BIOCHEMISTRY
Enzymes and Coenzymes

BIOB111

CHEMISTRY & BIOCHEMISTRY

Session 15

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Session Plan

- General Characteristics of Enzymes
- Enzyme Structure
- Enzyme Nomenclature
- Enzyme Function
- Enzyme Specificity
- Factors Affecting Enzyme Activity
- Enzyme Inhibition
- Regulation of Enzyme Activity
- Medical Uses of Enzymes

http://highered.mheducation.com/sites/0073522732/student_view0/chapter4/animation_-_enzyme_action.html

NOTE: **Vitamins** are discussed in detail in the Nutrition Modules in your further studies.

General Characteristics of Enzymes

- **ENZYME**
  - Usually a **protein**, acting as **catalyst in specific biochemical reaction**

- Each cell in the human body contains 1,000s of different enzymes
  - Every reaction in the cell requires its own specific enzyme

- Most enzymes are globular proteins
  - A few enzymes are made of RNA
    - Catalyze biochemical reactions involving nucleic acids

- Enzymes undergo all the reactions of proteins
  - Enzymes denaturation due to pH or temperature change
    - A person suffering high fever runs the risk of denaturing certain enzymes

Animation of enzyme at work

http://highered.mheducation.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

http://bcs.whfreeman.com/webpub/Ektron/pol1e/Animated%20Tutorials/at0302/at_0302_enzyme_catalysis.html

Enzyme Structure

- **SIMPLE ENZYMES**

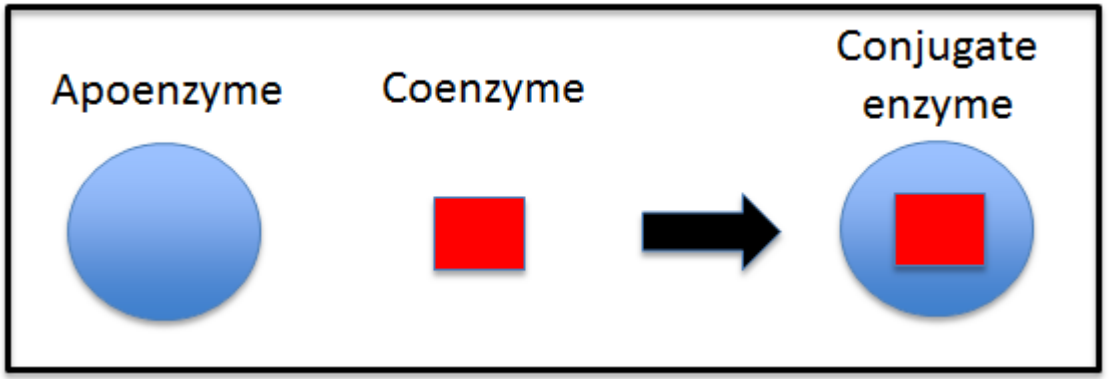
  Composed only of protein

- **CONJUGATED ENZYMES**

  Composed of:

  – **Apoenzyme**
    - Conjugate enzyme without its cofactor
    - Protein part of a conjugated enzyme

  – **Coenzyme (Cofactor)**
    - Non-protein part of a conjugated enzyme



- The apoenzyme can't catalyze its reaction without its cofactor.
  – The combination of the apoenzyme with the cofactor makes the conjugated enzyme functional.
- **Holoenzyme** = apoenzyme + cofactor
  – The **biochemically active** conjugated enzyme.

Coenzymes and cofactors

- Coenzymes provide additional chemically reactive functional groups besides those present in the amino acids of the apoenzymes
  - Are either small organic molecules or inorganic ions

- Metal ions often act as additional cofactors (Zn^{2+}, Mg^{2+}, Mn^{2+} & Fe^{2+})
  - A metal ion cofactor can be bound directly to the enzyme or to a coenzyme

- **COENZYME**
  - A small organic molecule, acting as a cofactor in a conjugated enzyme
    - Coenzymes are derived from vitamins or vitamin derivatives
      - Many vitamins act as coenzymes, esp. B-vitamins

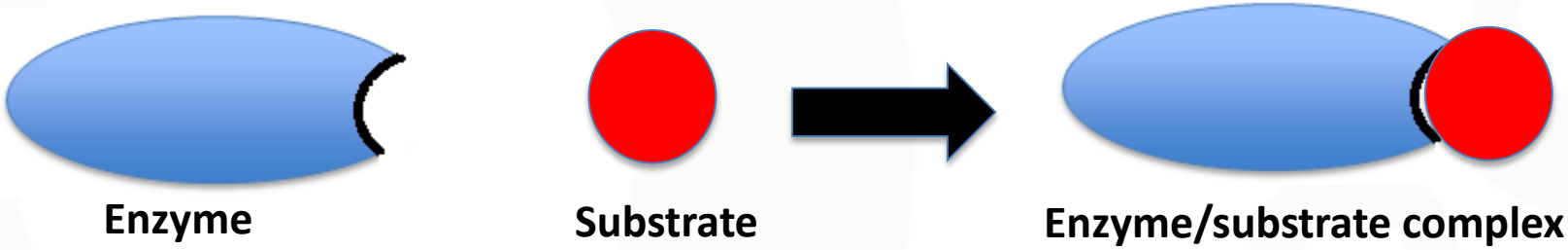
Enzyme definitions

Term	Definition
Enzyme (simple)	Protein only enzyme that facilitates a chemical reaction
Coenzyme	Compound derived from a vitamin (e.g. NAD^+) that assists an enzyme in facilitating a chemical reaction
Cofactor	Metal ion (e.g. Mg^{2+}) that that assists an enzyme in facilitating a chemical reaction
Apoenzyme	Protein only part of an enzyme (e.g. isocitrate dehydrogenase) that requires an additional coenzyme to facilitate a chemical reaction (not functional alone)
Holoenzyme	Combination of the apoenzyme and coenzyme which together facilitating a chemical reaction (functional)

Enzyme Nomenclature

- Enzymes are named according to the
  type of reaction they catalyze and/or their substrate
- **Substrate** = the reactant upon which the specific enzyme acts
  - Enzyme physically binds to the substrate

- **Suffix of an enzyme –ase**
  - *Lactase*, *amylase*, *lipase* or *protease*
    - Denotes an enzyme
- Some digestive enzymes have the suffix **–in**
  - *Pepsin*, *trypsin & chymotrypsin*
    - These enzymes were the first ones to be studied
- **Prefix** denotes the type of reaction the enzyme catalyzes
  - *Oxidase: redox reaction*
  - *Hydrolase: Addition of water to break one component into two parts*
- **Substrate identity** is often used together with the reaction type
  - *Pyruvate carboxylase*, *lactate dehydrogenase*



Enzyme Class	Reaction Catalyzed	Examples in Metabolism
Oxidoreductase	Redox reaction (reduction & oxidation)	Examples are **dehydrogenases** catalyse reactions in which a substrate is oxidised or reduced
Transferase	Transfer of a functional group from 1 molecule to another	**Transaminases** which catalyze the transfer of amino group or kinases which catalyze the transfer of phosphate groups.
Hydrolase	Hydrolysis reaction	**Lipases** catalyze the hydrolysis of lipids, and proteases catalyze the hydrolysis of proteins
Lyase	Addition / removal of atoms to / from double bond	**Decarboxylases** catalyze the removal of carboxyl groups
Isomerase	Isomerization reaction	**Isomerases** may catalyze the conversion of an aldose to a ketose, and mutases transfer functional group from one atom to another within a substrate.
Ligase	Synthesis reaction (Joining of 2 molecules into one, forming a new chemical bond, coupled with ATP hydrolysis)	**Synthetases** link two smaller molecules are form a larger one.

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6 Major Classes of Enzymes

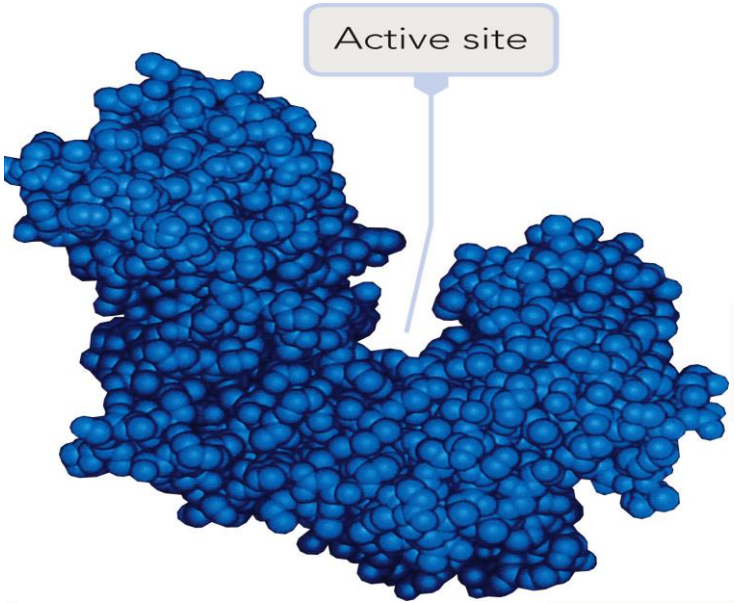
Based on the type of reaction they catalyze

The table explains the functions of enzymes and how they are classified and named.

Enzyme Active Site

- **Active site**
  - The specific portion of an enzyme (location) where the substrate binds while it undergoes a chemical reaction

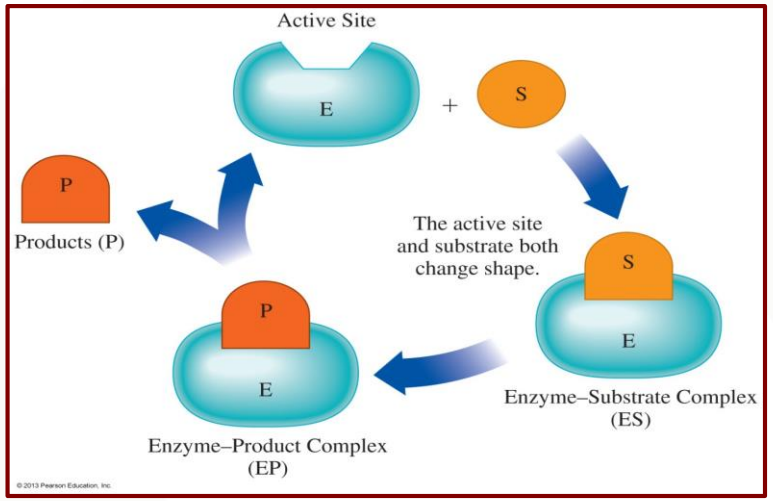
- The active site is a 3-D ‘crevice-like’ cavity formed by secondary & tertiary structures of the protein part of the enzyme
  - Crevice formed from the folding of the protein
    - Aka binding cleft
  - An enzyme can have more than only one active site
  - The amino acids R-groups (side chain) in the active site are important for determining the specificity of the substrate



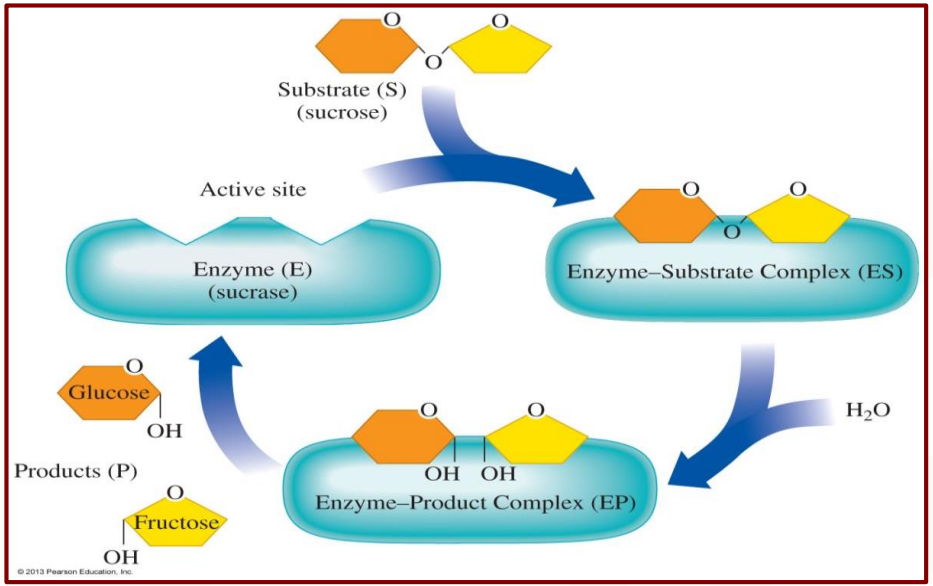
Stoker 2014, Figure 21-2 p750

Enzyme – Substrate Complex

- When the substrate binds to the enzyme active site an **Enzyme-Substrate Complex** is formed temporarily
  - Allows the substrate to undergo its chemical reaction much faster



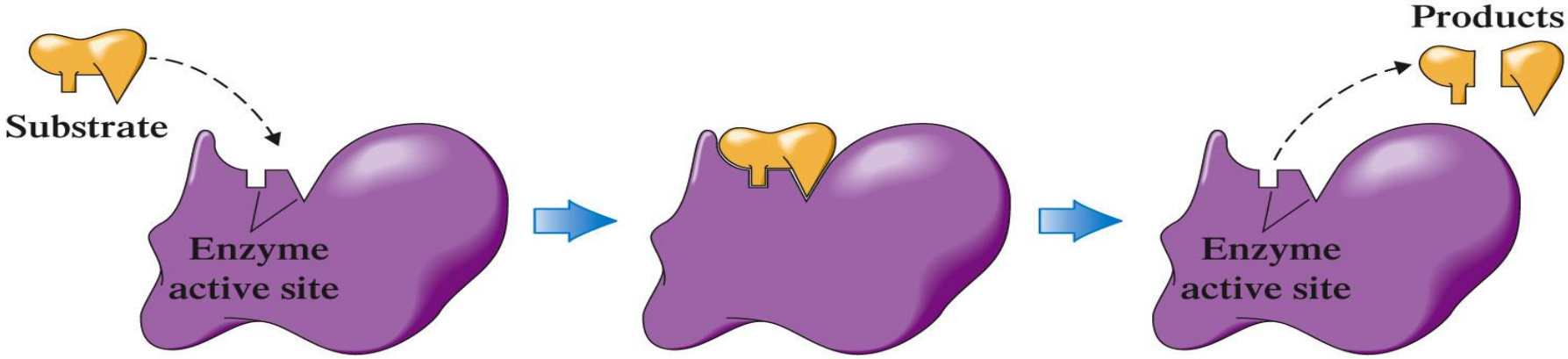
Timberlake 2014, Figure 4, p.738



Timberlake 2014, Figure 3, p.737

Lock & Key Model of Enzyme Action

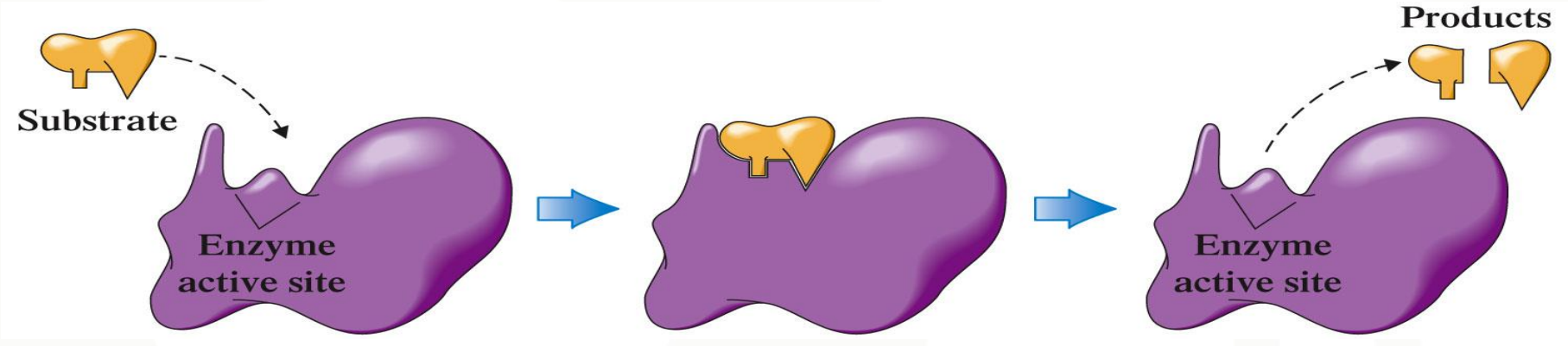
- The active site is fixed, with a rigid shape (LOCK)
- The substrate (KEY) must fit exactly into the rigid enzyme (LOCK)
- Complementary shape & geometry between enzyme and substrate
  - Key (substrate) fits into the lock (enzyme)
- Upon completion of the chemical reaction, the products are released from the active site, so the next substrate molecule can bind



Stoker 2014, Figure 21-3 p750

Induced Fit Model of Enzyme Action

- Many enzymes are flexible & constantly change their shape
  - The shape of the active site changes to accept & accommodate the substrate
    - Conformation change in the enzyme's active site to allow the substrate to bind
    - Analogy: a glove (enzyme) changes shape when a hand (substrate) is inserted into it



Enzyme Specificity

- **Absolute Specificity**
  - An enzyme will catalyze a particular reaction for only one substrate
  - Most restrictive of all specificities
    - Not common
      - ***Catalase*** has absolute specificity for hydrogen peroxide (H_2O_2)
      - ***Urease*** catalyzes only the hydrolysis of urea

- **Group Specificity**
  - The enzyme will act only on similar substrates that have a specific functional group
    - ***Carboxypeptidase*** cleaves amino acids one at a time from the carboxyl end of the peptide chain
    - ***Hexokinase*** adds a phosphate group to hexoses

Enzyme Specificity

- **Linkage Specificity**
  - The enzyme will act on a particular type of chemical bond, irrespective of the rest of the molecular structure
  - The most general of the enzyme specificities
    - ***Phosphatases*** hydrolyze phosphate–ester bonds in all types of phosphate esters
    - ***Chymotrypsin*** catalyzes the hydrolysis of peptide bonds
- **Stereochemical Specificity**
  - The enzyme can distinguish between stereoisomers
  - Chirality is inherent in an active site (as amino acids are chiral compounds)
    - ***L-Amino-acid oxidase*** catalyzes reactions of L-amino acids but not of D-amino acids

Attempt Socrative questions: 1 to 4

Google Socrative and go to the student login

<u>Room name:</u>

City name followed by 1 or 2 (e.g. PERTH1)

1 for 1st session of the week and 2 for 2nd session of the week

Factors Affecting Enzyme Activity

Enzyme activity

- Measure of the rate at which an enzyme converts substrate to products in a biochemical reaction

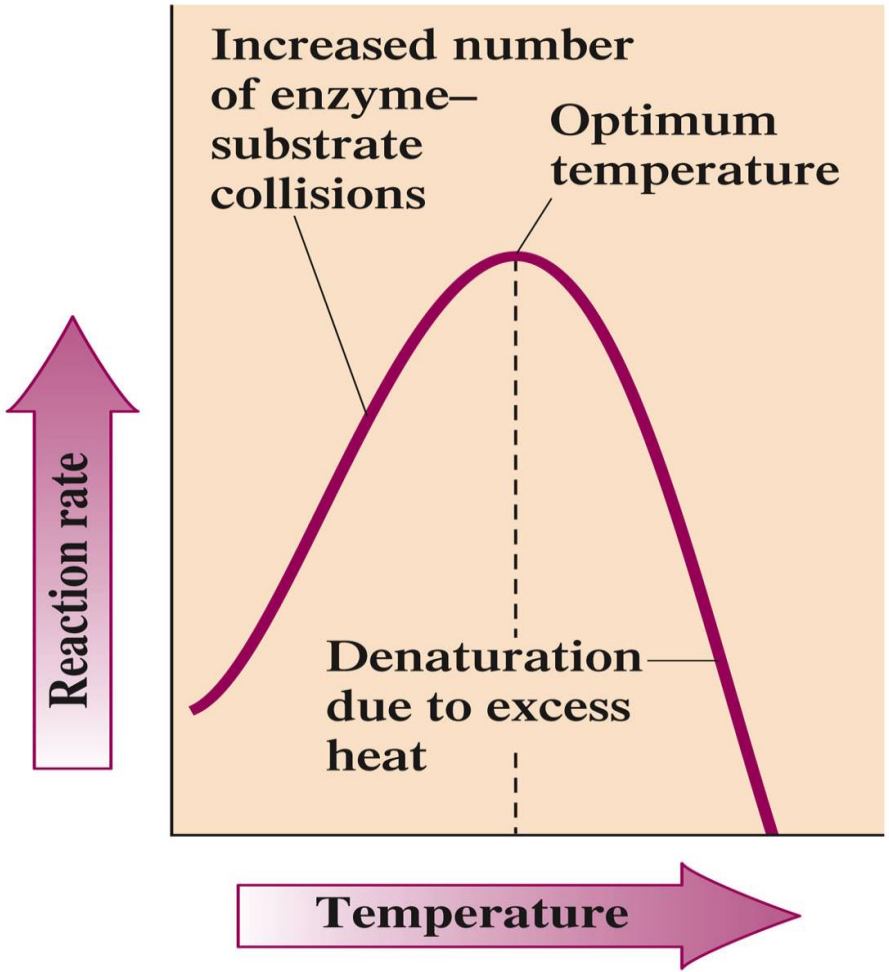
4 factors affect enzyme activity:

- Temperature
- pH
- Substrate concentration: [substrate]
- Enzyme concentration: [enzyme]

Temperature (t)

Stoker 2014, Figure 21-6 p753

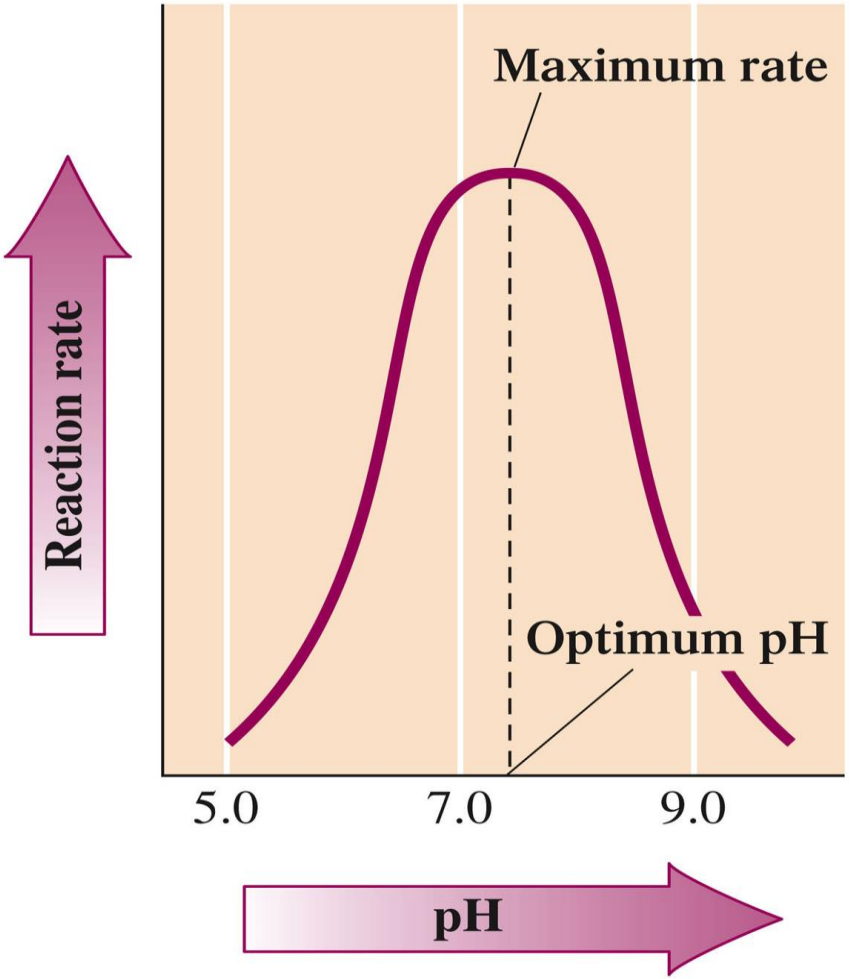
- With increased **t** the E_{KIN} increases
  - More collisions
  - Increased reaction rate
- ***Optimum temperature*** ***(t_{OPT})*** is the **t,** at which the enzyme exhibits maximum activity
  - The t_{OPT} for human enzymes = 37^0C
- When the **t** increases beyond t_{OPT}
  - Changes in the enzyme's tertiary structure occur, inactivating & denaturing it (e.g. fever)
- Little activity is observed at low **t**



pH

- ***Optimum pH*** ***(pH_{OPT})*** is the **pH**, at which the enzyme exhibits maximum activity
- Most enzymes are active over a **very narrow pH range**
  - Protein & amino acids are properly maintained
  - Small changes in pH (low or high) can result in enzyme denaturation & loss of function
- Each enzyme has its characteristic ***pH_{OPT}***, which usually falls within physiological pH range 7.0 - 7.5
- ***Digestive enzymes are exceptions:***
  - ***Pepsin*** (in stomach) – ***pH_{OPT}*** = 2.0
  - ***Trypsin*** (in SI) – ***pH_{OPT}*** = 8.0

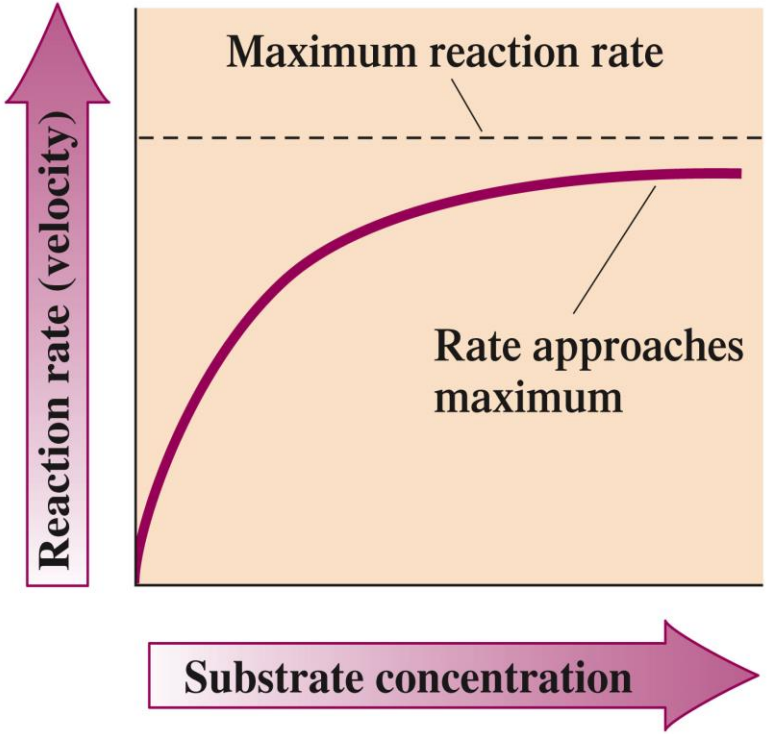
Stoker 2014, Figure 21-7 p753



Substrate Concentration

Stoker 2014, Figure 21-8 p754

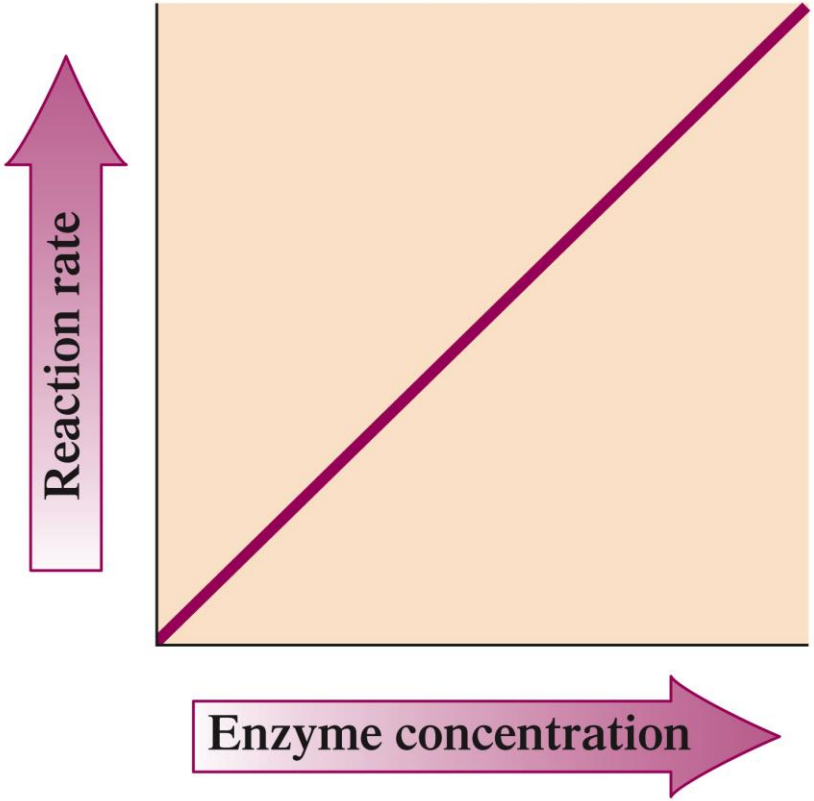
- If [enzyme] is kept constant & the [substrate] is increased
  - The reaction rate increases until
    a ***saturation point*** is met
    - At saturation the reaction rate stays the same even if the [substrate] is increased
  - **At saturation point** substrate molecules are bound to all available active sites of the enzyme molecules
- Reaction takes place at the active site
  - If they are all active sites are occupied the reaction is going at its maximum rate
    - Each enzyme molecule is working at its maximum capacity
  - The incoming substrate molecules must "wait their turn"



Enzyme Concentration

Stoker 2014, Figure 21-9 p755

- If the [substrate] is kept constant & the [enzyme] is increased
  - The reaction rate increases
  - The greater the [enzyme], the greater the reaction rate
- **RULE:**
  - The rate of an enzyme-catalyzed reaction is always directly proportional to the amount of the enzyme present
- ***In a living cell:***
  - The [substrate] is much higher than the [enzyme]
    - Enzymes are not consumed in the reaction
    - Enzymes can be reused many times



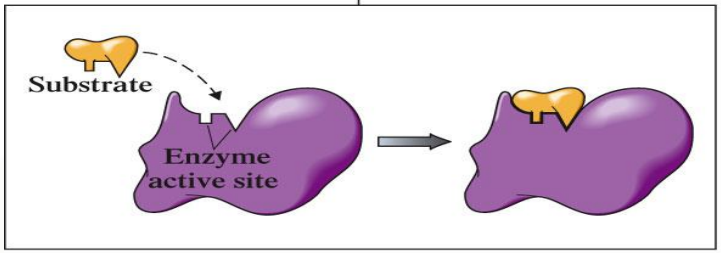
Stoker 2014, p756

THE MECHANISM OF ENZYME ACTION

Formation of an enzyme–substrate complex as an intermediate species provides an alternative pathway, with lower activation energy, through which a reaction can occur.

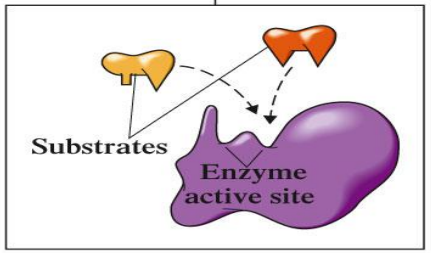
Lock-and-Key Model

The active site has a fixed geometric shape. Only a substrate with a matching shape can fit into it.



Induced-Fit Model

The active site has a flexible shape that can change to accept a variety of related substrates. Enzymes vary in their degree of specificity for substrates.



FACTORS THAT AFFECT THE RATE OF ENZYME ACTIVITY

Temperature

Reaction rate increases with temperature until the point at which the protein is denatured and activity drops sharply.

pH

Maximum enzymatic activity is possible only within a narrow pH range; outside this pH range, the protein is denatured and activity drops sharply.

Concentration of Substrate

Reaction rate increases with substrate concentration until full saturation occurs; then the rate levels off.

Concentration of Enzyme

Reaction rate increases with increasing enzyme concentration, assuming enzyme concentration is much lower than that of substrate.

Participation+

Key concept: function of an enzyme

ENDEAVOUR

What is the function of an enzyme in a chemical reaction?

What happens to the enzymes when the body temperature rises from 37°C to 42°C?

If an enzyme has broken down and is non-functional, what would happen to the chemical reaction normally facilitated by the enzyme? Explain.

Attempt Socrative questions: 5 and 6

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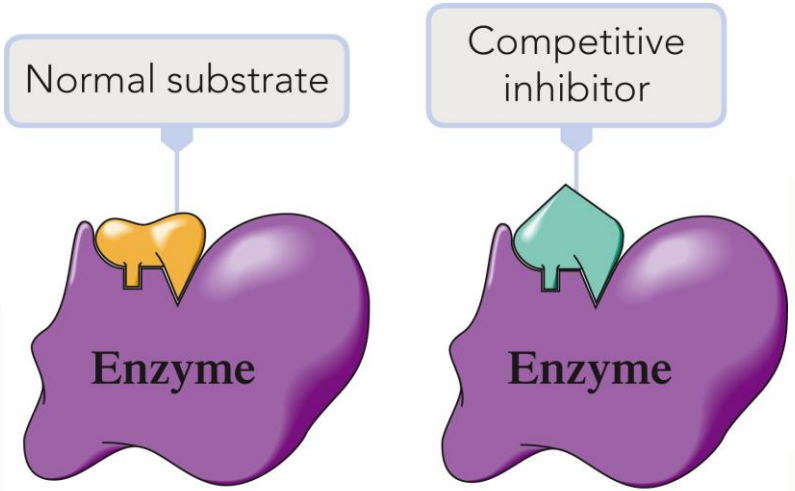
Enzyme Inhibition

- **ENZYME INHIBITOR**
  - A substance that slows down or stops the normal catalytic function of an enzyme by binding to the enzyme

- ***Three types of inhibition:***
  - Reversible competitive inhibition
  - Reversible non-competitive inhibition
  - Irreversible inhibition

Reversible Competitive Inhibition

- A ***competitive inhibitor*** resembles the substrate
  - Inhibitor competes with the substrate for **binding to the active site of the enzyme**
  - If an inhibitor is bound to the active site:
    - Prevents the substrate molecules to access the active site
      - Decreasing / stopping enzyme activity
- The binding of the competitive inhibitor to the active site is a reversible process
  - Add much more substrate to outcompete the competitive inhibitor
- ***Many drugs are competitive inhibitors:***
  - **Anti-histamines** inhibit *histidine decarboxylase*, which converts histidine to histamine

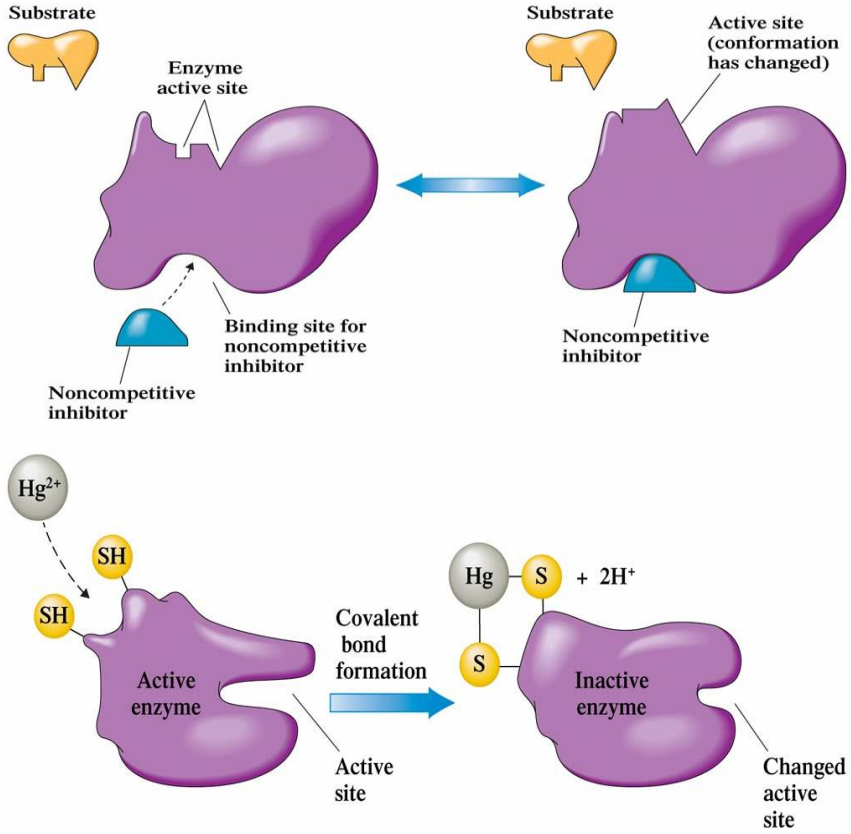


Stoker 2014, Figure 21-11 p758

Stoker 2004, Figure 21.11, p.634

Reversible Noncompetitive Inhibition

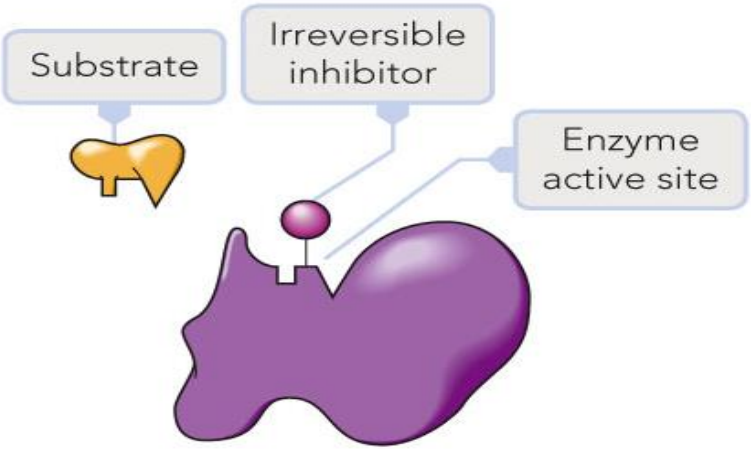
- A **non-competitive inhibitor** decreases enzyme activity by **binding to a site** on the enzyme **other than the active site**
  - The non-competitive inhibitor alters the tertiary structure of the enzyme & the active site
    - Decreasing enzyme activity
    - Substrate cannot fit into active site
  - Process can be reversed only by lowering the [non-competitive inhibitor]
- **Example:**
  - **Heavy metals** Pb^{2+} & Hg^{2+} bind to –SH of Cysteine, away from active site
    - Disrupt the secondary & tertiary structure



Stoker 2004, Figure 21.12, p.634

Irreversible Inhibition

- An ***irreversible inhibitor*** inactivates an enzyme by **binding to** its **active site by a strong covalent bond**
  - **Permanently deactivates the enzyme**
  - Irreversible inhibitors do not resemble substrates
- Addition of excess substrate doesn't reverse this process
  - Cannot be reversed
- **Chemical warfare** (nerve gases)
- **Organophosphate insecticides**

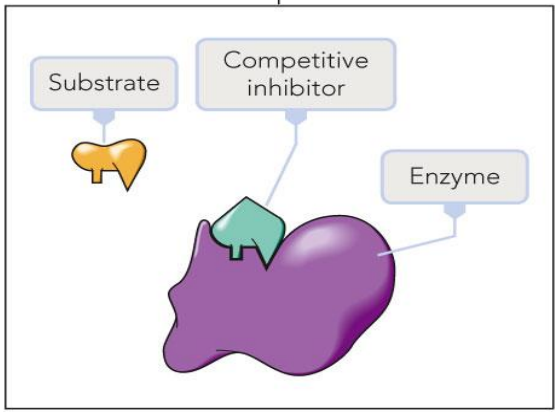


ENZYME INHIBITORS

Substances that bind to an enzyme and stop or slow its normal catalytic activity

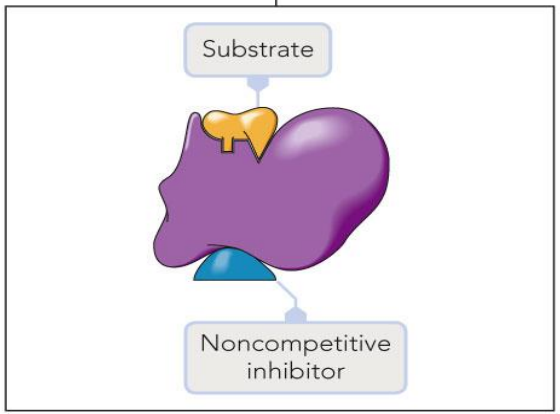
Competitive Enzyme Inhibitor

A molecule closely resembling the substrate. Binds to the active site and temporarily prevents substrates from occupying it, thus blocking the reaction.



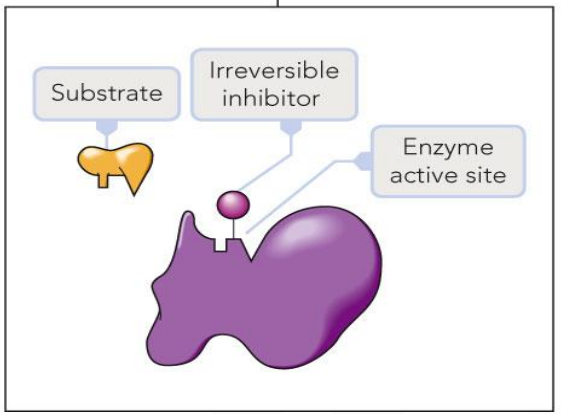
Noncompetitive Enzyme Inhibitor

A molecule that binds to a site on an enzyme that is not the active site. The normal substrate still occupies the active site but the enzyme cannot catalyze the reaction due to the presence of the inhibitor.



Irreversible Enzyme Inhibitor

A molecule that forms a covalent bond to a part of the active site, permanently preventing substrates from occupying it.



Stoker 2014, p760

Attempt Socrative questions: 7 to 9

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Allosteric Enzymes

- Allosteric enzymes have a quaternary structure
  - Are composed of 2 or more protein chains
  - Possess 2 or more binding sites

- ***2 types of binding sites:***

  - One binding site for the substrate
    - Active site

  - Second binding site for a regulator molecule
    - Regulatory site

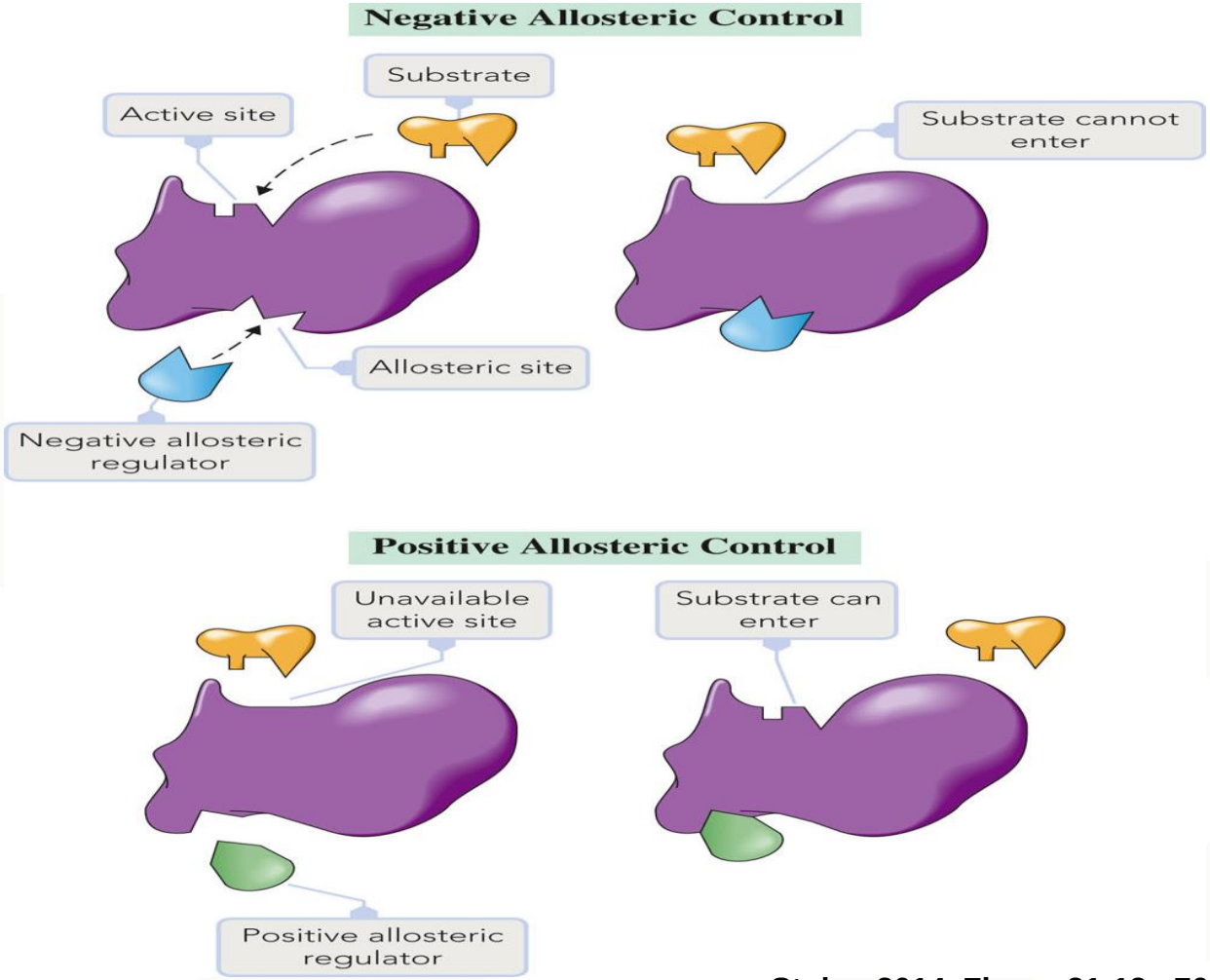
- Active & regulatory binding sites are distinct from each other in shape & location

- Binding of a regulator molecule to its regulatory site causes changes in 3-D structure of the enzyme & the active site

  - Binding of a **Positive regulator** up-regulates enzyme activity
    - Enhances active site, more able to accept substrate

  - Binding of a **Negative regulator** (non-competitive inhibitor)
    down-regulates enzyme activity
    - Compromises active site, less able to accept substrate

The different effects of Positive & Negative regulators on an Allosteric enzyme



Stoker 2014, Figure 21-13 p762

Feedback Control

- A process in which activation or inhibition of one of the earlier reaction steps in a reaction sequence is controlled by a product of this reaction sequence.
  - One of the mechanisms in which allosteric enzymes are regulated
  - Most biochemical processes proceed in several steps & each step is catalyzed by a different enzyme
    - The product of each step is the substrate for the next step / enzyme.

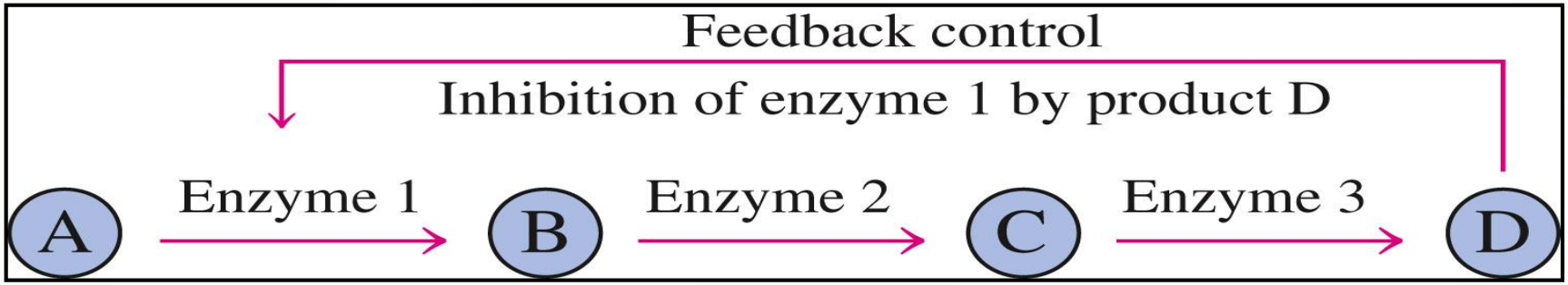
Example:
The degradation of glucose through a metabolic pathway can be *regulated* in several ways

The enzyme PFK is allosterically inhibited by the product *ATP*

Glycolysis (makes ATP) is slowed when cellular *ATP* is in excess

Observe animation of feedback control

http://highered.mheducation.com/sites/0072507470/student_view0/chapter2/animation__feedback_inhibition_of_biochemical_pathways.html



Reaction 1: converts reagent A into product B

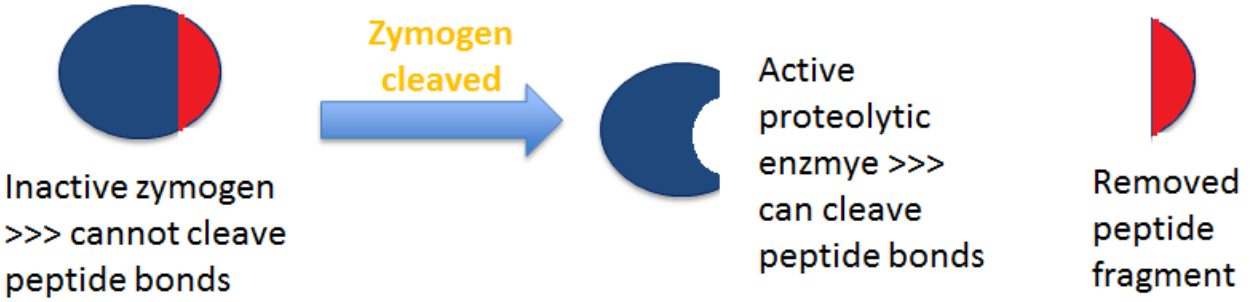
Reaction 2: converts reagent B into product C

Reaction 3: converts reagent C into product D

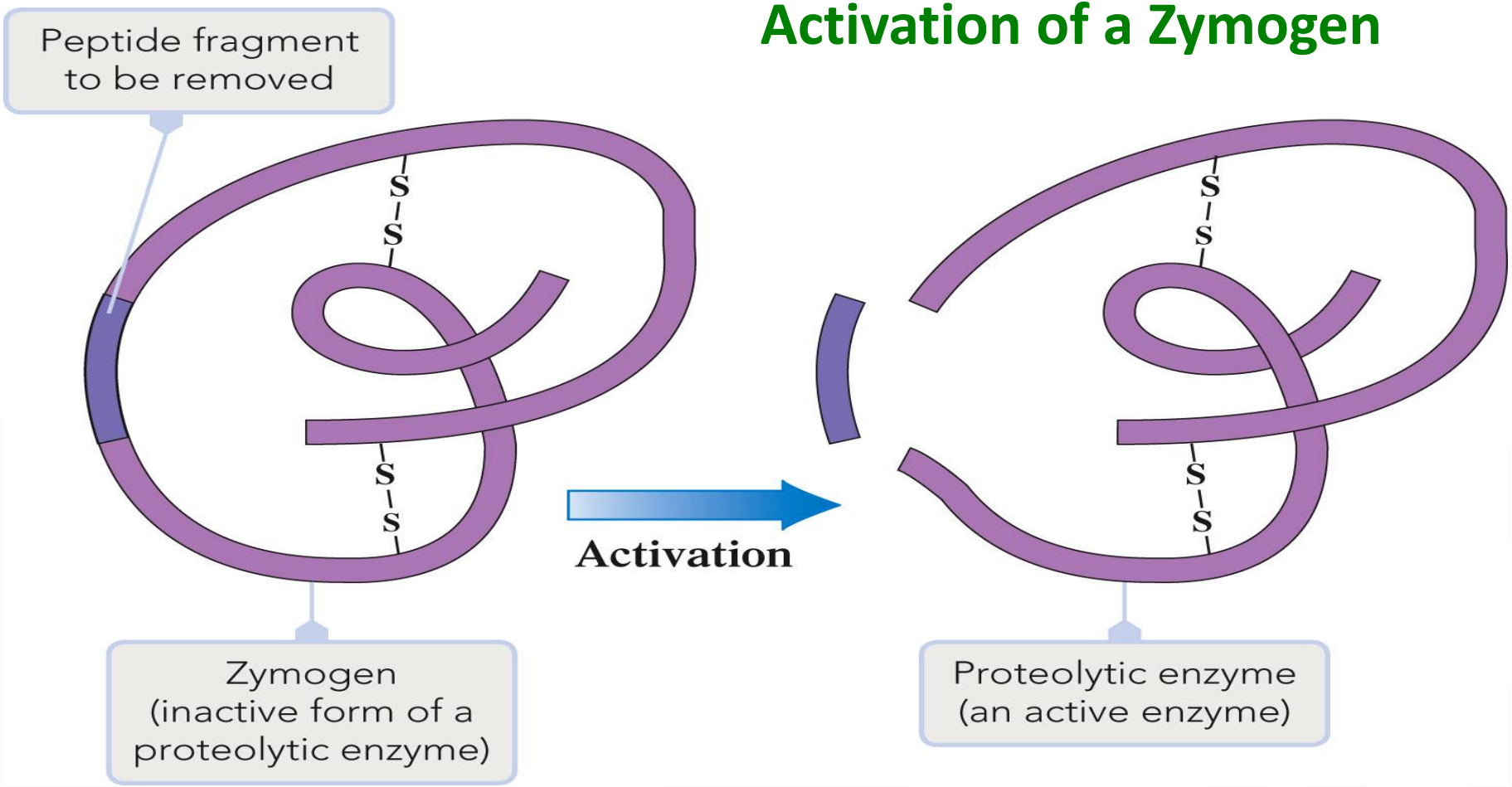
Proteolytic Enzymes & Zymogens

- 2nd mechanism of allosteric enzyme regulation
  - Production of an enzyme in an inactive form
  - Activated when required (right time & place)
    - Activated aka “turned on”
- **Proteolytic enzymes** catalyze breaking of peptide bond in proteins
  - To prevent these enzymes from destroying the tissues, that produced them, they are released in **an inactive form** = **ZYMOGENS**

- Most digestive & blood-clotting enzymes are proteolytic
  - Blood clotting enzymes break down proteins within the blood so that they can form the clot
    - Platelets interspersed with tangled protein (collagen and thrombin)
- Activation of a zymogen requires the removal of a peptide fragment from the zymogen structure
  - Changing the 3-D shape & affecting the active site
    - E.g. Pepsiongen (zymogen)
      >>> Pepsin (active proteolytic enzyme)



Activation of a Zymogen



Stoker 2014, Figure 21-14 p763

Covalent Modification of Enzymes

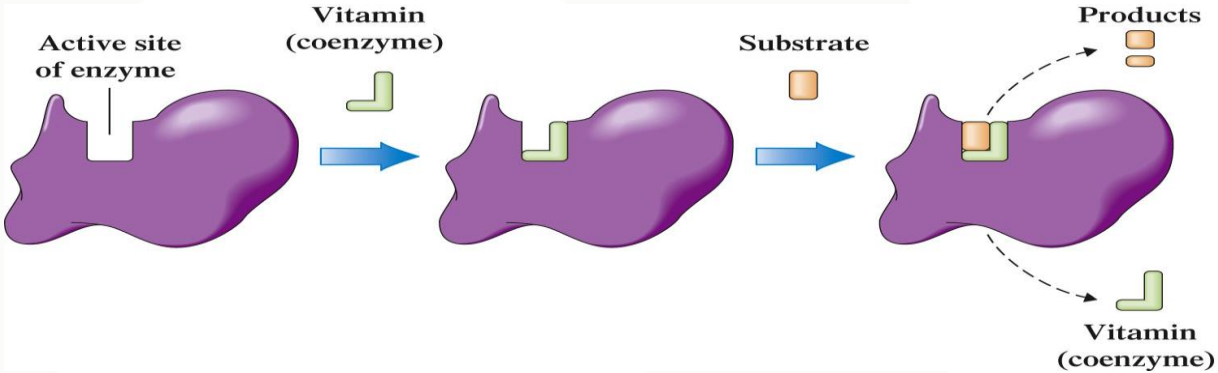
- Covalent modifications are the 3rd mechanism of enzyme activity regulation
  - A process of altering enzyme activity by covalently modifying the structure of the enzyme
    - Adding / removing a group to / from the enzyme

- Most common covalent modification = addition & removal of phosphate group:
  - Phosphate group is often derived from an ATP molecule
    - Addition of phosphate = **phosphorylation** is catalyzed by a ***Kinase*** enzyme
    - Removal of phosphate = **dephosphorylation** is catalyzed by a ***Phosphatase*** enzyme
  - ***Glycogen synthase:*** involved in synthesis of glycogen
    - Deactivated by phosphorylation
  - ***Glycogen phosphorylase:*** involved in breakdown of glycogen
    - Activated by phosphorylation.

Vitamins as Coenzymes

Stoker 2014, Figure 21-20 p779

- Many enzymes require B vitamins as coenzymes
  - Allow the enzyme to function
- Coenzymes serve as temporary carriers of atoms or functional groups
  - Coenzymes provide chemical reactivity that the apoenzyme lacks
  - Important in metabolism reactions to release energy from foods
    - E.g. redox reactions where they facilitate oxidation or reduction
- B vitamins don't remain permanently bonded to the apoenzyme
  - After the catalytic action the vitamin is released & can be repeatedly used by various enzymes
  - This recycling reduces the need for large amounts of B vitamins



Participation+

Key concept: sites with enzymes, coenzymes

ENDEAVOUR

Why is an enzymes active site important to the function of the enzyme?

Why is the enzymes regulatory binding site important for controlling the activity of the enzyme?

Why are coenzymes (derived from vitamins) important to the function of some enzymes?

Attempt Socrative questions: 10 to 13

Google Socrative and go to the student login

Room name:

City name followed by 1 or 2 (e.g. PERTH1)

1 for 1st session of the week and 2 for 2nd session of the week

Drugs Inhibiting Enzyme Activity

- Many prescription drugs inhibit enzymes

- **ACE Inhibitors**
  - Inhibit Angiotensin-Converting Enzyme
    - Lowers blood pressure

- **Sulfa drugs**
  - Antibiotics acting as competitive inhibitors of bacterial enzymes
    - Involved in conversion of PABA to Folic acid
      - Deficiency of folic acid retards bacterial growth, eventually killing them

- **Penicillin's**
  - β-lactam antibiotics inhibit *transpeptidase*
    - Transpeptidase enzyme strengthens the cell wall
      - Forms peptide cross links between polysaccharides strands in bacterial cell walls
      - Without transpeptidase enzyme (inhibited by Penicillin) >>> weakened cell wall, bacteria dies

Medical Uses of Enzymes

- Enzymes can be used in diagnosis & treatment of certain diseases

- ***Lactate dehydrogenase*** **(LDH)** is normally not found in high levels in blood, as it is produced in cells
  - Increased levels of LDH in the blood indicate myocardial infarction (MI) (Heart attack)

  - **Tissue plasminogen activator (TPA)** activates the enzyme *plasminogen* that dissolves blood clots
    - Used in the treatment of MI

- There is no direct test to measure urea in the blood
  - ***Urease*** converts urea into ammonia, which is easily measured & is used as urea indicator
    - Blood Urea Nitrogen (BUN) is used to measure kidney function

  - High urea levels in the blood indicate kidney malfunction

Isoenzymes

- Isoenzyme catalyze the same reaction in different tissues in the body
  - e.g. *lactate dehydrogenase* (LDH) consists of 5 isoenzymes
    - Each isoenzyme of LDH has the same function
      - Converts lactate to pyruvate
    - LDH_1 isoenzyme is more prevalent in heart muscle
    - LDH_5 form is found in skeletal muscle & liver
- Isoenzymes can be used to identify the damaged or diseased organ or tissue
  - It is a marker for a particular location
- If LDH_1 isoenzyme was found in the blood >>> indicates heat muscle damage

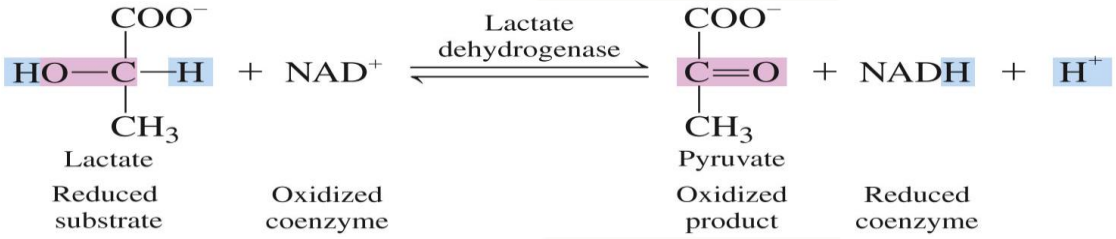


Table 21.3 Selected Blood Enzyme Assays Used in Diagnostic Medicine

Enzyme	Condition Indicated by Abnormal Level
lactate dehydrogenase (LDH)	heart disease, liver disease
creatine phosphokinase (CPK)	heart disease
aspartate transaminase (AST)	heart disease, liver disease, muscle damage
alanine transaminase (ALT)	heart disease, liver disease, muscle damage
gamma-glutamyl transpeptidase (GGTP)	heart disease, liver disease
alkaline phosphatase (ALP)	bone disease, liver disease

Stoker 2014, Table 21-3 p768

Table 21.7 Selected Important Coenzymes in Which B Vitamins Are Present

B Vitamin	Coenzymes	Groups Transferred
thiamin	thiamin pyrophosphate (TPP)	aldehydes
riboflavin	flavin mononucleotide (FMN) flavin adenine dinucleotide (FAD)	hydrogen atoms
niacin	nicotinamide adenine dinucleotide (NAD^+) nicotinamide adenine dinucleotide phosphate ($NADP^+$)	hydrogen atoms
pantothenic acid	coenzyme A (CoA)	acyl groups
vitamin B_6	pyridoxal-5-phosphate (PLP) pyridoxine-5′-phophate (PNP) pyridoxamine-5′-phosphate (PMP)	amino groups
biotin	biotin	carbon dioxide (carboxyl group)
folate	tetrahydrofolate (THF)	one-carbon groups other than CO_2
vitamin B_{12}	methylcobalamin	methyl groups, hydrogen atoms

Stoker 2014, Table 21-7 p780

Readings & Resources

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