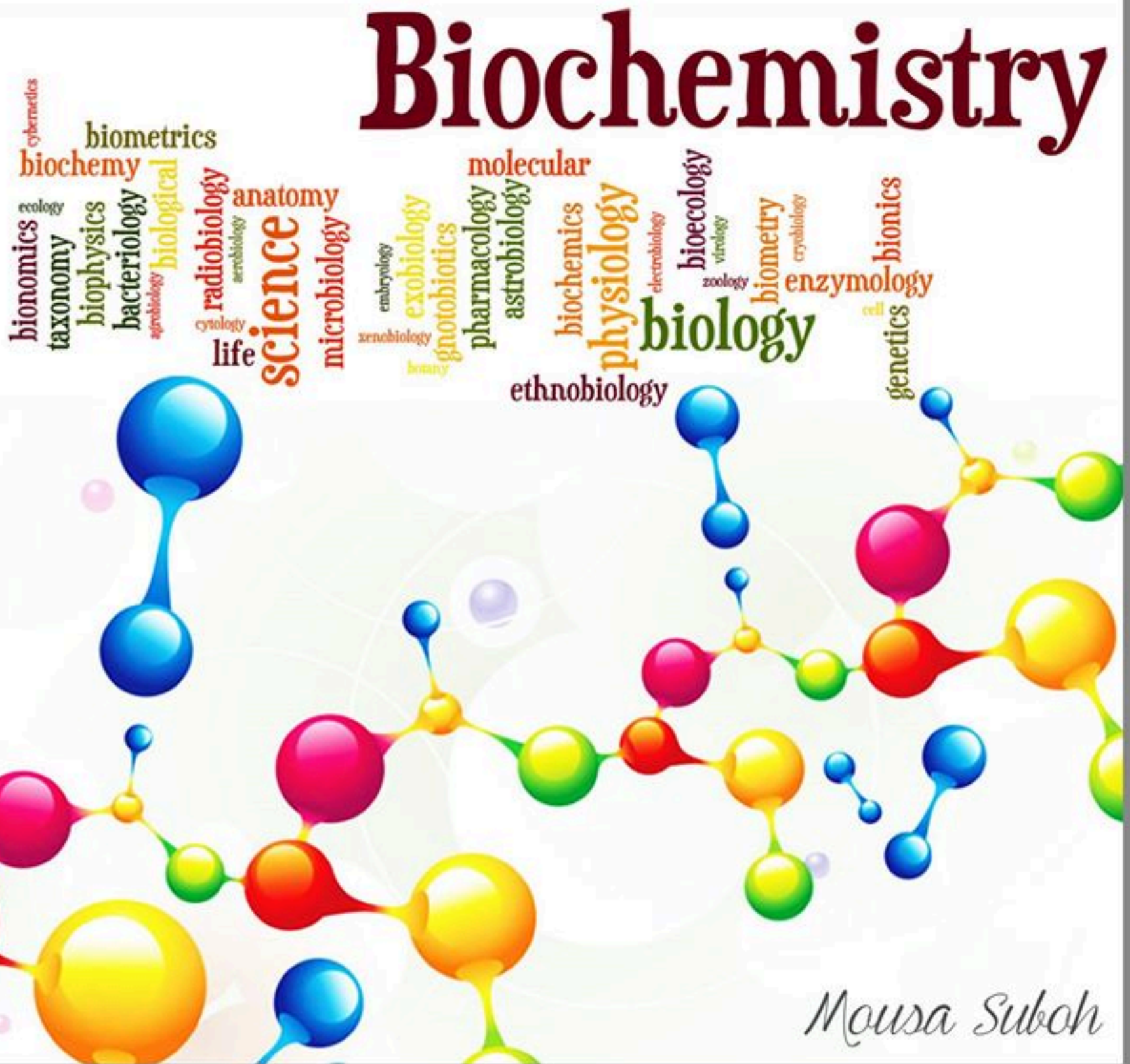
Slide : Enzymes part 4

Dr. Name : Dr.Nafez Abu Tarboosh

Sections : 1,2,3

■ Slide □ Sheet

Medical Committee
The University of Jordan



Nafith Abu Tarboush
DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

ENZYMES

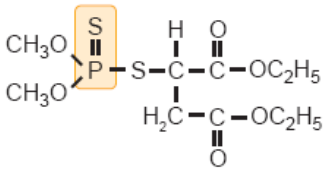
2. Inhbition

2.1 MECHANISM-BASED INHIBITORS

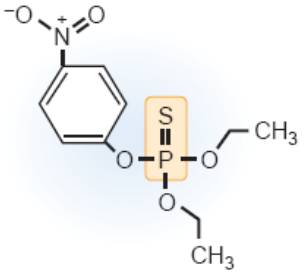
- Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction
- The term includes:
- A. Covalent inhibitors
- B. Transition state analogs
- C. Heavy metals

- The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme

2.1.A. Covalent Inhibitors



Malathion



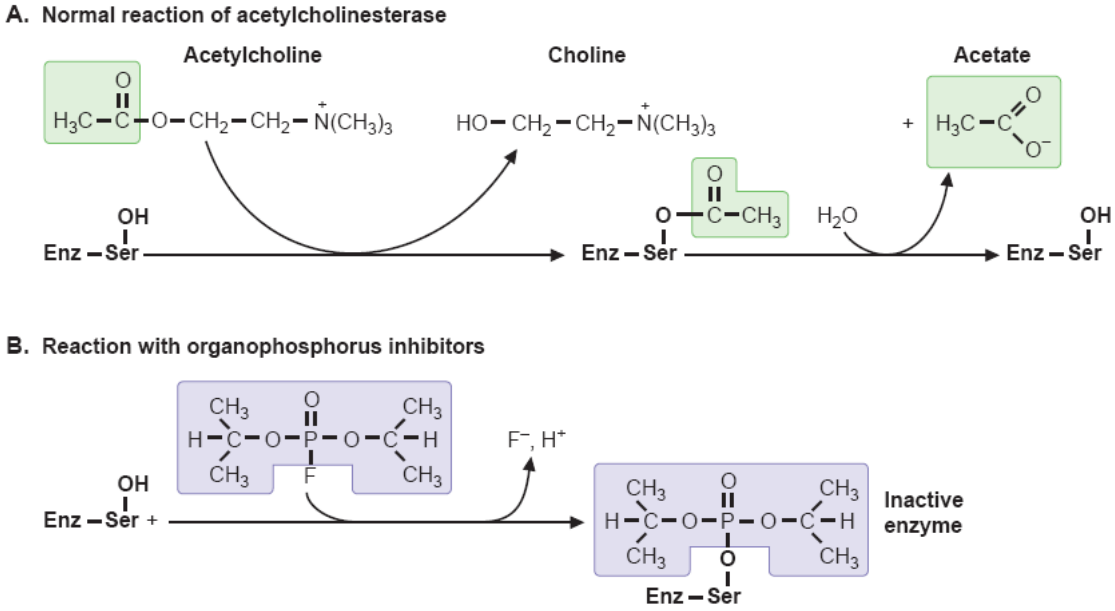
Parathion

- Covalent or extremely tight bonds with aactive site mino acids
- Amino acids are targeted by drugs & toxins

- The lethal compound [DFP] is an organophosphorus compound that served as a prototype for:
  - The nerve gas sarin
  - The insecticides malathion & parathion

Sarin

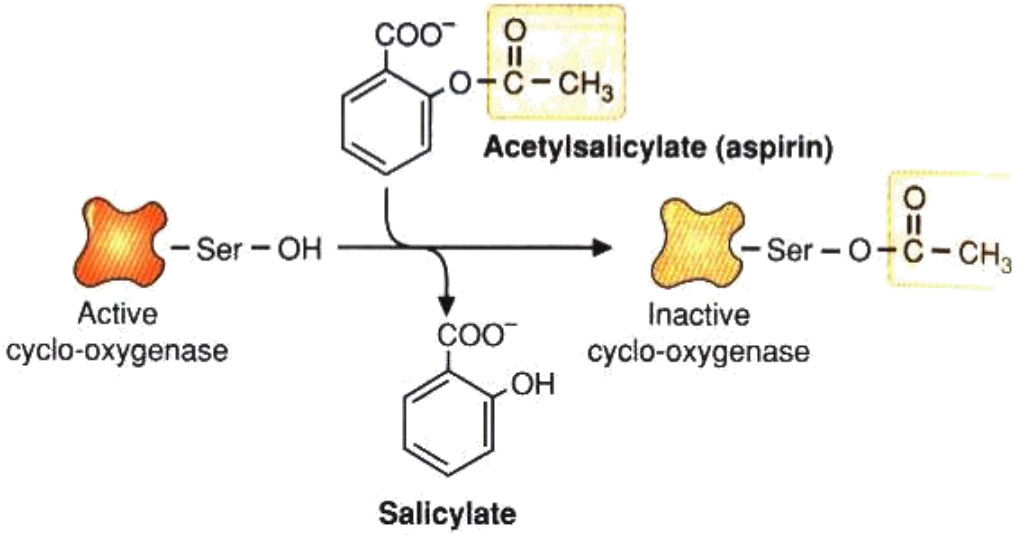
- DFP also inhibits other enzymes that use serine (ex. serine proteases), but the inhibition is not as lethal



2.1.A. Covalent Inhibitors

- **Aspirin (acetylsalicylic acid): covalent acetylation of an active site serine in the enzyme prostaglandin endoperoxide synthase (cyclooxygenase)**

- **Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme**

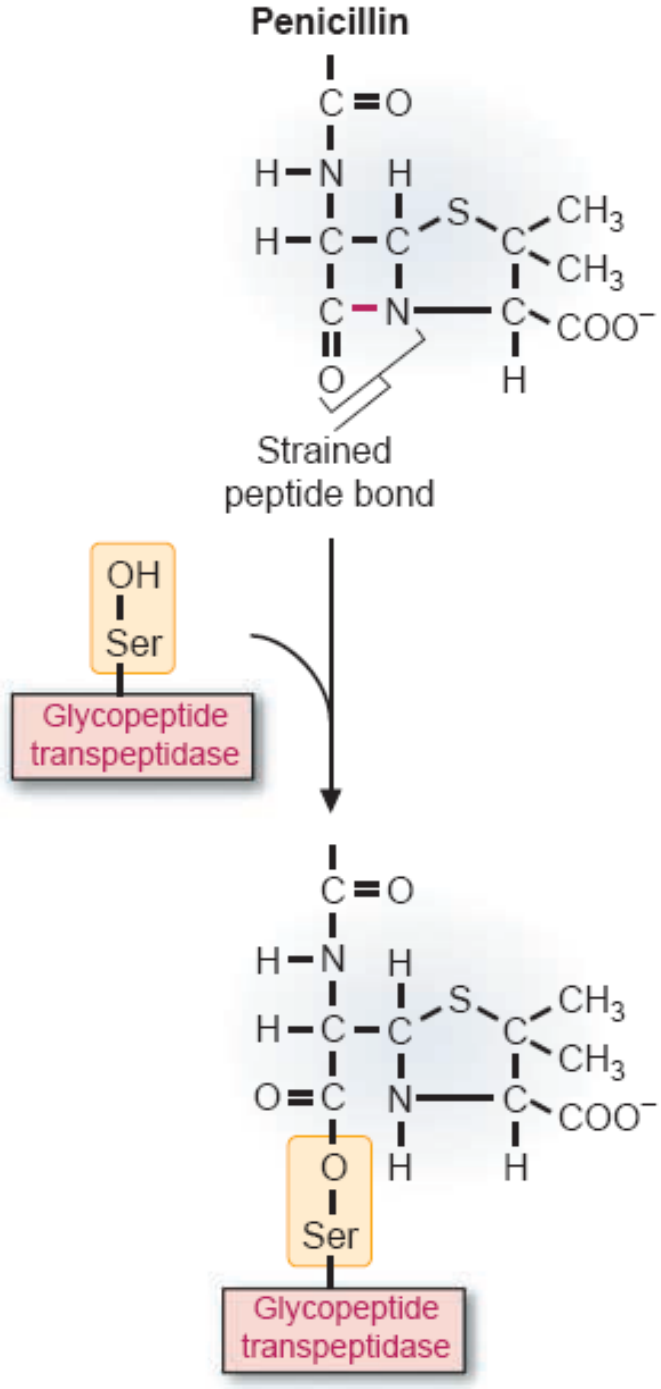


2.1.B. Transition-State Analogs & Compounds that Resemble Intermediate Stages of the Reaction

- Transition-state analogs: extremely potent inhibitors (bind more tightly)
- Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure)
- Substrate analogs: even though they bind more tightly than substrates

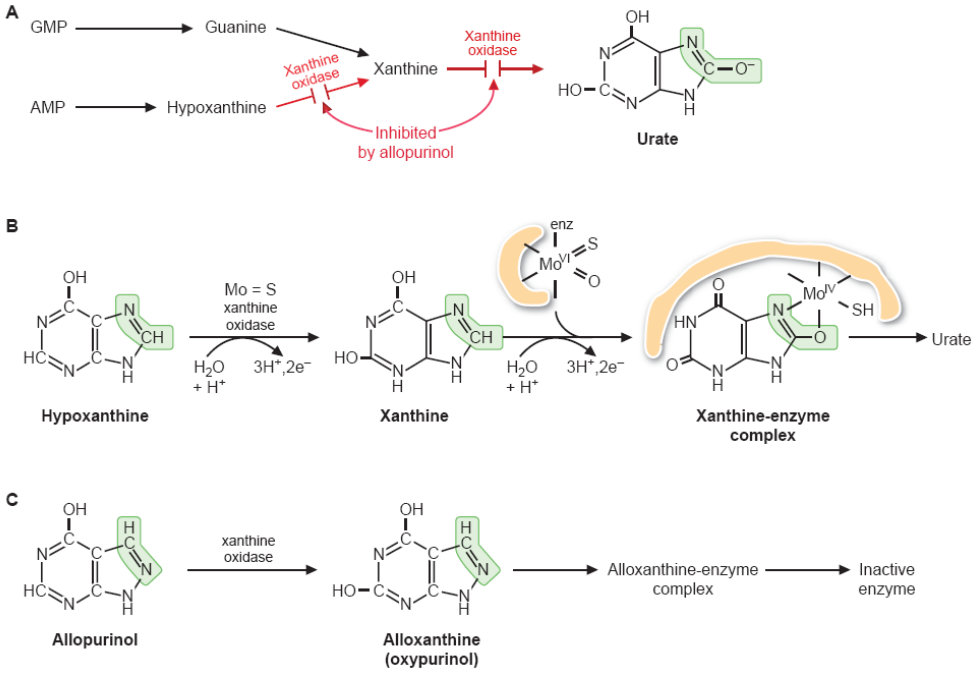
2.1.B.1 PENICILLIN

- A transition-state analog to *glycopeptidyl transferase*
- Required by bacteria for synthesis of the cell wall
- The reaction is favored by the strong resemblance between the peptide bond in the β-lactam ring of penicillin & the transition-state complex of the natural transpeptidation reaction
- Inhibitors that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed *suicide inhibitors*



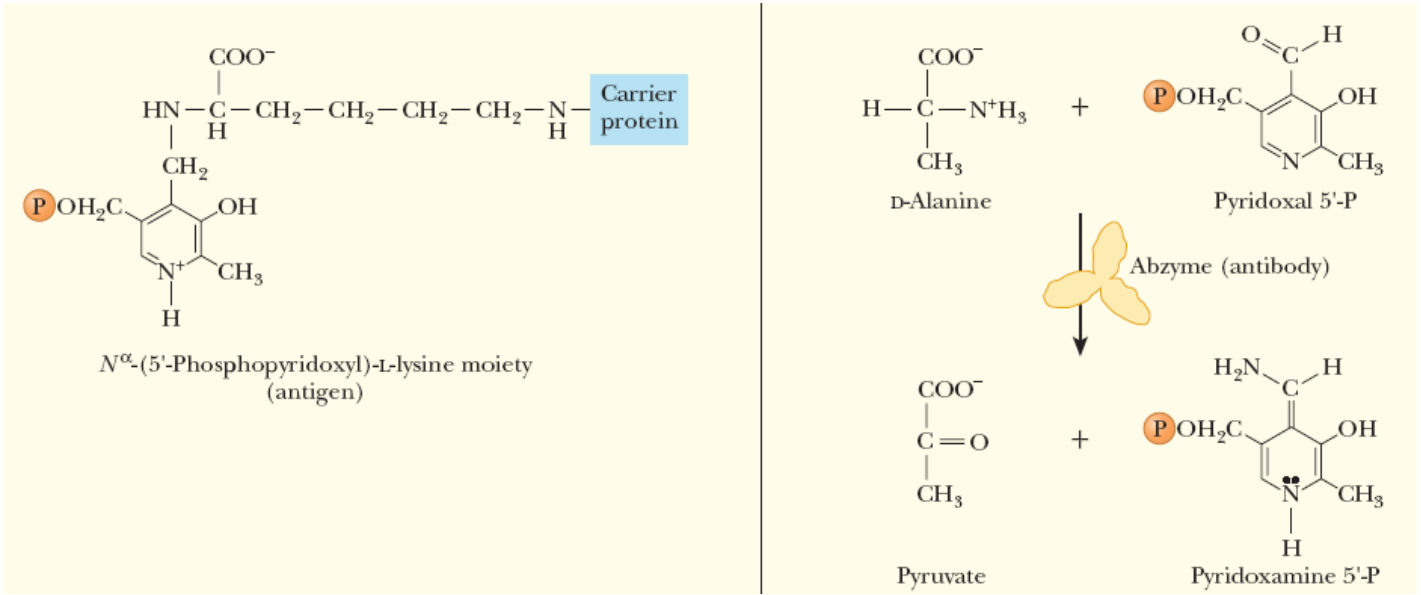
2.1.B.2 ALLOPURINOL

- A drug used to treat gout
- Decreases urate production by inhibiting xanthine oxidase
- The enzyme commits suicide by converting the drug to a transition-state analog
- The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the oxidation reactions
- Xanthine oxidase oxidizes the drug allopurinol to oxypurinol, a compound that binds very tightly to a molybdenum–sulfide complex in the active site



2.1.B.3. Abzymes

- An antibody that is produced against a transition-state analog & that has catalytic activity similar to that of a naturally occurring enzyme
- An abzyme is created by injecting a host animal with a transition-state analogue of a reaction of interest
- The host animal makes antibodies to the foreign molecule, & these antibodies have specific binding points that mimic an enzyme surrounding a transition state



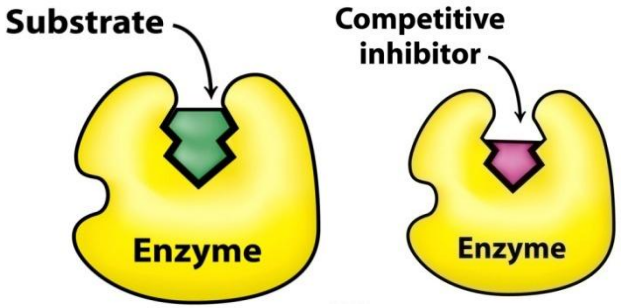
2.1.C. Heavy Metals

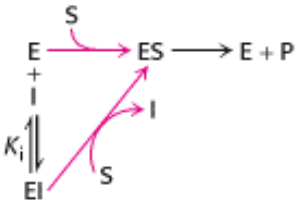
- Tight binding of a metal to a functional group in an enzyme
- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe)
- Relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity
- Mercury: binds to so many enzymes, often at reactive sulfhydryl groups in the active site
  - It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity
- Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme, such as calcium, iron, or zinc
  - Its developmental & neurologic toxicity may be caused by its ability to replace Ca^{+2} in several regulatory proteins that are important in the central nervous system and other tissues

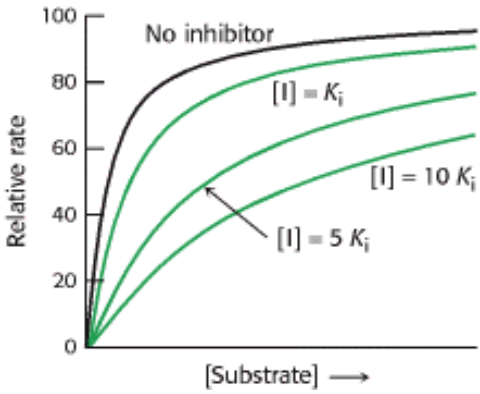
2.2 Reversible Inhibitors

- Characterized by a rapid dissociation of the enzyme-inhibitor complex
- Usually these inhibitors bind through non-covalent interactions & inhibitor maintains a reversible equilibrium with the enzyme
- Reversible inhibitors can be divided into two classes: competitive & noncompetitive
- The double-reciprocal plots are highly useful for distinguishing among these inhibitors

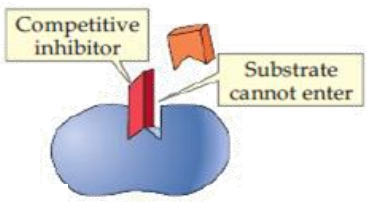
2.2.A. Competitive inhibition

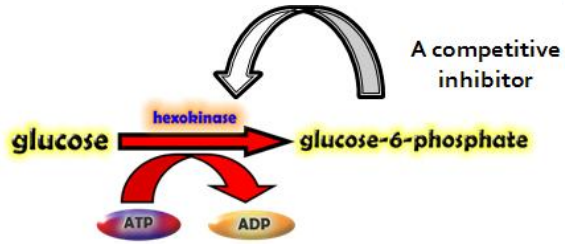


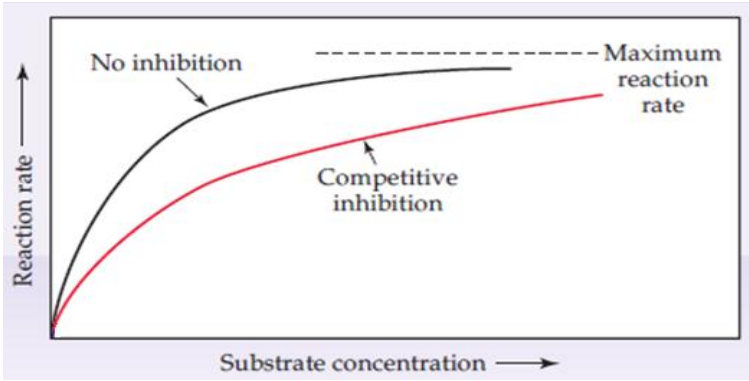


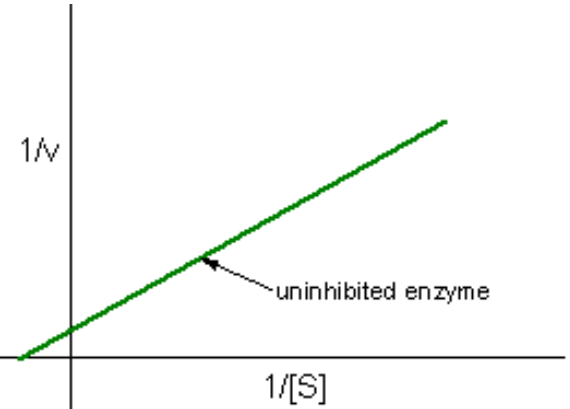


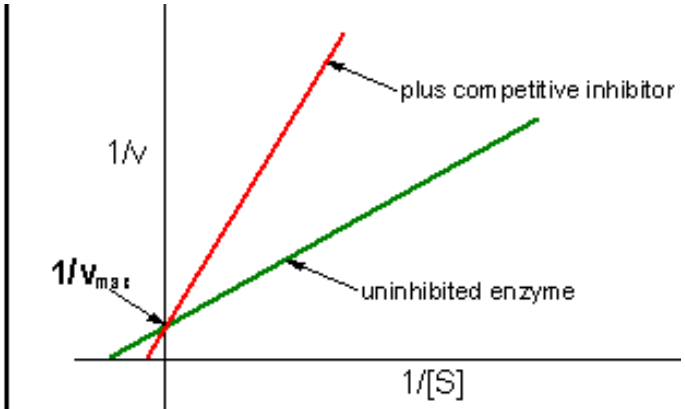
- The inhibitor competes with substrate
- Increasing [S] can overcome the inhibition (V_{max})
- Does K_M change?
- Significance (ex. Hexokinase)

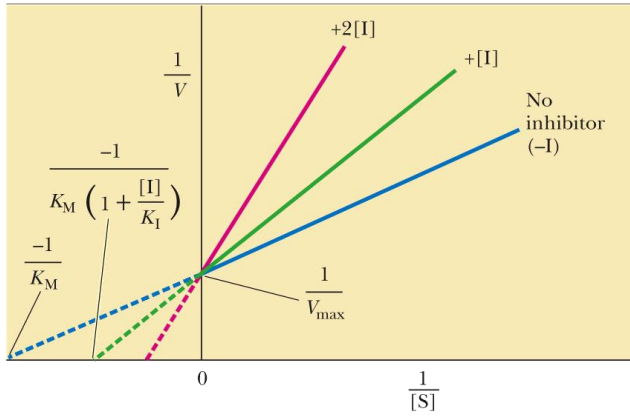






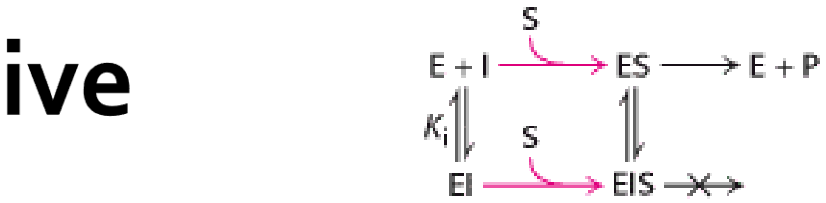


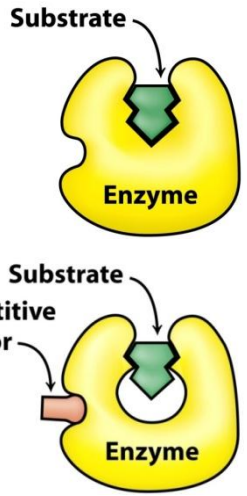


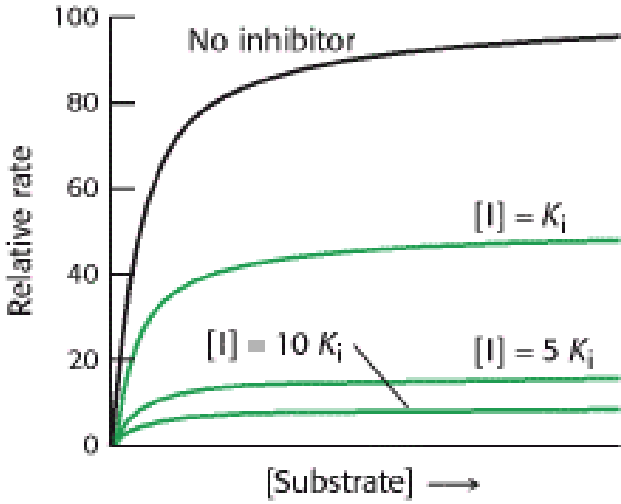


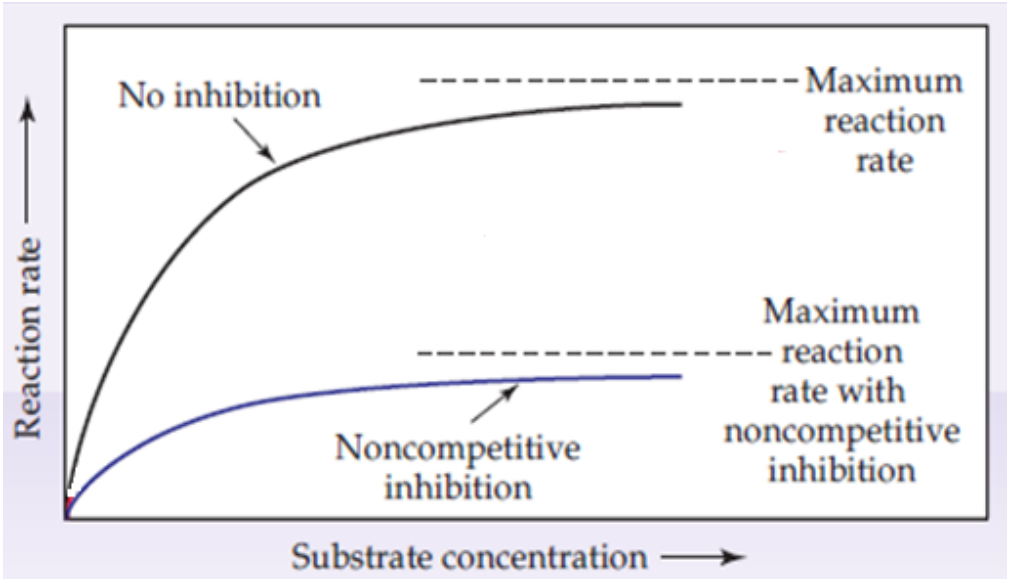
2.2.B. Noncompetitive inhibition

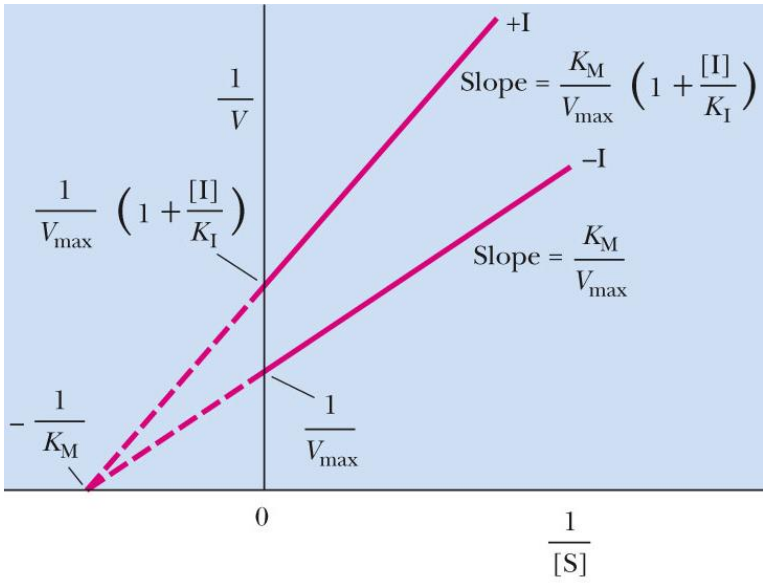
- The inhibitor binds at a site other than the active site
- The complex does not proceed to form product or has a lower efficiency
- V_{max} vs. K_M
- Can we reach V_{max}?











3. REGULATION THROUGH CONFORMATIONAL CHANGES

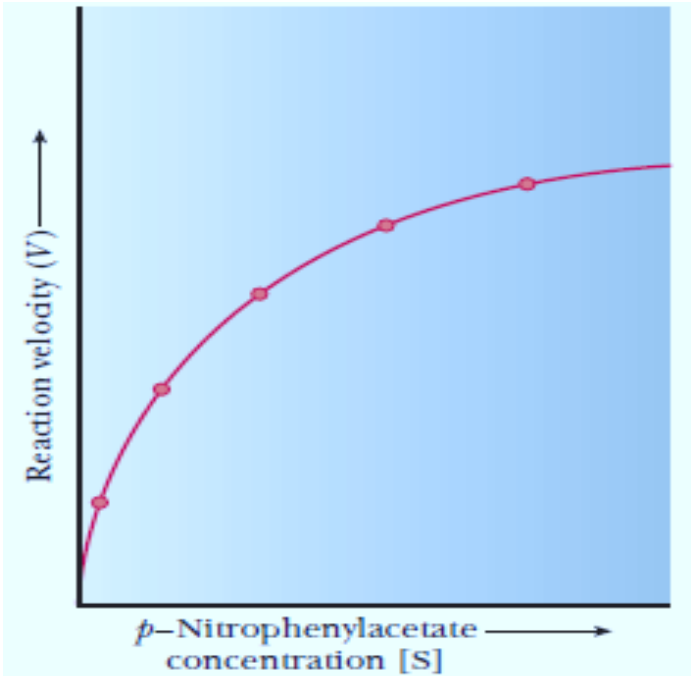
- **These regulatory mechanisms include**
  A. **Allosteric activation and inhibition;**
  B. **Phosphorylation or other covalent modification;**
  C. **Protein-protein interactions between regulatory & catalytic subunits or between two proteins;**
  D. **Proteolytic cleavage**

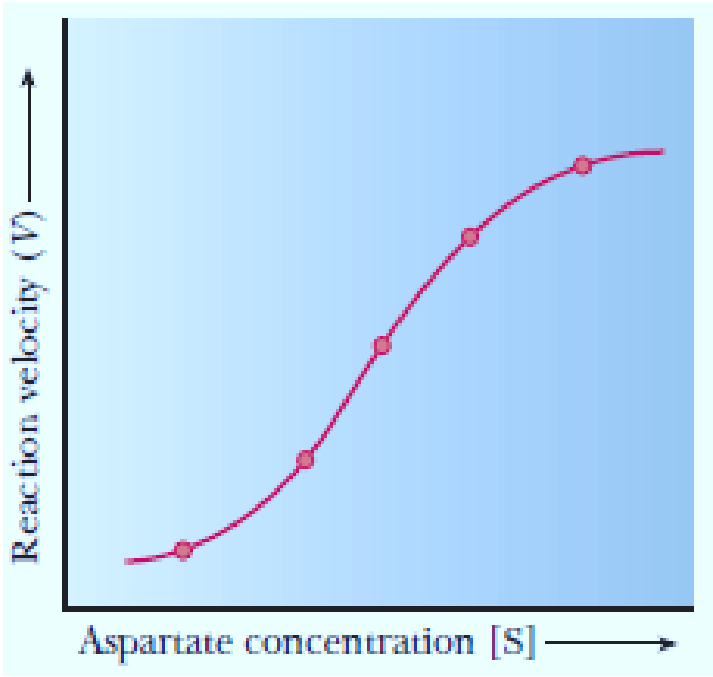
- **These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation**

3.A. Not all enzymes follow Michaelis-Menten equation; Chymotrypsin vs. ATCase

- **Chymotrypsin: Specificity for aromatic residues mainly. Also, hydrolysis of ester bonds**
- **Aspartate transcarbamoylase (ATCase): synthesis of CTP & UTP for RNA and DNA synthesis**

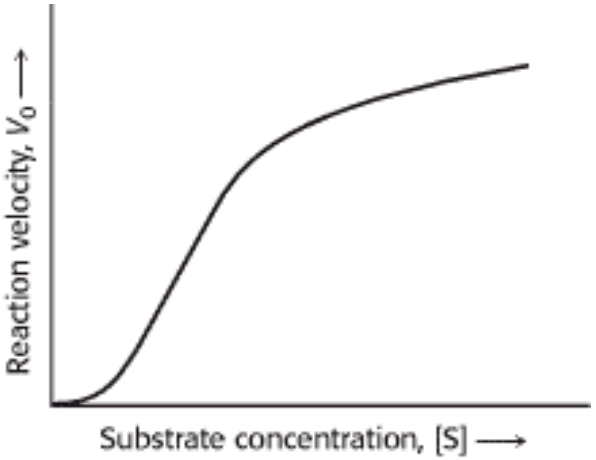
$$\text{Carbamoyl phosphate} + \text{Aspartate} \rightarrow \text{Carbamoyl aspartate} + HPO_4^{2-}$$

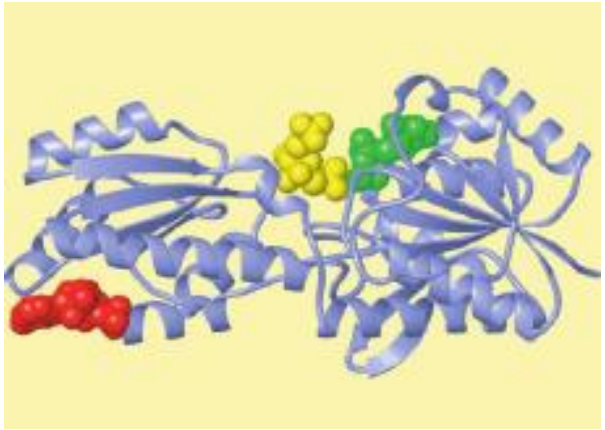


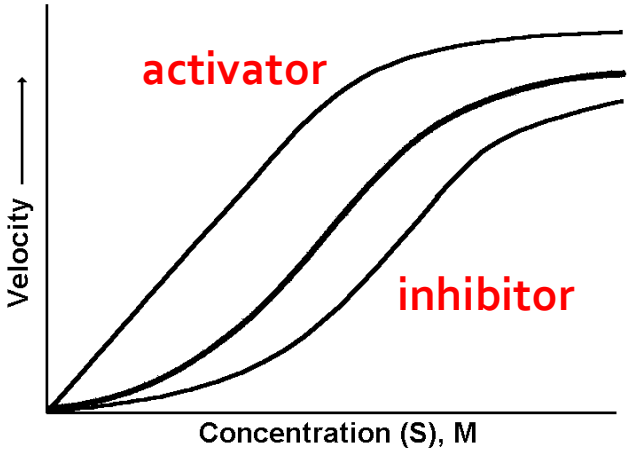


Allosteric regulation

- What are allosteric enzymes? A multi-subunit enzyme with catalytic subunit(s) and regulatory subunit(s)
- Binding triggers a conformational change in the active site
- The Michaelis-Menten model can't explain the kinetic properties
- The effect of the modulators (allosteric modifiers)
- Homotropic vs. heterotropic
- The substrate concentration at half of the V_{max} is called ($K_{0.5}$)

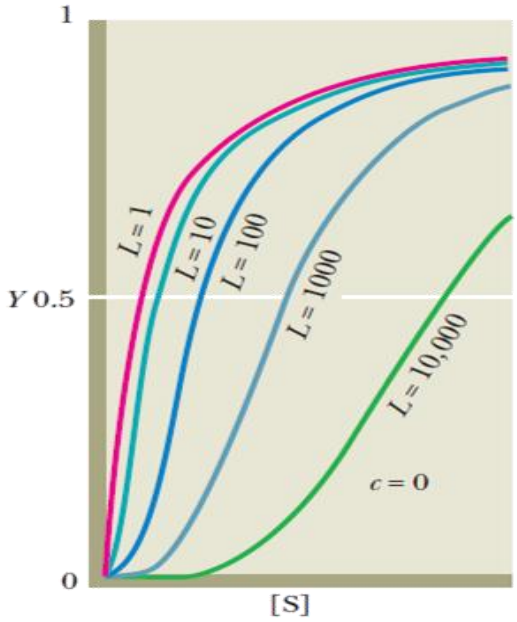


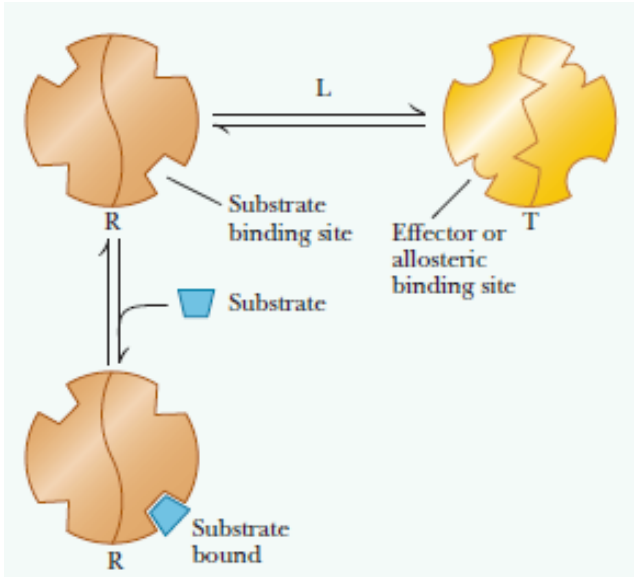


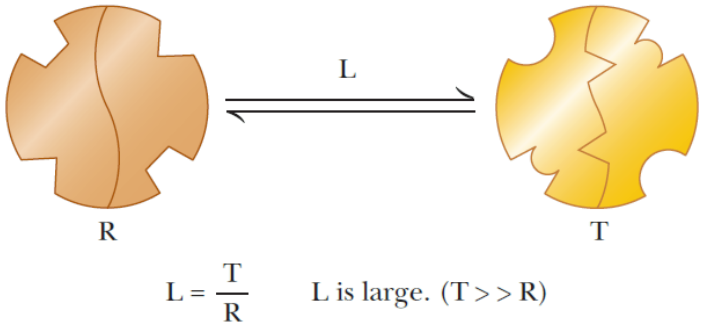


How do allosteric enzymes work? The Concerted Model

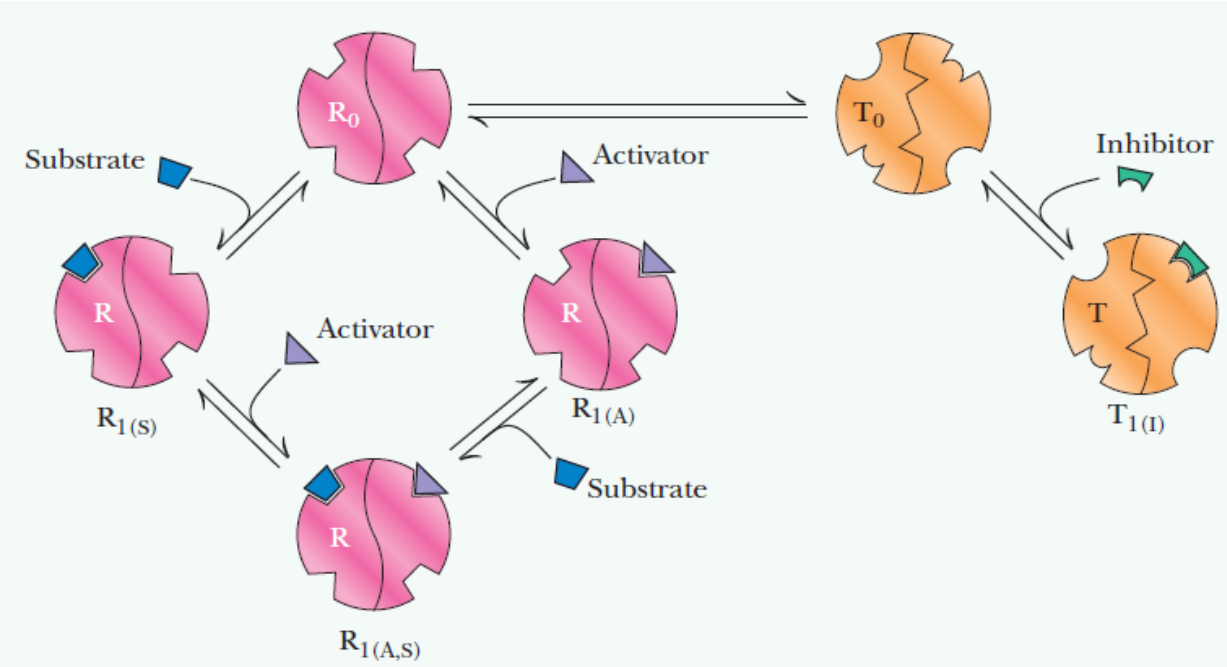
- Two conformations, active R (relaxed), binds substrate tightly, and inactive T (tight), binds substrate less tightly
- Both subunits change conformation at the same time
- The equilibrium ratio of the (T/R) is called L and is assumed to be high
- As L (T/R) increases, the shape becomes more sigmoidal

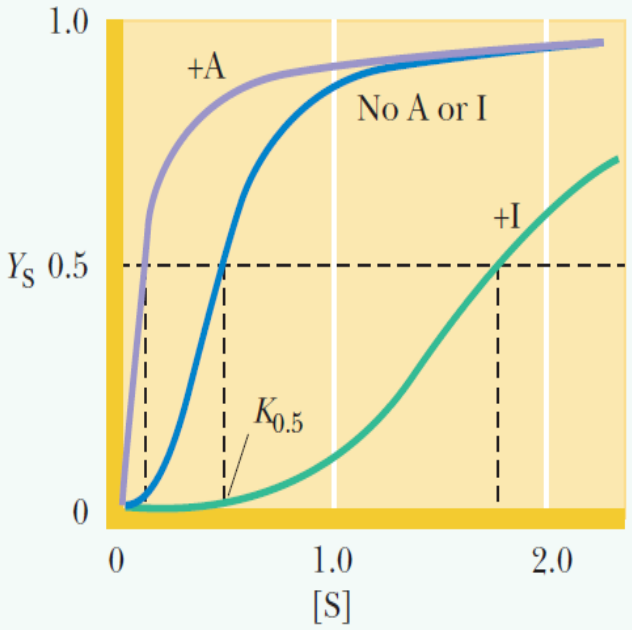






Allosteric inhibition & activation according to the concerted model



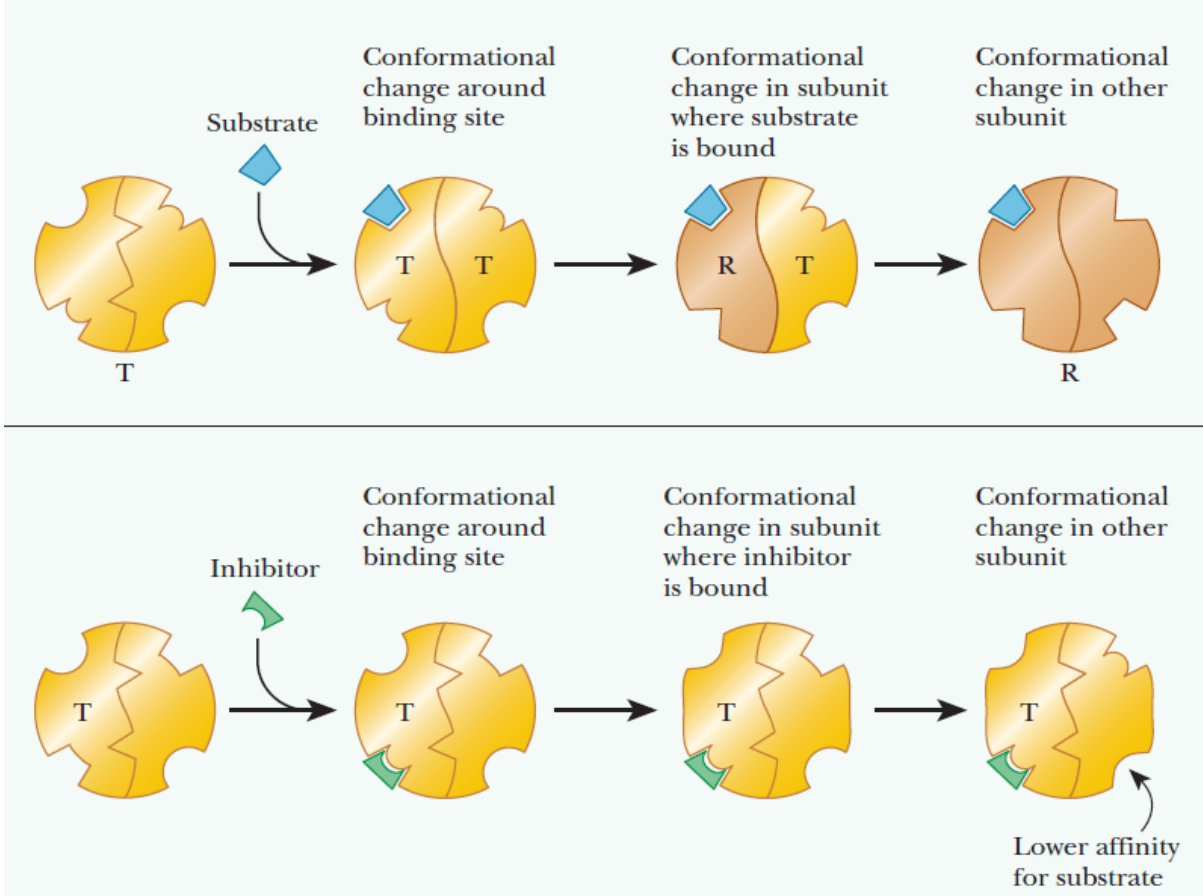


Either substrate concentration or activator concentration must be increased to overcome the effects of the allosteric inhibitor

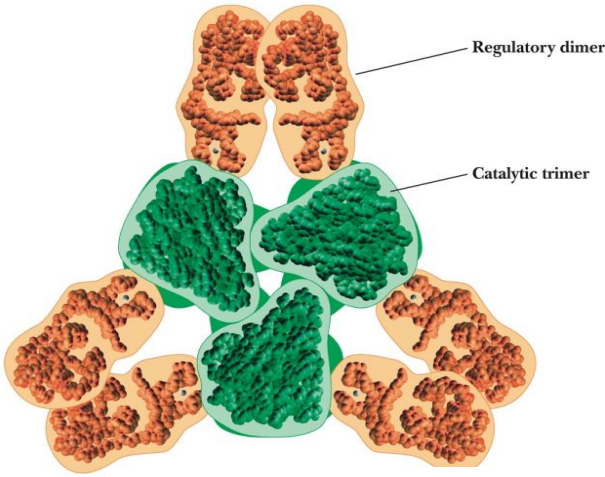
How do allosteric enzymes work? The sequential model

- **Binding of substrate induces conformational change from the T form to the R form (induced-fit theory of substrate binding)**

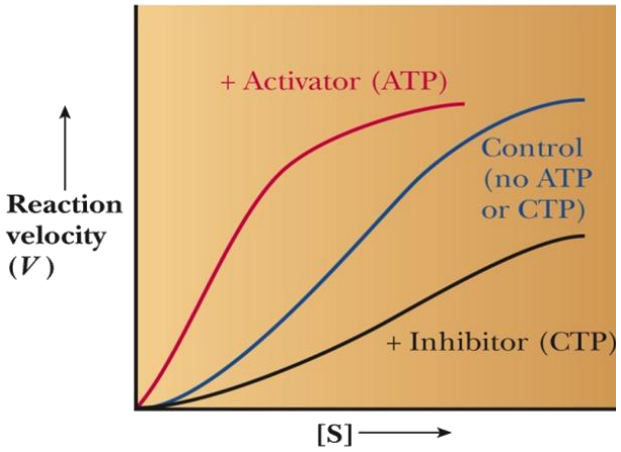
- **The net result is to favor the R state with activator and the T state with inhibitor**

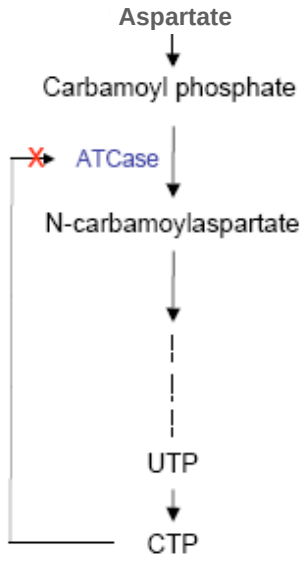


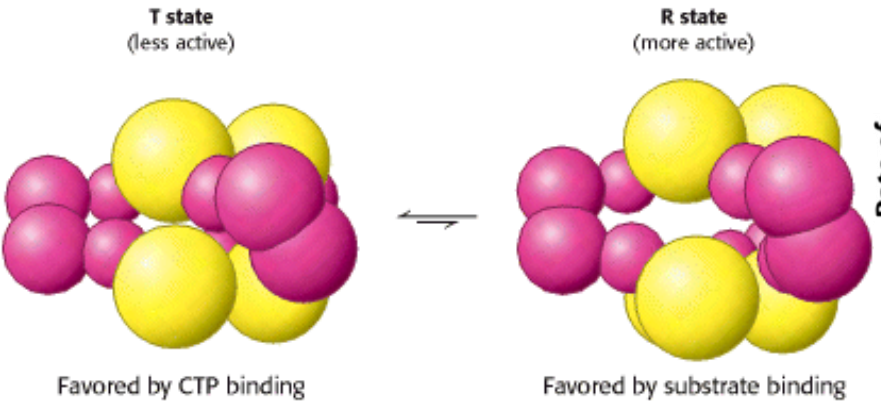
Allosteric regulation - ATCase

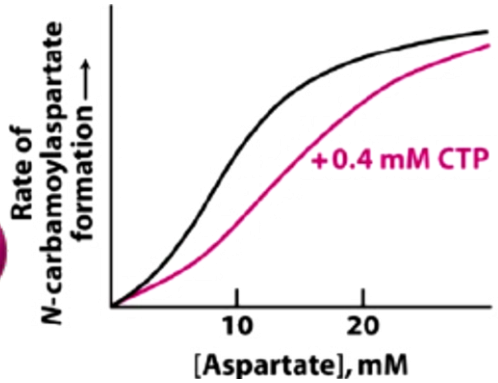


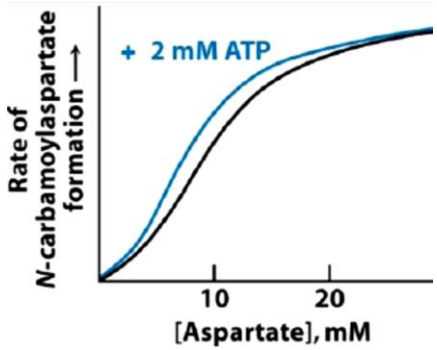
- ATCase and Hb are allosteric proteins (cooperative behavior)
- Catalytic can be separated from regulatory (hyperbolic)
- Cooperativity in relation to substrate
- CTP is an inhibitor of ATCase (feedback inhibition), ATP is an activator





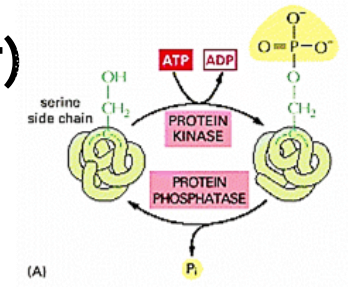


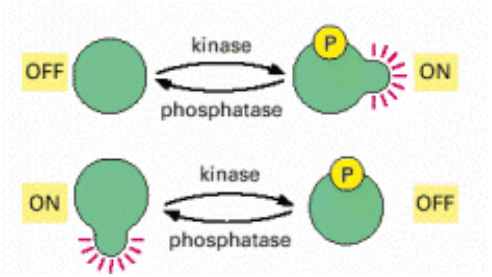




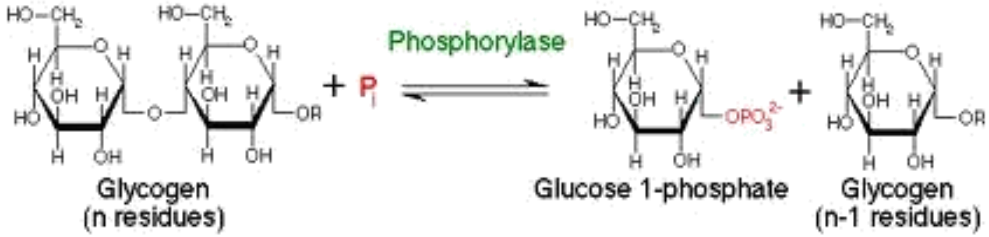
3.B. Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION

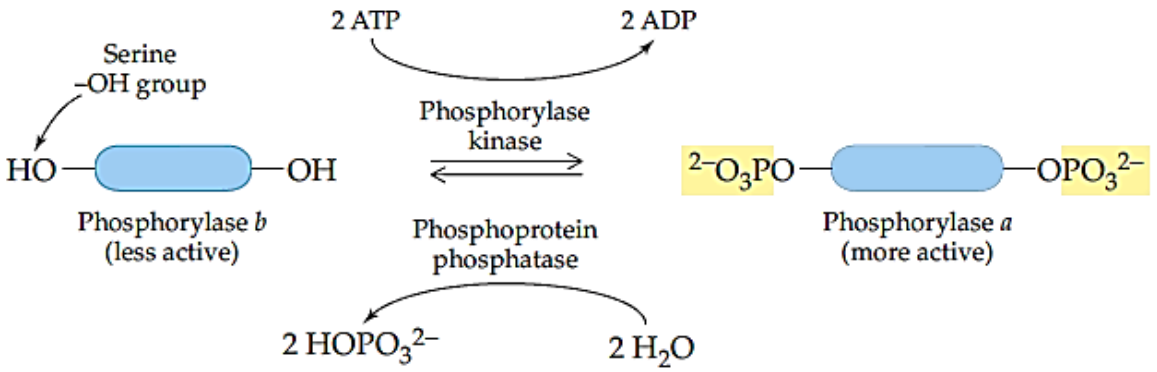
- Rapid and transient regulation of enzyme activity - reversible
- Phosphorylation: (Ser, Thr, & Tyr)
  - ✓ Mostly, ATP is the donor
  - ✓ Kinases vs. phosphatases





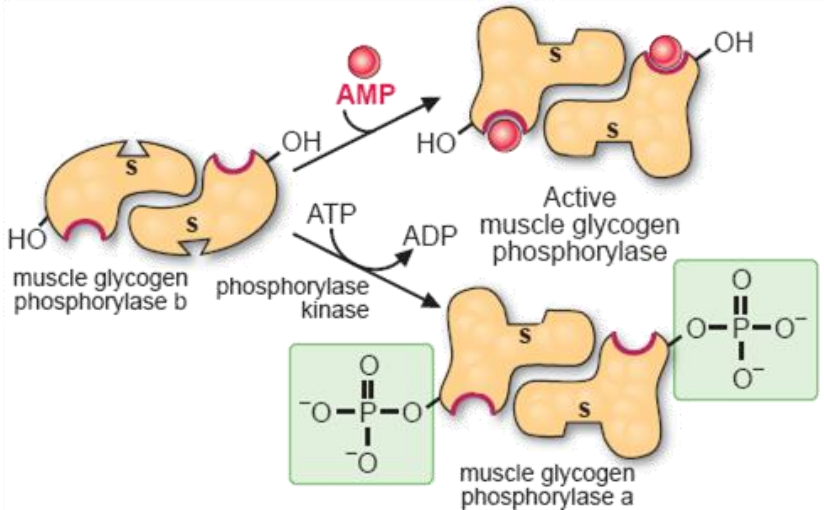
- ✓ Phosphorylation does not mean always activation of enzymes
- ✓ Glycogen phosphorylase-reaction (two forms; a & b). Ser is away from the active site





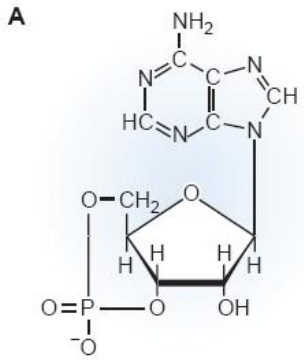
3.B. Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION

- AMP is an allosteric activator (muscular contraction)
- Glycogen phosphorylase kinase links the activation of muscle glycogen phosphorylase to:
  - ✓ Changes in the level of the hormone adrenaline in the blood
- It is regulated through phosphorylation by:
  - ✓ Protein kinase A
  - ✓ Activation of Ca^{+2}-calmodulin during contraction

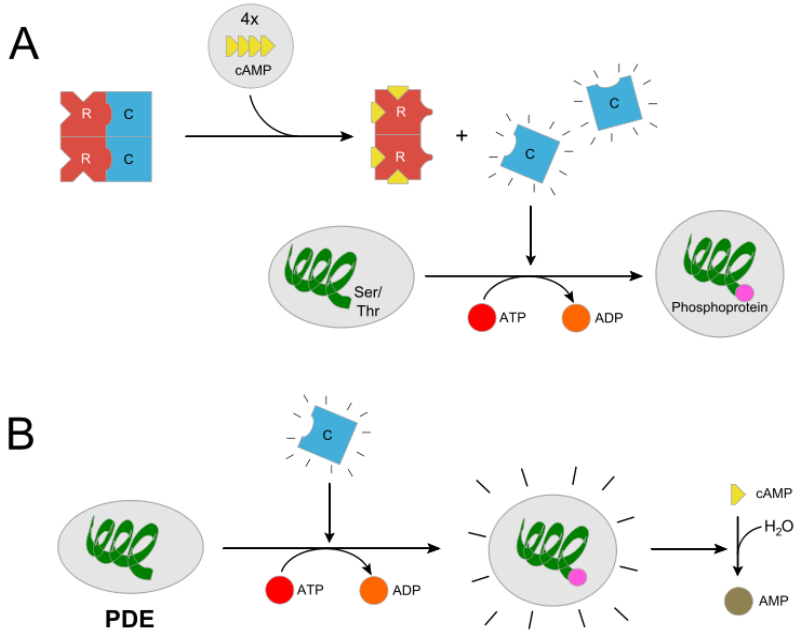


- Others:
  - ✓ Adenylation (AMP, Tyr), Uridylation (UMP), ADP-ribosylation, Methylation (carboxylate), Acetylation (acetyl CoA)

Protein kinase A (PKA)



- Protein kinase A (PKA): refers to a family of enzymes whose activity is dependent on cellular levels of cyclic AMP (cAMP)
- cAMP: referred to as a hormonal 2nd messenger
- Either dedicated or not
- Has several functions in the cell, including regulation of glycogen, sugar, & lipid metabolism

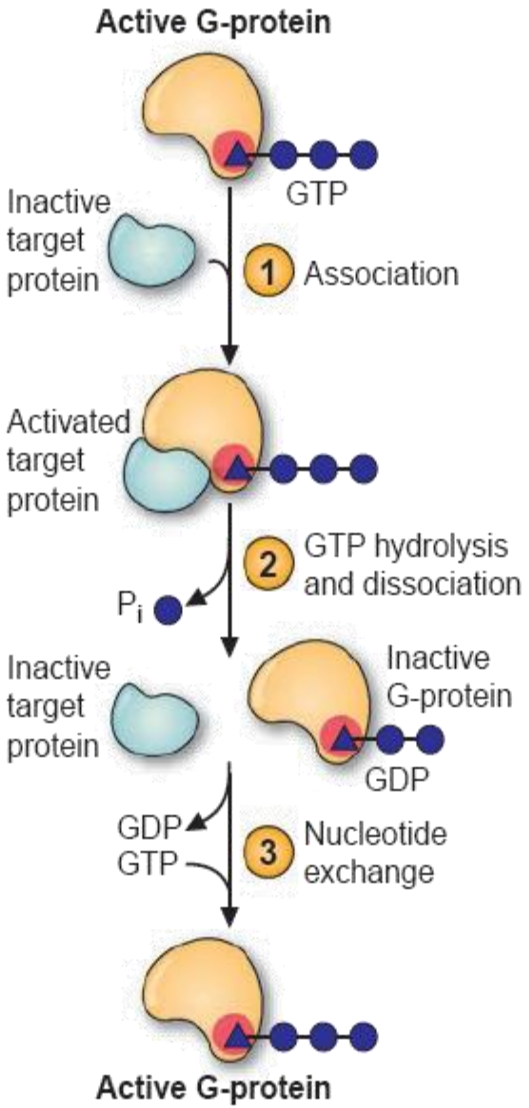


- Adrenaline (epinephrine) → ↑cAMP → activates protein kinase A → phosphorylates & activates glycogen phosphorylase kinase → phosphorylates & activates glycogen phosphorylase
- Phosphorylation cascade

3.C. Conformational Changes from Protein–Protein Interactions

- SMALL (MONOMERIC) G PROTEINS:
- Reversible protein association
- Small single-subunit proteins that bind & hydrolyze GTP
- Regulation by accessory proteins:

1) (GAPs [GTPase-activating proteins]
   - Increases the rate of GTP hydrolysis (dissociation of the G protein–target protein complex)
2) GEFs [guanine nucleotide exchange factors]
   - Increases the rate of GTP exchange for a bound GDP (activates G protein)
3) GDIs [GDP dissociation inhibitors])
   - Inhibit dissociation of GDP, thereby keeping the G protein inactive

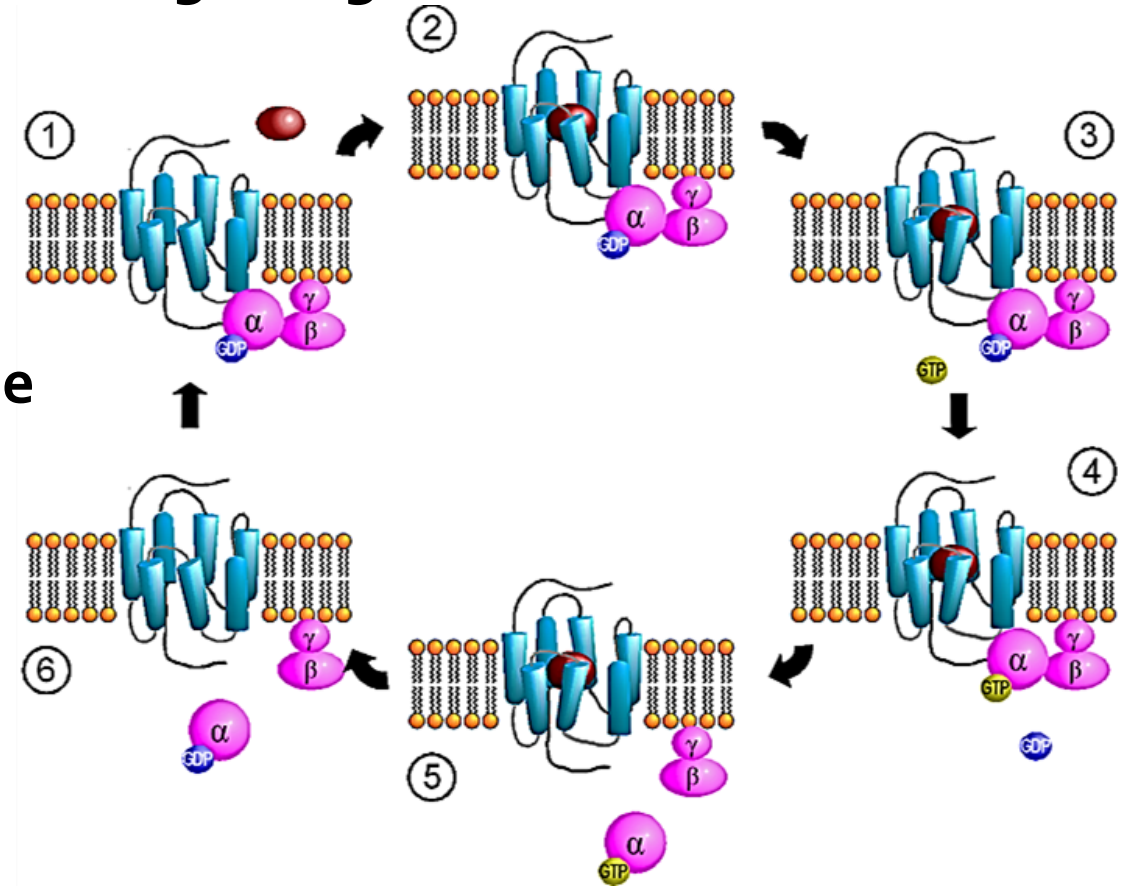


Regulation - Large regulatory molecules

- G protein: a family of trans-membrane proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors

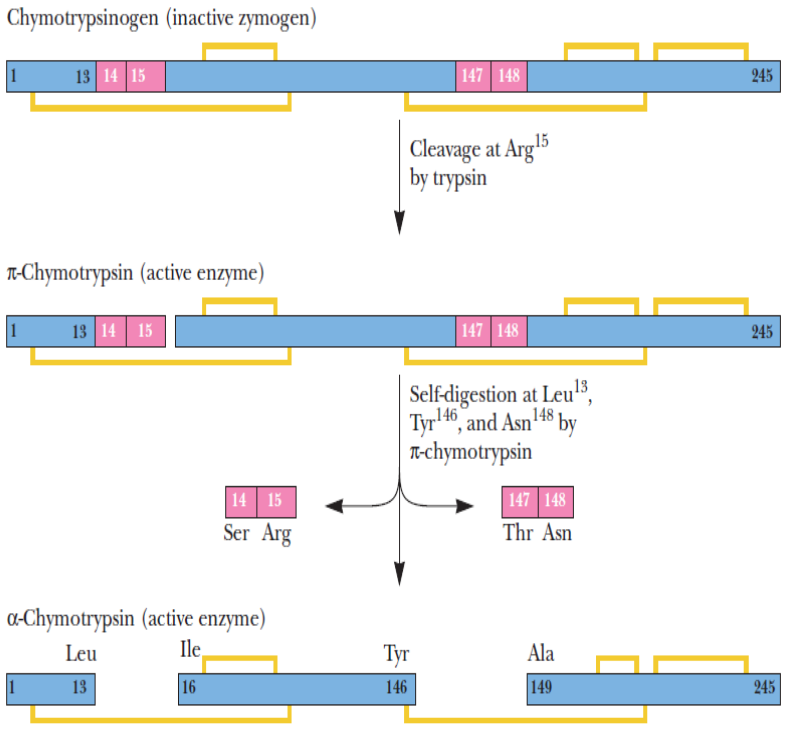
- When they bind guanosine triphosphate (GTP), they are 'on', and, when they bind guanosine diphosphate (GDP), they are 'off'

- α-Subunit can be stimulatory or inhibitory

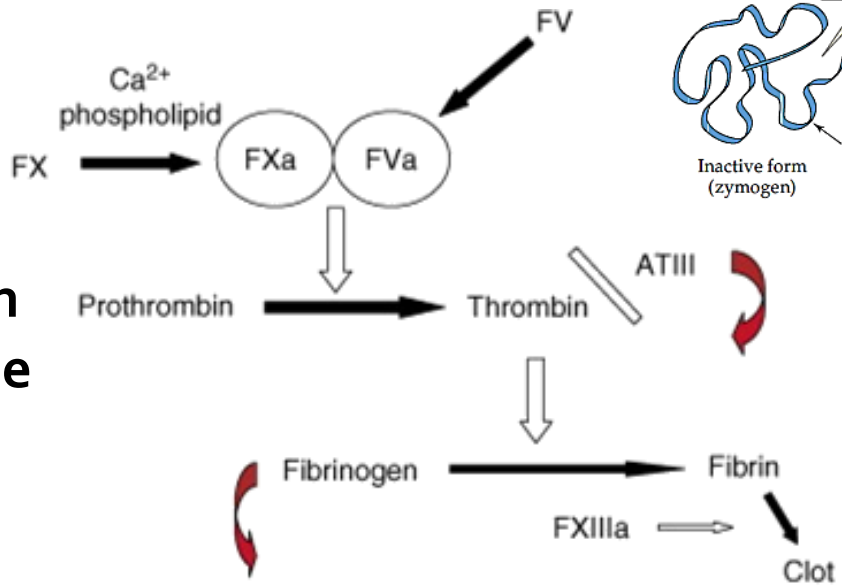


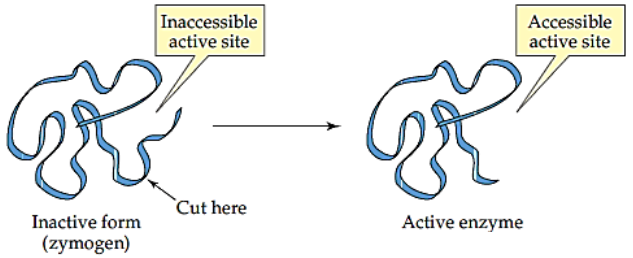
3.D. Proteolytic Cleavage Zymogens

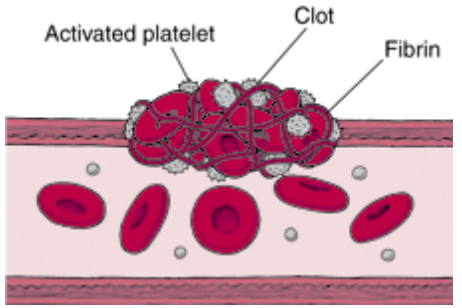
- Irreversible
- Trypsin, chymotrypsin, pepsin (trypsinogen, pepsinogen chymotrypsinogen)
  - ✓ Chymotrypsinogen: single polypeptide chain (245 residues), 5 (S—S) bonds



- Blood clotting
  - ✓ The soluble protein fibrinogen is converted to the insoluble protein fibrin







4. REGULATION THROUGH CHANGES IN AMOUNT OF ENZYME

A. **Regulated Enzyme Synthesis**

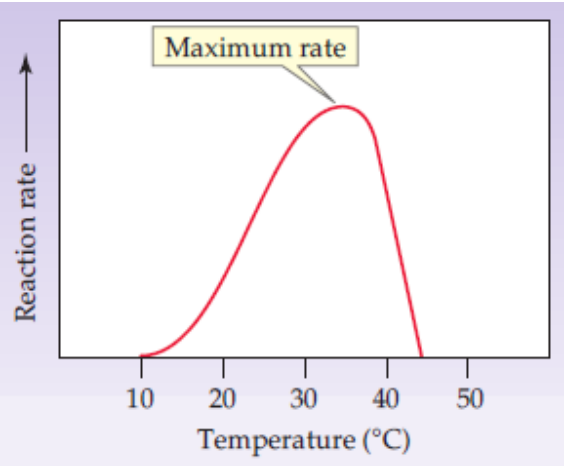
- Regulated by increasing or decreasing the rate of gene transcription (induction & repression)
  - Usually slow in humans (hours to days)
- Sometimes through stabilization of the messenger RNA

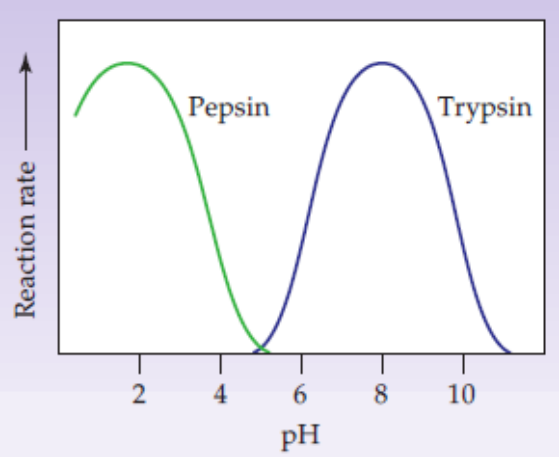
B. **Regulated Protein Degradation**

- Can be degraded with a characteristic half-life within lysosomes
- Also, via two specialized systems, proteosomes & caspases (highly selective & regulated)
  - During fasting or infective stress: gluconeogenesis increase & synthesis of antibodies (protein degradation increases)
  - Increased synthesis of ubiquitin

Effect of Temperature & pH Non-specific regulators

- Increase in T° increases the rate until reaches a max (≈ 50°)
- The maximum activity for most human enzymes occurs near 37°C
- Autoclave steam heating
- Hypothermia, metabolic reactions, cardiac surgery
- For pH, usually has a well defined optimum point, usually around physiological one
- Pepsin (2) vs. trypsin (8)
- Most enzymes have their maximum activity between (5-9)
- Extremes of pH denatures the protein





Extremozymes

Taq polymerase and PCR

Thermophiles (heat lovers)

Psychrophiles (cold lovers)

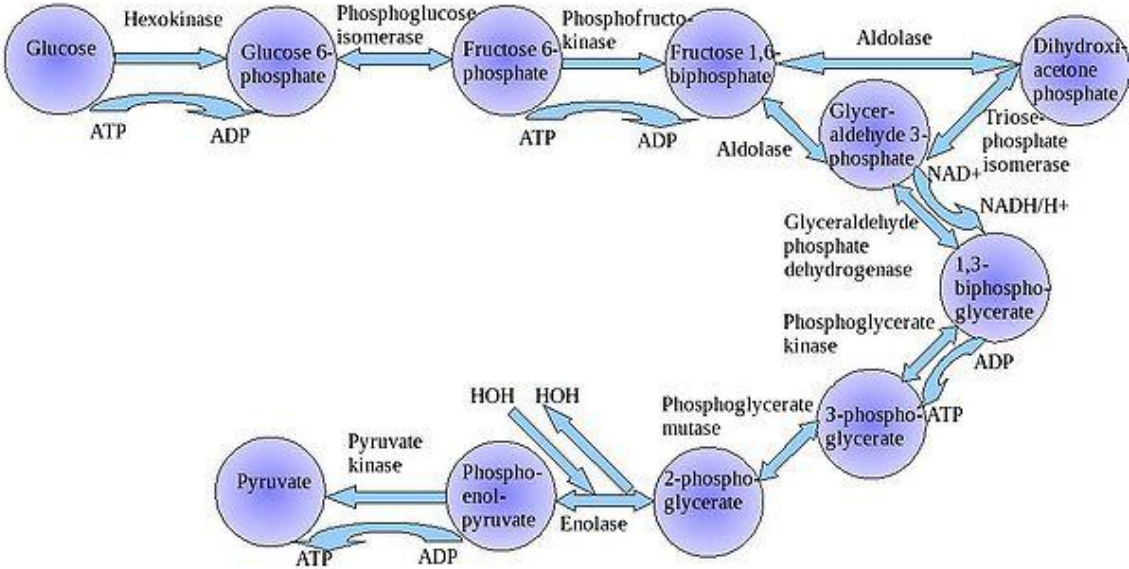
lipases and proteases

Biobleaching of paper pulp using heat-stable xylanases

REGULATION OF METABOLIC PATHWAYS

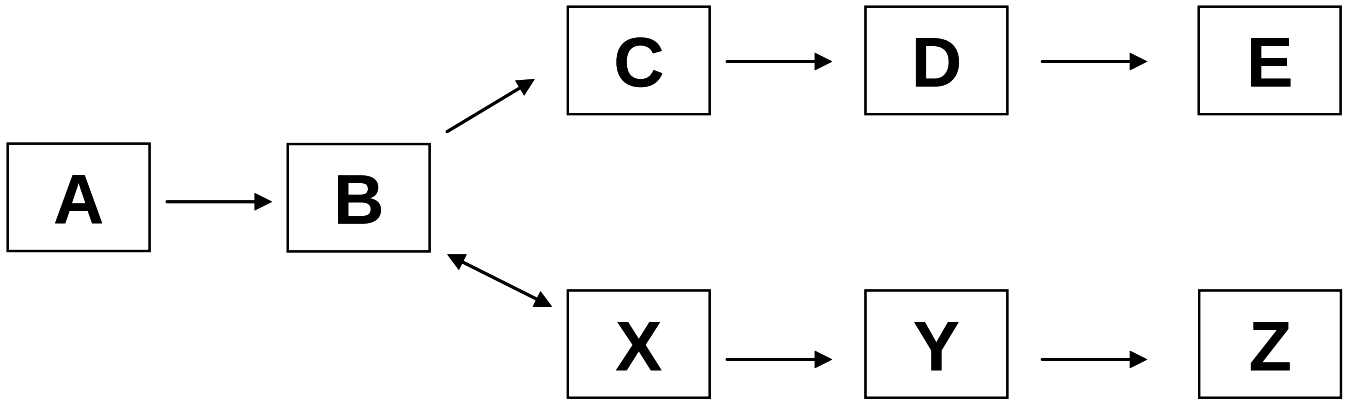
Principles of Pathway Regulation

- *1. COUNTERREGULATION OF OPPOSING PATHWAYS*
- Synthesis vs. degradation (a different regulatory enzyme)
- *2. TISSUE ISOZYMES OF REGULATORY PROTEINS*
- *3. REGULATION AT THE RATE-LIMITING STEP*
- Pathways are principally regulated at their rate-limiting step
- The slowest step & is usually not readily reversible
  - Changes in this step can influence flux through the rest of the pathway
- Usually the first committed step in a pathway
- Requirement for high amount of energy
- High Km values of enzyme towards its substrate



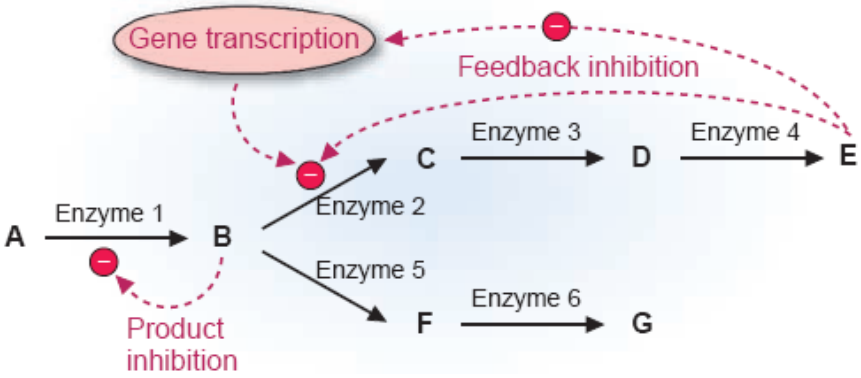
Principles of Pathway Regulation

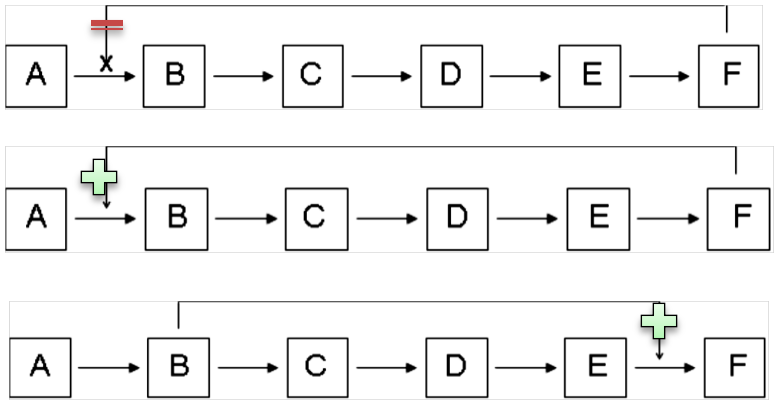
- ***4. The committed step***
- **A committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return**
- **Committed steps are exergonic reaction**
- **For example, the committed step for making product E is (B → C), not (A → B)**

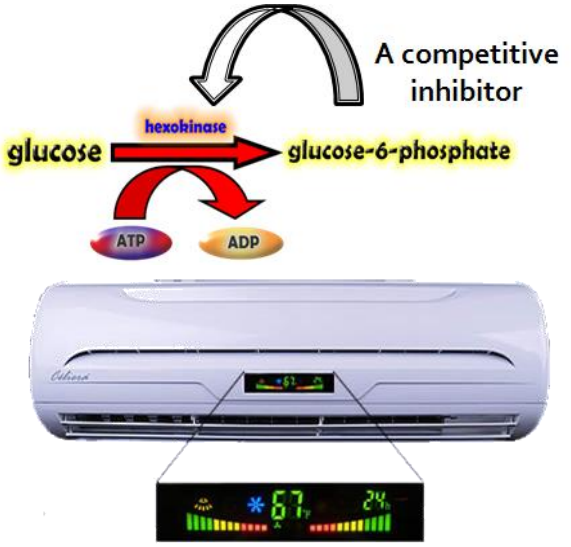


Principles of Pathway Regulation

- *5. FEEDBACK REGULATION*
- This type of regulation is much slower to respond to changing conditions than allosteric regulation
  - Negative feedback regulation (feedback inhibition)
  - Positive feedback regulation
  - Feed-forward regulation
    - Disposal of toxic compounds







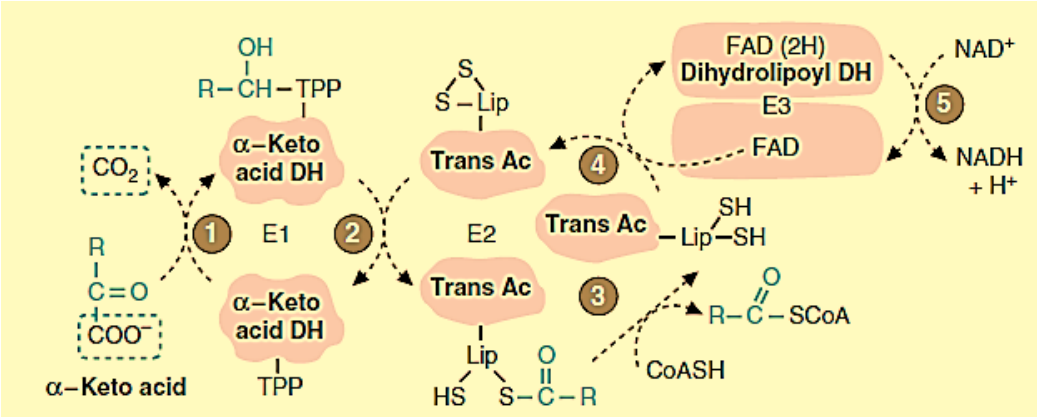
Principles of Pathway Regulation

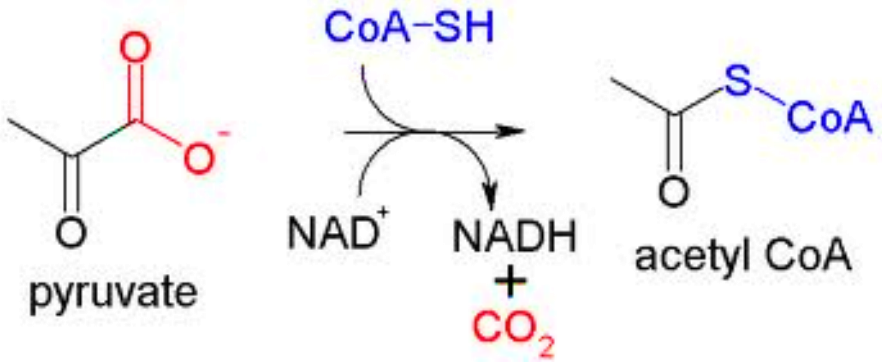
- ***6. Enzyme compartmentalization***
- **Both enzymes and their substrates are present in relatively small amount in a cell**
- **A mechanism by which rate of reactions become faster is their compartmentalization; reducing area of diffusion**
- **In this way, enzymes are sequestered inside compartments where access to their substrates is limited**
- **Lysosomes; proteins get transported to lysozymes**
- **Mitochondria; energy metabolic pathways**
- **Metabolism of fatty acids; synthesis (cytosol) vs. degradation (mitochondria)**

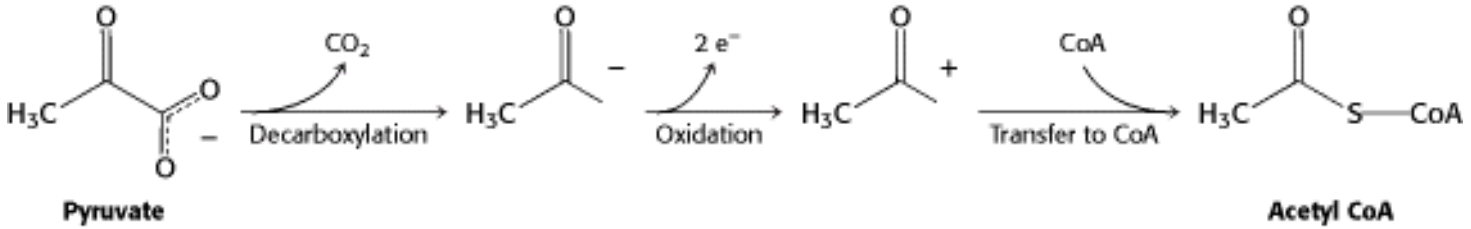
Principles of Pathway Regulation

- ***7. Enzyme complexing (A multienzyme complex)***

- **Complexing various enzymes that share one process**
- **Product of enzyme A pass directly to enzyme B**
- **Pyruvate dehydrogenase (mitochondria) 3 enzymes: decarboxylation, oxidation, & transfer of the resultant acyl group to CoA**







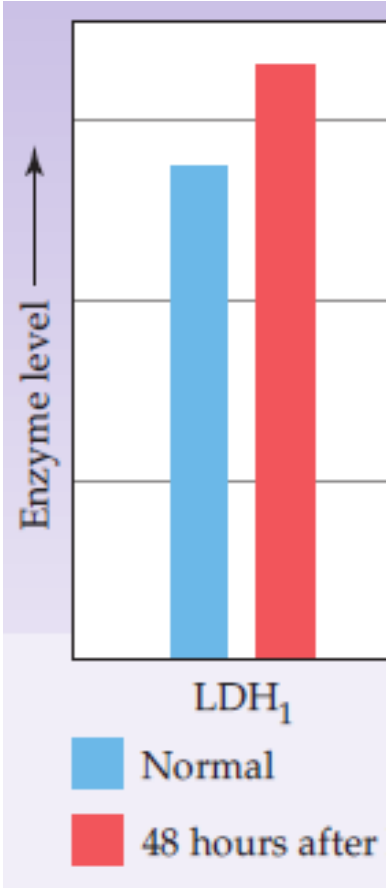
Ribozymes

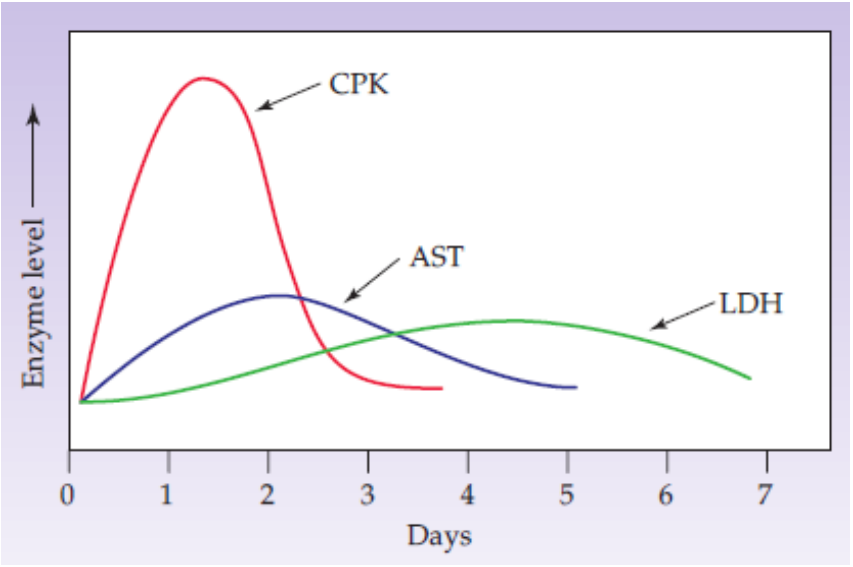
- A few enzymes with *RNA* components had been discovered, such as telomerase & *RN*ase P (cleaves extra nucleotides off the 5' ends of t*RNA* precursors)
- Some RNAs catalyzes its own self-splicing mechanisms
- More recently, it has been shown that RNAs can catalyze reactions involved in protein synthesis
- The catalytic efficiency of catalytic RNAs is less than that of protein enzymes
- The catalytic efficiency is greatly enhanced by the presence of protein subunits

Enzymes in Medical Diagnosis

Enzymes in Medical Diagnosis

- High vs. low serum conc., dependent on the tissue
- Heart attack; CPK, ALT, AST, LDH
- Isoenzymes of LDH, 1-5, 1 for heart attack, 4 for renal injury, 5 for acute hepatitis
- Creatine kinase (CK) (brain, heart, skeletal muscle)
- Brain type indicates a stroke or a brain tumor, whereas the heart type indicates a heart attack

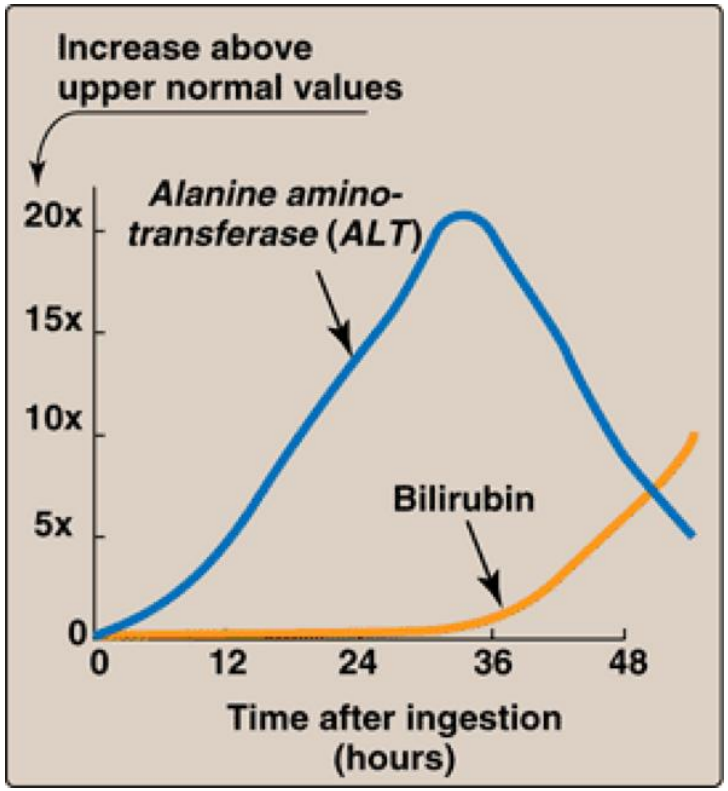




Aspartate transaminase (AST)	Damage to heart or liver
Alanine transaminase (ALT)	Damage to heart or liver
Lactate dehydrogenase (LH)	Damage to heart, liver, or red blood cells
Alkaline phosphatase (ALP)	Damage to bone and liver cells

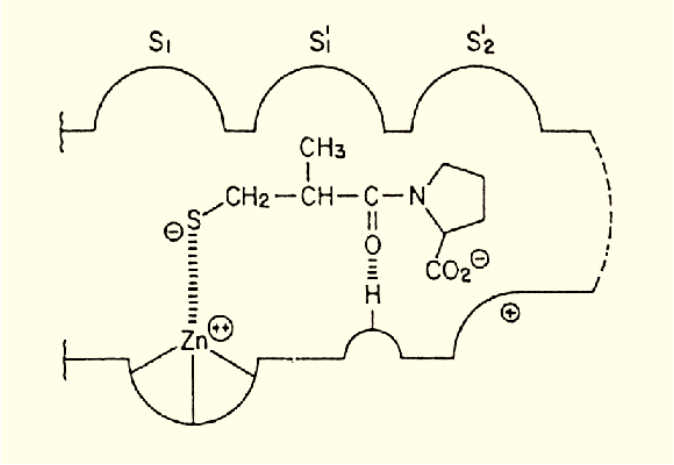
AST & ALT

- Present in highest conc. in cells from the liver, heart & skeletal muscles
- Rise in conditions that cause extensive cell necrosis, such as severe viral hepatitis & toxic injury
- ALT is more specific for liver disease
- Non-hepatic disease: Aminotransferases may be elevated but they are clinically different



Enzyme Inhibitors as Drugs

- **ACE (Angiotensin Converting Enzyme) inhibitors**
- **Angiotensin I, a decapeptide, Angiotensin II, an octapeptide (His and Leu)**



Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu —(Angiotensin-converting enzyme)→

Angiotensin I

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe + His-Leu

Angiotensin II