Lec 6 **Enzymes**

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Enzymes

- With the exception of a few catalytic RNAs, all known enzymes are proteins that act as catalysts to increase the rate of biochemical reactions
- Enzyme-catalyzed reactions have three basic steps: Enzyme catalysts bind reactants (substrates), convert them to products and release the products:
- Enzymes, provide speed, specificity, and regulatory control to reactions in the body.
- Enzymes return to their original form at the end of reaction
- Have M.Weight ranging from about 12,000 to more than 1 million
- Substrate is the substance upon which the enzyme act. The substrates are bound to specific substrate binding sites on the enzyme through interactions with the amino acid residues of the enzyme.

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Enzymes

- Each enzyme selective for its substrates and ensures that only specific products are formed
- An enzyme-catalyzed reaction is take place within the confines of a pocket on the enzyme called the active site
- **E + S E-SE-PE + P**

- Enzymes provide a means for regulating the rate of metabolic pathways in the body
- In some diseases, especially genetic disorders, there may be deficiency or a total absence of one or more enzymes
- Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in diagnosing certain illnesses
- Many drugs exert their biological effects through interactions with enzymes

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- If an enzyme is broken down into its component amino acids, its catalytic activity is always destroyed. Thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.

Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	*Examples of chemical groups transferred*	*Dietary precursor in mammals*
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

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▪Coenzyme act as transient carriers of specific functional groups

▪A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a prosthetic group (a non protein group forming part of or combined with a protein.)

▪Active enzyme together with its bound coenzyme and/or metal ions is called a (holoenzyme). The protein part of such an enzyme is called the apoenzyme or apoprotein

International Classification of Enzymes

Enzymes usually have both a common name and a systematic classification that includes a formal name and an Enzyme Commission (EC) number.

▪The common names for most enzymes derive from their ability to catalyze a specific chemical reaction. In general, an enzyme's name consists of a term that

identifies the type of reaction catalyzed followed by the suffix-**ase**. e.g dehydrogenases remove hydrogen atoms, proteases hydrolyze proteins, and isomerases catalyze rearrangements in configuration.

▪Systematic name: each enzyme has a unique name and code number that reflect the type of reaction catalyzed and the substrates involved. e.g "hexokinase" is designated "ATP:D-hexose-6-phosphotransferase E.C. **2.7.1.1**." This identifies hexokinaseas a member of class 2 (transferases), subclass 7(transfer of a phosphoryl group), sub-subclass 1(alcoholis the phosphoryl acceptor). Finally, the term "hexose-6" indicates that the alcohol phosphorylated is that of carbon six of a hexose.

International Classification of Enzyme

Biochemists have adopted a system for naming and classifying enzymes. This system divides enzymes into six classes

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Enzymes

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

1-Oxidoreductases: oxidation and reduction reactions
•dehydrogenases addition or removal of H

•peroxidases use as H_2O_2 as oxygen donor, forming H_2O

2-Transferases: transfer a chemical group from one substrate to another
•kinases transfer phosphate from ATP onto substrate

3-Hydrolases: hydrolysis(water splits the bond) of C-O, C-N, O-P and C-S bonds (e.g. esterases, proteases, phosphatases, deamidases)
4-Lyases: catalyze cleavage of C-C, C-O, C-N and other bonds by elimination, leaving double bonds, and also add groups to double bonds (e.g. dehydratases, hydratases, decarboxylases)
5-Isomerases: intramolecular rearrangements (catalyze geometric or structural changes within a single molecule
6-Ligase (Synthetases) : formation of bonds between two substrates (frequently linked to utilization of ATP)

What is a catalyst ?

▪The functional groups in the catalytic site of the enzyme activate the substrates and decrease

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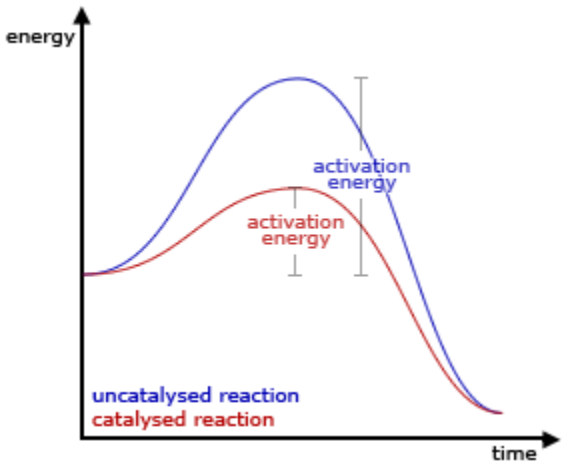
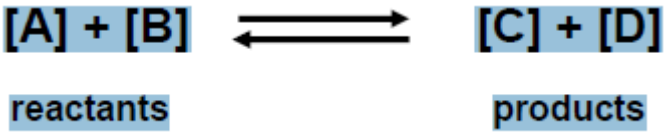
the energy needed to form the high-energy intermediate stage of the transition state complex

- The pressence of catalysis is specific stabilization of the transition state.

- lowers the activation energy
- increased rate of reaction
- is not consumed in the reaction
- does not affect the reaction equilibrium





Enzymes as catalysts

- The enzymatic catalysis of reactions is essential to living systems

- Protein–large organic compound made of amino acids arranged in a linear chain and folded into a 3-D structure

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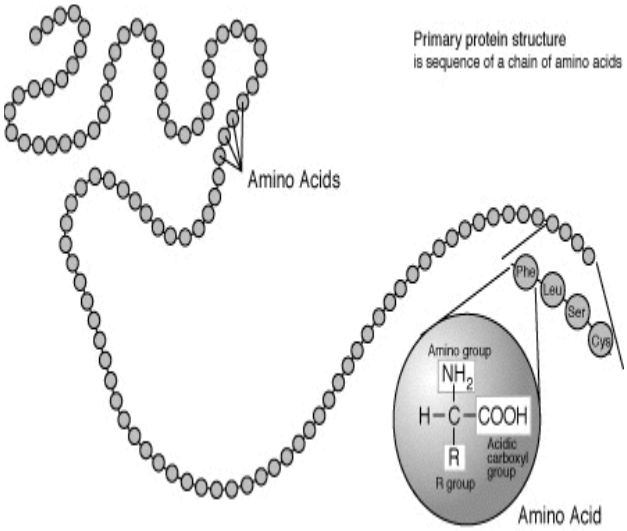
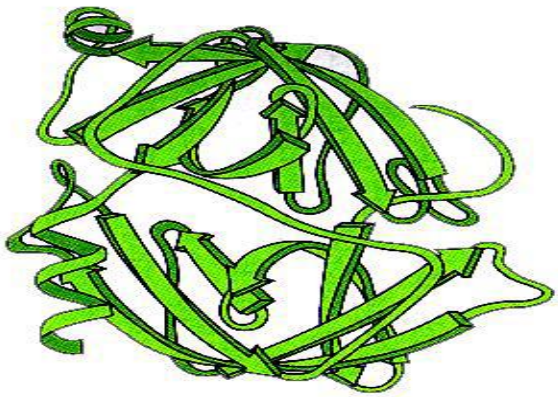
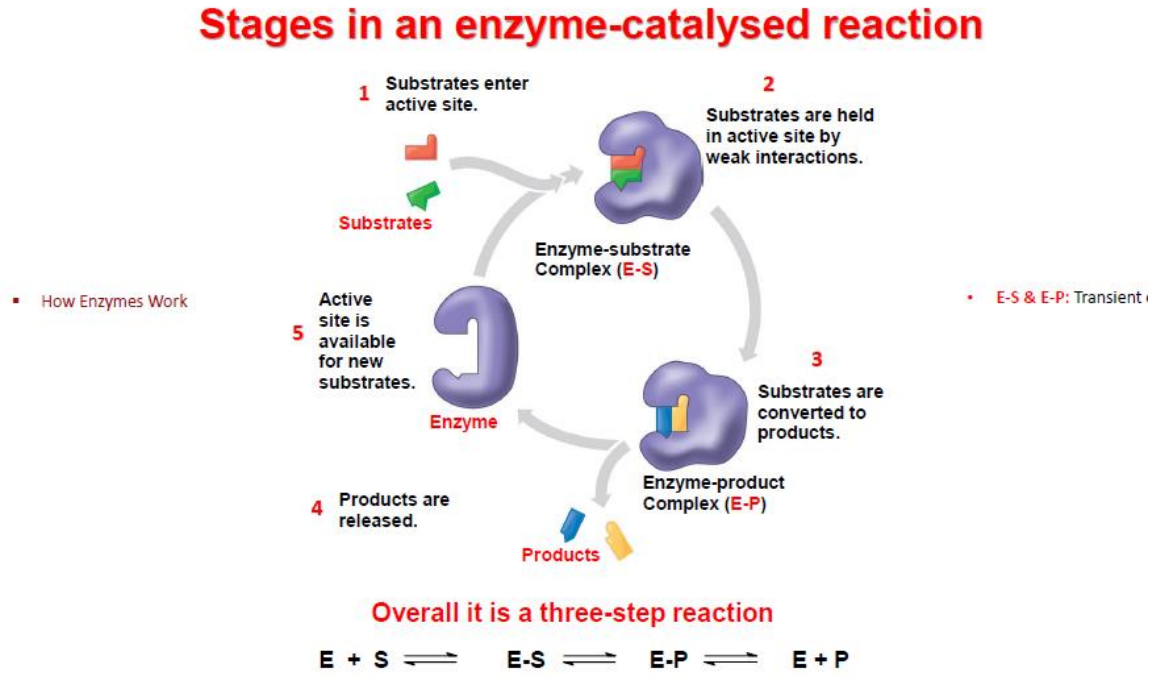
Enzymes

- Folding of the protein brings side-chains of various amino acids that may be far apart in the primary sequence into close juxtaposition, forming an active site





Stages in an enzyme-catalysed reaction



Properties of the active site

Positioning of substrate molecules in the most favourable relative orientation for the reaction to occur

- The active site is perfectly complementary to the transition state

Enzymes

- Amino acid side chains of the active site stabilize the electron distribution of the transition state

- The substrate is strained on binding to the active site

  ➢ lowers the activation energy

  ➢ increases the reaction rate

- The transition state is rapidly converted to the product(s)

- The products bind less tightly to the enzyme and are released

The active site of an enzyme –substrate binding site

Enzymes

- **Non-covalent interaction between the substrate and the amino side – chain of the enzyme**:
  - Basic groups (Lys, His, Arg) → **ionic bonds**
  - Acidic groups (Asp, Glu) → **ionic bonds**
  - **Hydrophobic interactions** (Ala, Leu, Ile, Val, Met)
  - **Hydrophilic interactions with –OH** or alcoholic groups (Ser,Thr, Tyr)
  - **Hydrophilic interactions with –SH** or thiol groups (Cys)
  - **Hydrophilic interactions with amide** groups (Asn, Gln)
  - **Aromatic interactions** (Phe, Tyr, Trp)

The active site of an enzyme –catalytic site

Reactive groups at the enzyme surface catalyse the reaction by:

- Donating or withdrawing electrons
- Stabilizing or generating free radical intermediates
- Forming temporary covalent bonds(a transition state intermediate)

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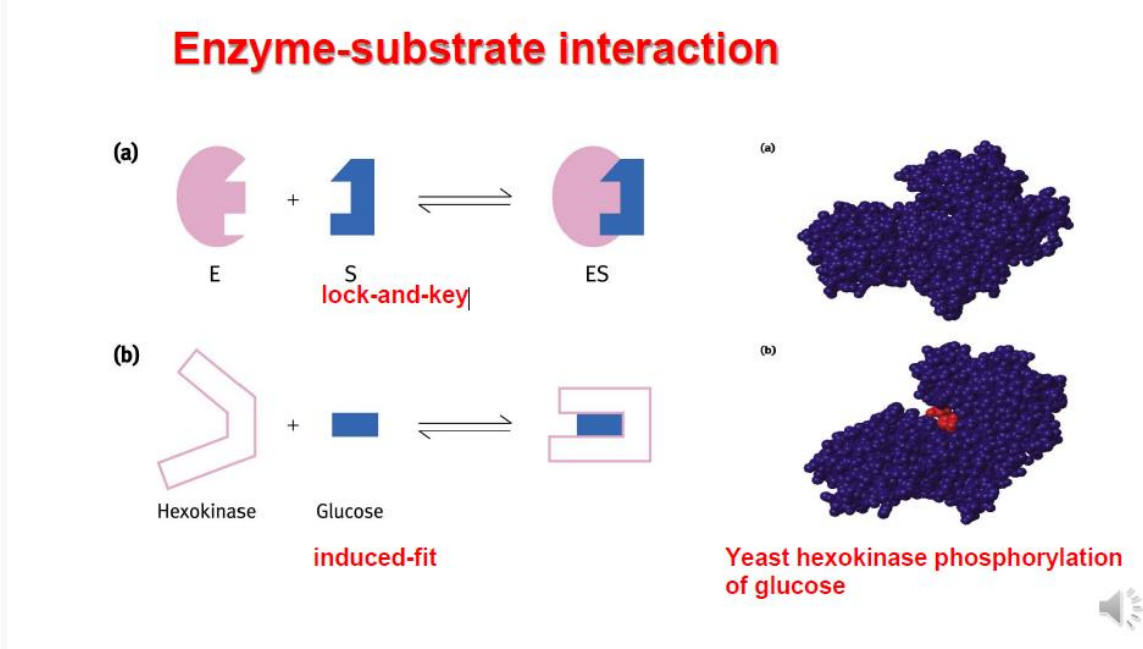
Enzyme-substrate interaction



lock-and-key model

The substrate binding site contains amino acid residues arranged in a complementary three-dimensional surface that “recognizes” the substrate and binds it through multiple hydrophobic interactions, electrostatic interactions, or hydrogen bonds. The amino acid residues that bind the substrate can come from very different parts of the linear amino acid sequence of the enzyme. In the lock-and-key model, the complementarity between

the substrate and its binding site is compared to that of a key fitting into a rigid lock.

Induced fit model

As the substrate binds, enzymes undergo a conformational change ("induced fit") that repositions the side chains of the amino acids in the active site and increases the number of binding interactions . The induced fit model for substrate binding recognizes that the substrate binding site is a dynamic surface created by the flexible overall three-dimensional structure of the enzyme.

- In addition to reactive groups from amino acids enzymes may contain non-proteinmolecules also called **cofactors and coenzyme**:

- Metal group (e.g. hexokinase **Mg^{2+}**)
- Coenzyme–tightly but not covalently bound organic molecule (**NAD**)
- Prosthetic group–covalently bound organic molecule (heme)

Enzymes

- Enzyme **with** prosthetic group = holoenzyme–**catalytically active**

- Enzyme protein **without** prosthetic group
= apoenzyme–**catalytically inactive**

Units of enzyme activity – catalytic activity

- How much substrate can be converted (product formed) in a given time

- Number of micromoles (µmol) of substrate converted to product per minute under standard optimized conditions at 30°C

- 1enzyme unit (EU) = 1 µmol min^{-1}

Specificity

What does specificity mean ?

- The ability of an enzyme to select just one substrate and distinguish this substrate from a group of very similar compounds is referred to as specificity, e.g. Glucokinase catalyzes the transfer of a phosphate from ATP to carbon 6 of glucose

- Enzymes catalyse only one specific reaction. The enzyme converts this substrate to just one product

- Shape, charge and conformation of the substrate are critically important for binding to an enzyme

- Specificity and speed of enzyme catalyzed reactions result from the unique sequence of specific amino acids that form the three-dimensional (3D) structure of the enzyme.

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Specific activity

- Activity of an enzyme per milligram (mg) of total protein (expressed in µmolmin-1mg-1)

- Specific activity gives a measurement of the purity of the enzyme.

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The reaction rate

- Generation of the reaction product with time
- Measured at a fixed enzyme concentration
- Defined temperature and pH

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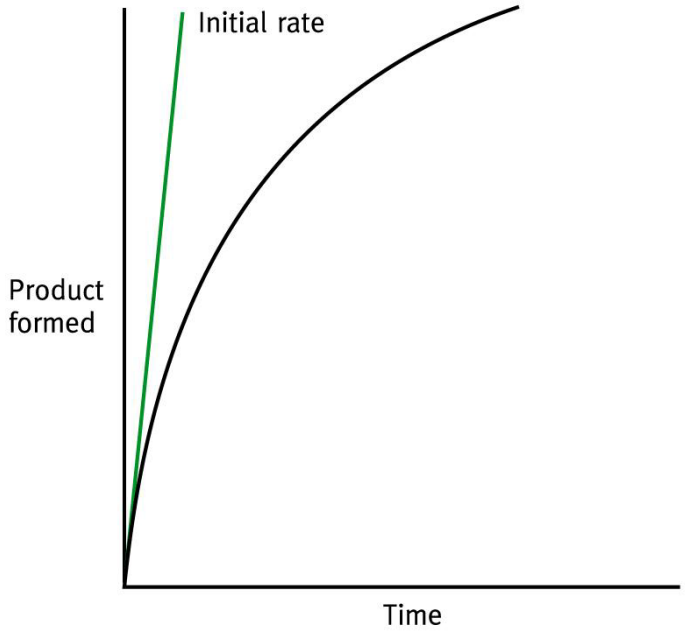
Enzymes



Why is it hyperbolic ?

- Accumulation of product
- Depletion of substrate
- Denaturation of enzyme

Factors affecting enzyme activity

- pH
- Temperature
- Concentration of enzyme
- Concentration of substrate
- Covalent modification of enzyme
- Inhibitors and activators

pH

- pH is a measure of the acidity or alkalinity of a solution

Enzymes

- Every enzyme has an optimum pH (or pH range) at which it has maximal activity
  - **Neutral = pH 7.00**
  - **Acidic< 7.00 < Basic**

PH dependence of an enzyme catalyzed reaction

Ionization state of amino acid side chains depends on the pH of the solution

- Binding of the substrate and catalysis depend on pH
- e.g. pH optima for phosphatases in the blood plasma

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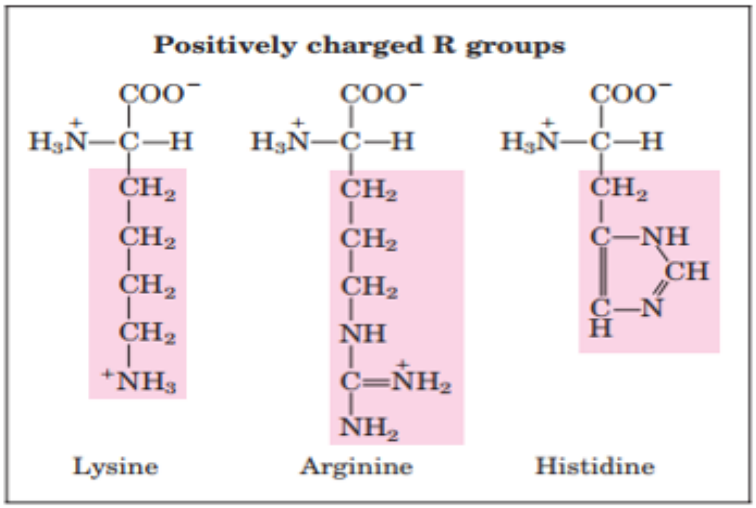
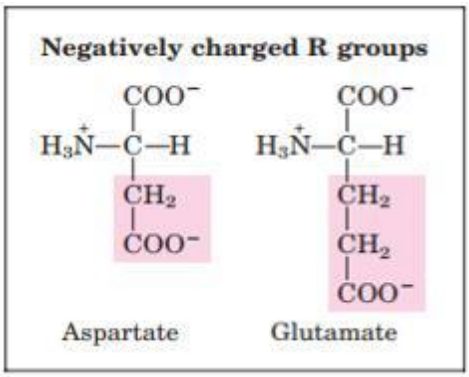
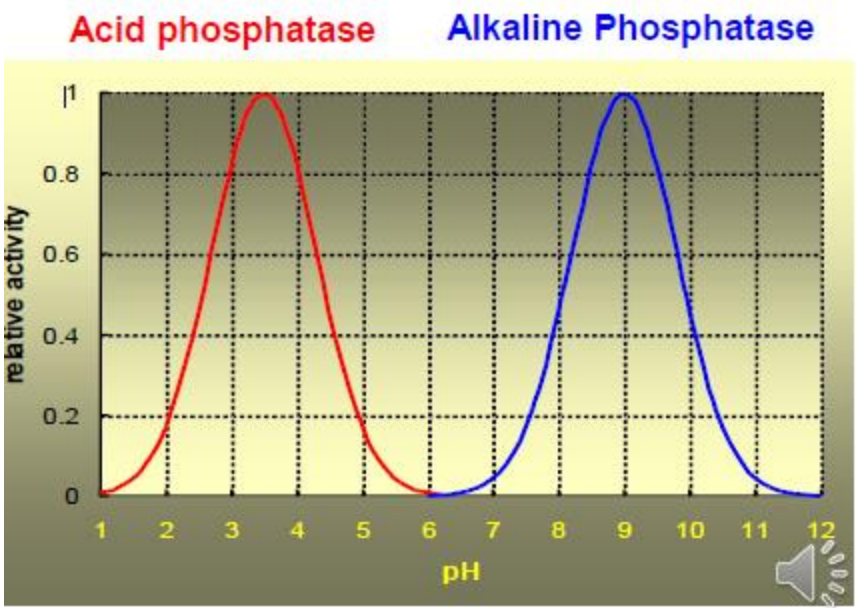
Enzymes







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Temperature dependence of an enzyme-catalysed reaction

- Most human enzymes function optimally at a temperature of approximately 37℃
- Chemical reactions proceed faster at higher temperatures:
  - ➢ molecules move faster, greater chance to collide
  - ➢ electrons gain activation energy easier
- Denaturation of the enzyme → loss of hydrogen bonding → unfolding → precipitation → loss of activity
- The temperature optimum depends on the time of incubation

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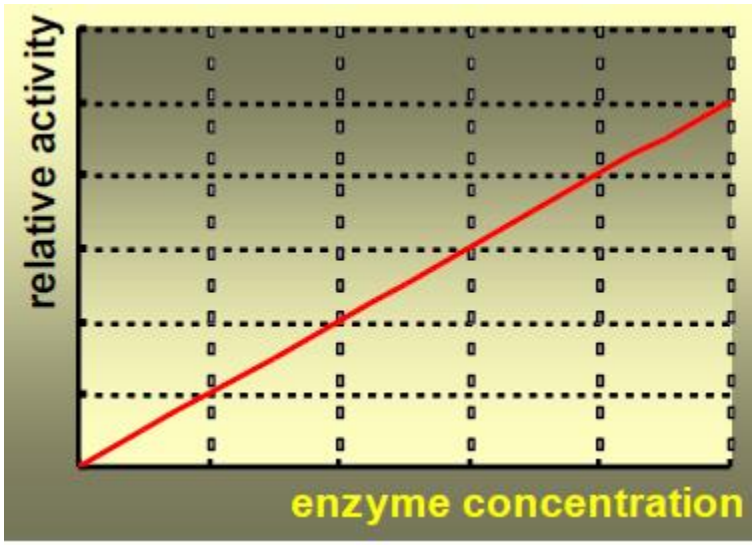
Enzymes

The effect of varyfying the amount of enzyme

- Predictable linear increase in product formation with increasing amount of enzyme



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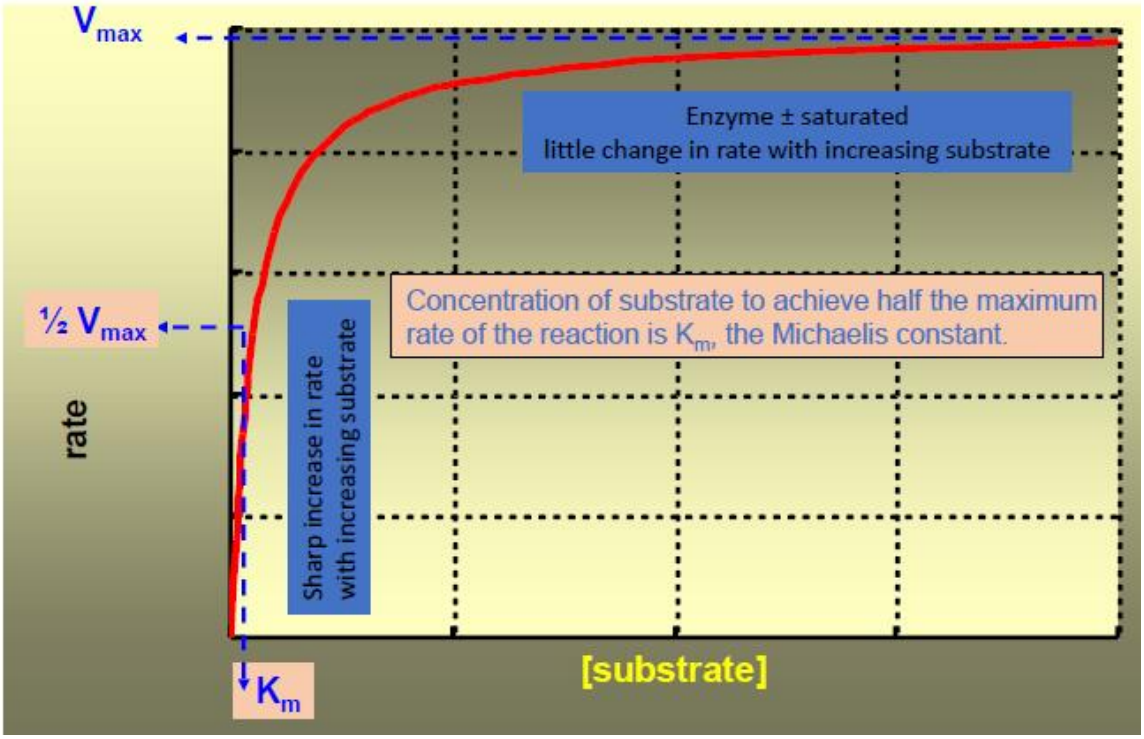
Substrate concentration dependence of an enzyme catalyzed reaction

At a constant concentration of enzyme ,the reaction rate increases with increasing substrate concentration until a maximal velocity is reached

- K_m is an important characteristic of enzyme-substrate interactions and is independent of enzyme and substrate concentrations



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Kinetic Properties of Enzyme

The equations of enzyme kinetics provide a quantitative way of describing the dependence of enzyme rate on substrate concentration.

Michaelis-Menten equation

- The Michaelis-Menten model of enzyme kinetics applies to a simple reaction in which the enzyme and substrate form an enzyme–substrate complex (ES) that can dissociate back to the free enzyme and substrate.

- Relates the velocity (v) to the concentration of substrate [S] and the two parameters K_m and V_{max}

Michaelis-Menten equation (single-substrate reaction)

m

- Describes the dependence of rate of reaction on concentration of substrate at steady state(ES formation balanced by its removal) and vast molar excess of substrate over enzyme [S]>>[E].

v rate of reaction

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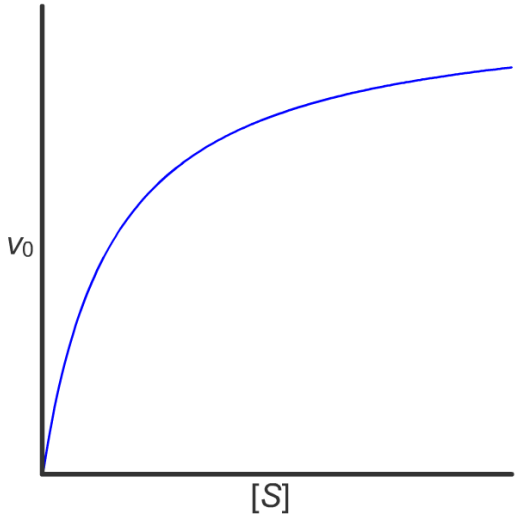
Enzymes

Michaelis-Menten equation:

$$v_0 = \frac{V_{\max}[S]}{K_M + [S]}$$



v	rate of reaction
V_{max}	maximal rate of reaction
[S]	concentration of substrate
K_m	Michaelis constant

K_m, the Michaelis constant

- Low K_m corresponds to high affinity for the substrate
- High K_m corresponds to low affinity for the substrate
- Enzymes with a low K_m compared with the concentration of substrate [S]in the cell act at their maximum rate

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Enzymes

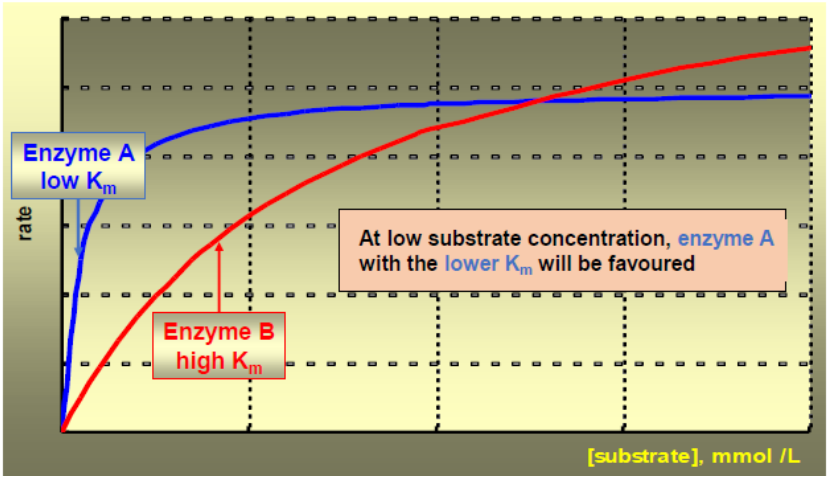
- modest changes in the concentration of substrate [S]have no effect on the rate of reaction

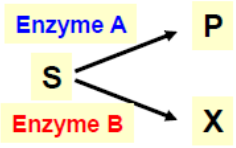
- Enzymes with a high K_m+ small change in the concentration of substrate [S]

- large change in the rate of reaction

- Typical $K_m$values:
  -Pyruvate carboxylase 60 µM for ATP
  -Chymotrypsin 5 mM for peptide substrate
  -Protein kinase 12 µM for ATP

The relevance of K_m: two enzymes "competing" for substrate [S]





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Experimental determination of K_m and V_{max}
The Lineweaver-Burk double reciprocal plot

- Is transformations of the Michaelis-Menten Equation

- The **K_m** and **V_{max}** for an enzyme can be visually determined from a plot of $1/v_0$ versus 1/S, called a Lineweaver-Burkor a double reciprocal plot

- More accurate determination of **V_{max}**

- Distinguishing between certain types of enzymatic reaction mechanisms and in analyzing enzyme inhibition

- (v_0 = Initial velocity)

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

(v_0 = Initial velocity)

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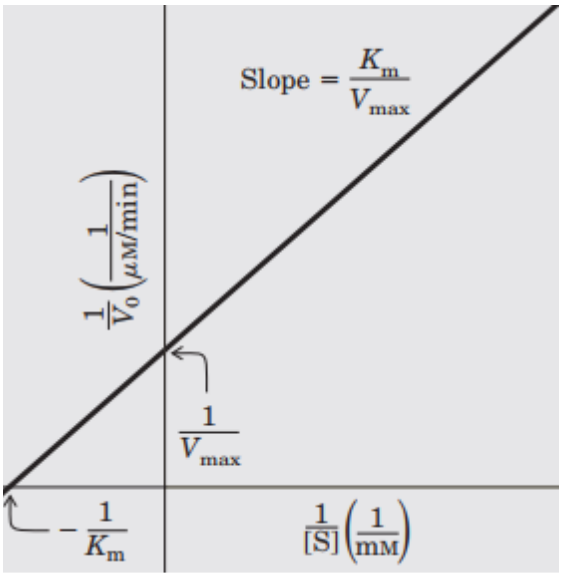

Most Biochemical Reactions Include Multiple Substrates

- Most reactions in biological systems usually include two substrates and two products

- Multiple substrate reactions can be divided into two classes: sequential displacement and double displacement

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Enzymes with two substrates (sequential displacement)

$A + B \rightleftharpoons C + D$

- All substrates must bind to the enzyme before any product is released. Consequently, a ternary complex of the enzyme and both substrates forms
- **Sequential reaction** – each substrate binds in turn

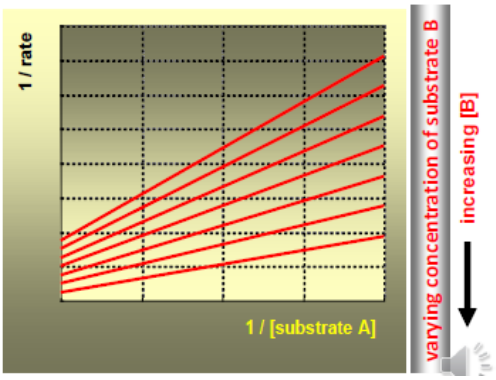
(ternary complex = complex containing three different molecules A-E-B)

$A + E \rightleftharpoons A\text{-}E$

$A\text{-}E + B \rightleftharpoons A\text{-}E\text{-}B \rightleftharpoons C\text{-}E\text{-}D \rightleftharpoons C\text{-}E + D$

$C\text{-}E \rightleftharpoons E + C$

converging lines



Enzymes with two substrates (double displacement)

In double-displacement, or Ping-Pong, reactions, one or more products are released before all substrates bind the enzyme

- The defining feature of double-displacement reactions is the existence of a substituted enzyme intermediate, where

the altered enzyme forms a second complex with another substrate molecule, and the second product leaves

- Substrate 1may transfer a functional group to the enzyme (to form the covalently modified E), which is subsequently transferred to substrate 2. This is called a Ping-Pong or double-displacement mechanism.

- Reactions that shuttle amino groups between amino acids and α -ketoacids are classic examples of double-displacement mechanisms.

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Enzymes with two substrates (double displacement)

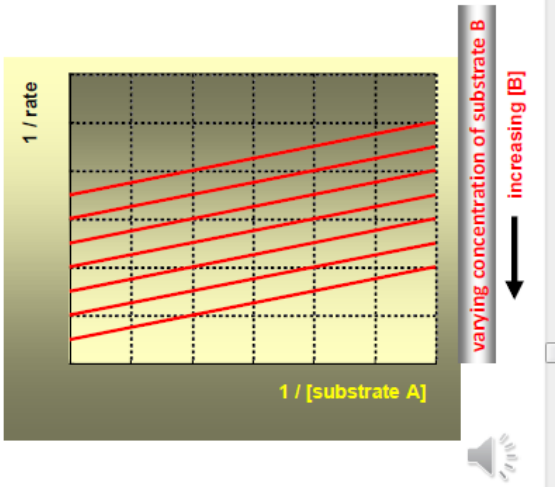
- The Michaelis Menten equation is also applicable to bisubstrate reactions, which occur by ternary-complex or Ping-Pong (double-displacement) pathways
- Ping-pong reaction – one substrate reacts, and modifies enzyme, then second substrate reacts with modified enzyme

$$A + B \rightleftharpoons C + D$$

$$A + E \rightleftharpoons A\text{-}E \rightleftharpoons C\text{-}E^* \rightleftharpoons C + E^*$$

$$B + E^* \rightleftharpoons B\text{-}E^* \rightleftharpoons D\text{-}E \rightleftharpoons D + E$$

parallel lines



Allosteric enzymes

- Allosteric enzymes-enzymes with cooperative substrate binding
- contain binding sites “other” (“allo”) than substrate binding sites.
- often in multi-subunit complex, more than one active site in the complex.

- binding of substrate to the active site of the first subunit leads to change in conformation facilitating binding of substrate to the other active sites

Enzyme inhibitors

- Decrease the enzyme's ability to bind substrate or/and lower the enzyme's catalytic activity

- Many drugs and toxic agents act by inhibiting enzymes

Type of enzyme inhibitors

- Reversible inhibitors

- Irreversible inhibitors (inactivators)

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Enzyme inhibitors

Reversible inhibitors	Irreversible inhibitors
▪ Non-covalent binding to enzyme	▪ Tightly Bind to enzyme covalently
▪ Many are relatively unspecific	▪ Many are substrate analogues
▪ Mechanism: blocking substrate binding or hindering catalytic steps	▪ Undergo part of reaction
	▪ Transition state covalent intermediate does not break down

Irreversible inhibitors

Dissociates very slowly from its target enzyme because it has become tightly bound to the enzyme

▪Some irreversible inhibitors are important drugs. Penicillin acts by covalently modifying the enzyme transpeptidase, there by preventing the synthesis of bacterial cell walls and thus killing the bacteria. Aspirin acts by covalently modifying the enzyme cyclooxygenase, reducing the synthesis of inflammatory signals.

Enzymes

Competitive inhibitor

- Competes with the substrate for binding at the active site
- Inhibition is a function of the relative affinities of the substrate and the inhibitor for binding the enzyme
- Inhibition is a function of the relative concentrations of substrate and inhibitor

$$E + S + I \rightleftharpoons E\text{-}S \rightleftharpoons E\text{-}P \rightleftharpoons E + P$$
$$E + S + I \rightleftharpoons E\text{-}I$$

- V_{max} is unchanged, K_m is increased
- If enough substrate is added, it overcomes the inhibitor

Competitive inhibitor

- Methotrexate is a structural analog of tetrahydrofolate, a coenzyme for the enzyme dihydrofolate reductase, which plays a role in the biosynthesis of purines and pyrimidines. It binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis. It is used to treat cancer

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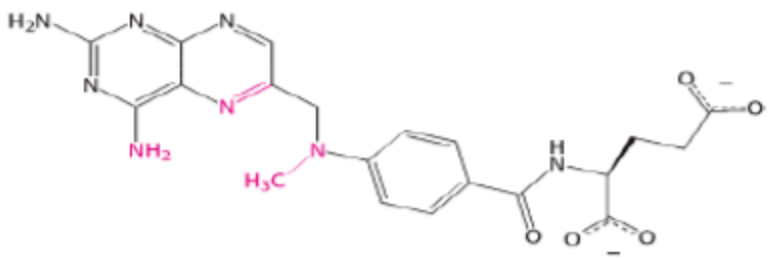
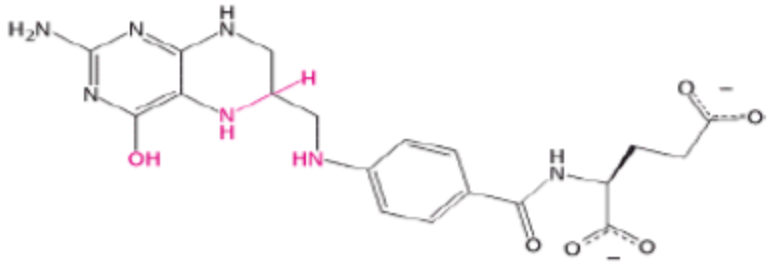
Enzymes



Methotrexate



Tetrahydrofolate

Non-competitive inhibitor

- Binds to the enzyme at a position separate from the active site
- No competition for binding with the substrate
- The apparent affinity for the substrate is unchanged, but the rate of reaction is slowed

$$E + S + I \rightleftharpoons E\text{-}S + I \rightleftharpoons E\text{-}S\text{-}I \overset{\text{slow}}{\rightleftharpoons} E\text{-}P\text{-}I \rightleftharpoons E + P + I$$

K_m is unchanged, V_{max} is decreased

- Adding more substrate has no effect on the rate of reaction

Mixed inhibitors

- Mixed inhibitors do not bind in the active site

- Inhibitor can bind prior to substrate or to the enzyme-substrate complex

- Mixed inhibitors distort the substrate binding site which affects:-apparent substrate affinity -catalytic turn-over (slowing catalysis)

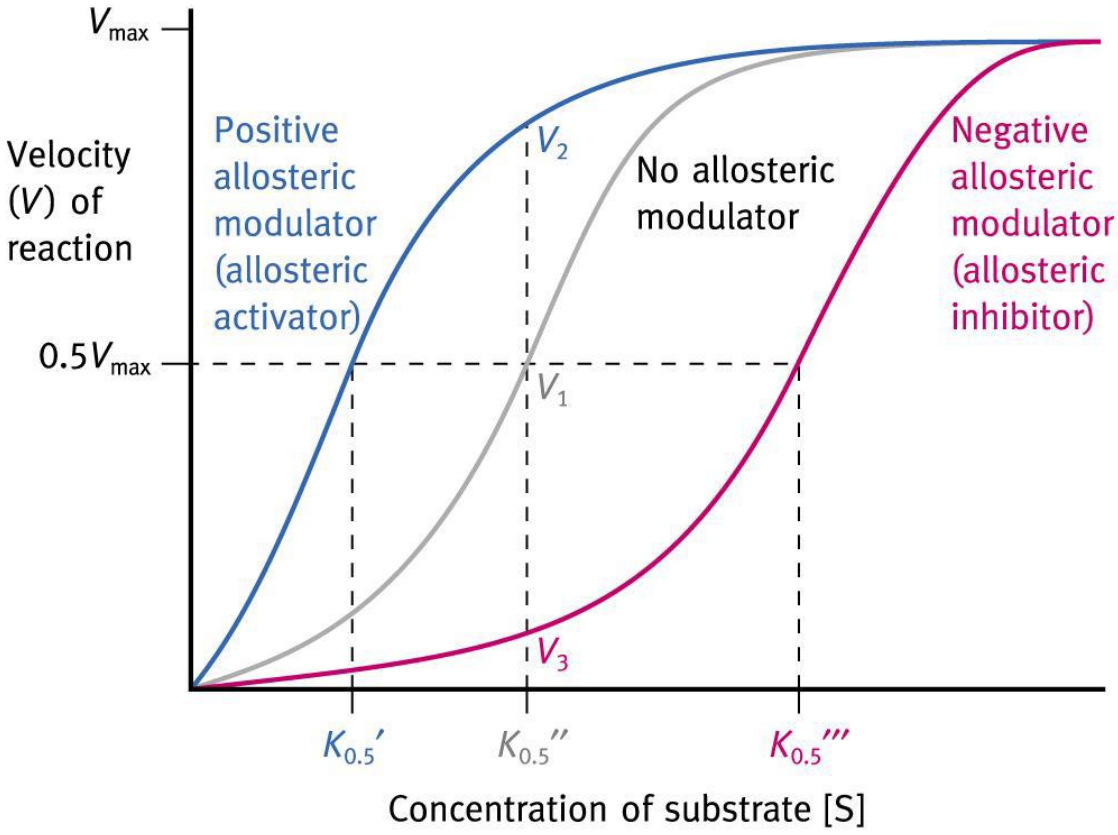
- Mixed inhibitors can either:
-increase or decrease K_m
-decreaseV_{max}

Allosteric inhibitors

- Increase the K_m and hence lower the apparent affinity of the enzyme for it is substrate

Lec 6 **Enzymes**

- A decrease in the substrate affinity leads to a decrease of enzyme activity (at subsaturating levels of substrate present in the cell)



Lec 6

Enzymes