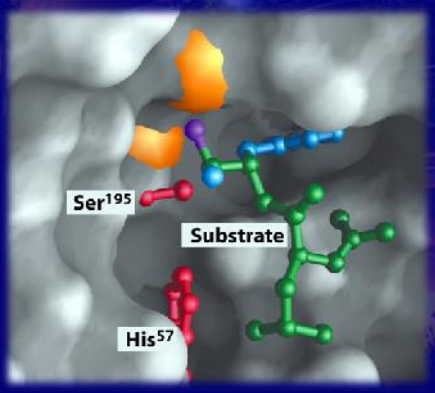
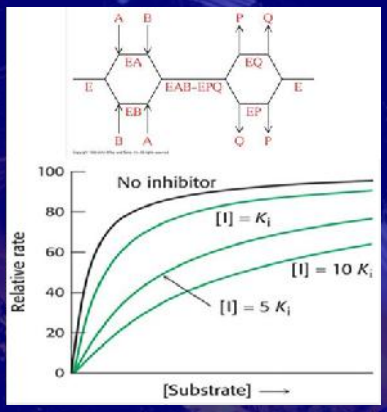
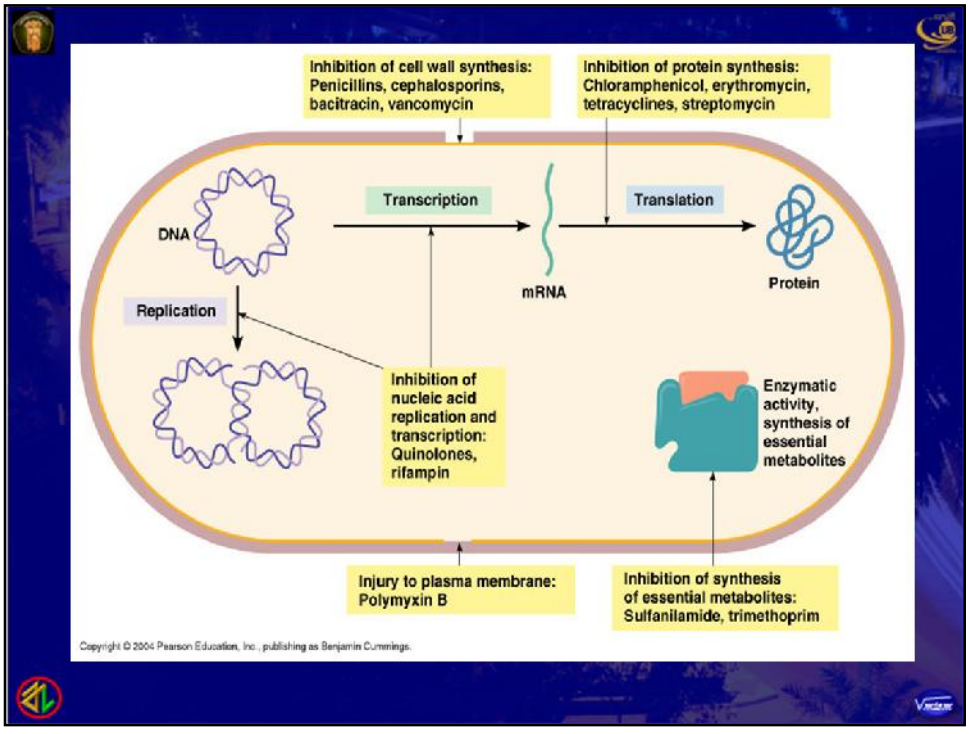
LECTURE 4: REACTION MECHANISM & INHIBITORS



Chymotrypsin





LECTURE OUTCOMES

After mastering the present lecture materials, students will be able to

1. to explain reaction mechanisms of between enzyme and substrate
2. to explain the influence of irreversible inhibitors on enzymatic reactions
3. to explain the influence of reversible inhibitors on enzymatic reactions
4. to explain competitive, uncompetitive, noncompetitiive inhibition of enzymatic reactions
5. to calculate K_M and Vmax of reactions catalyzed by enzymes with the presence of inhibitors

LECTURE LAYOUT

1. REACTION MECHANISMS
   - 1.1 Sequential Reactions
   - 1.2 Ping-Pong Reactions
   - 1.3 Kinetics of Bi-Bi Reactions

2. REACTON INHIBITION
   - 2.1 Irreversible Inhibition
   - 2.2 Reversible Inhibition
     - 2.2.1 Competitive inhibition,
     - 2.2.2 Uncompetitive inhibition.
     - 2.2.3 Noncompetitive inhibition
   - 2.3 Feedback Inhibition

1. REACTION MECHANISMS

- Most biochemical reactions are not simple-, single-substrate reactions, but typically involve two or three substrates that combine to release multiple products.
- Yet, enzymatic reactions involving two substrates and yielding two products account for ~60% of known biochemical reactions.
- Bi-Bi reactions, transferring groups catalyzed by enzymes, fall under two major mechanistic classifications.

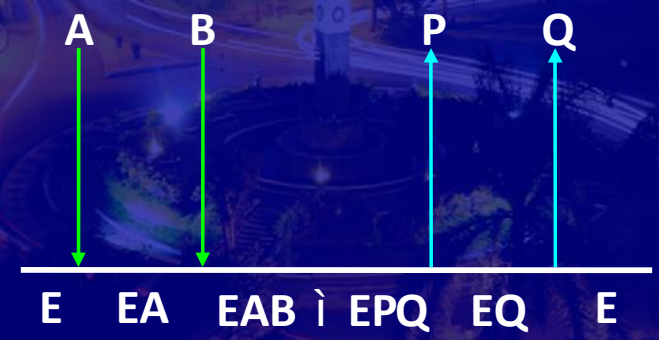
- **Sequential reactions**
- **Ping pong reactions**

1.1 Sequential reactions

- Reactions in which all substrates combine with the enzyme before a reaction can occur and products be released are known as *sequential reactions* which is also called single-displacement reactions**.**
- Sequential reactions can be further classified into 2 types :

  (*a*) **Ordered Mechanism** is that with a compulsory order of substrate addition to the enzyme

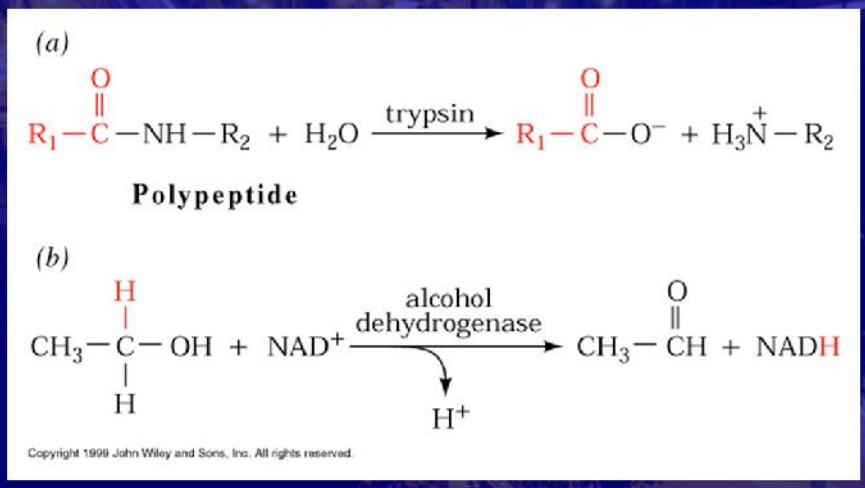
(*b*) **Random mechanism** is that with no preference for the order of substrate addition

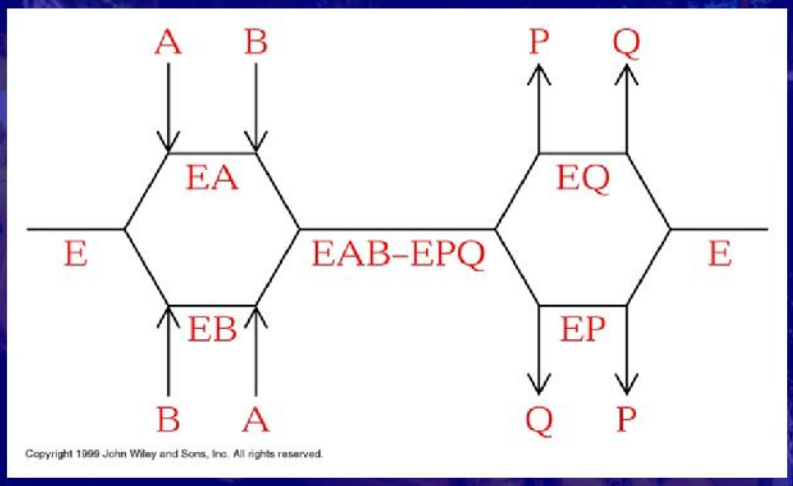


Sequential Reactions: Ordered Mechanism

All substrates must combine with enzyme before reaction can occur

Bisubstrate reactions

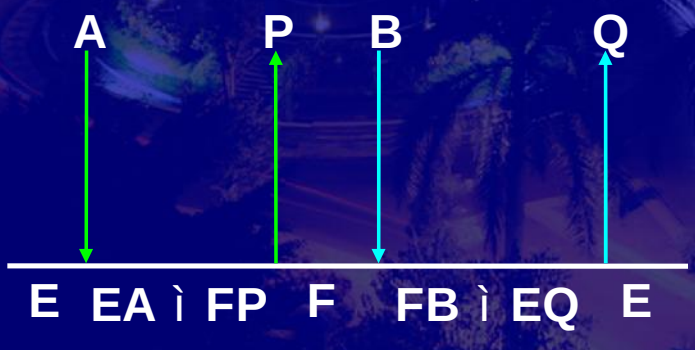




Sequential Reactions: Random Mechanism

1.2 Ping pong reactions

- Ping pong reactions are those in which one or more products are released before all substrates have been added.



1.3 Kinetics of Bi-Bi Reactions

- tSteady state kinetic measurements can be utilized to distinguish among the foregoing bisubstrate mechanisms. For doing so, one must first derive their *rate equations*.
- This can be done in much the same way as for single-substrate-enzymes, *i.e.*, solving a set of simultaneous linear equations consisting of an equation expressing the steady state condition for each kinetically distinct enzyme complex plus one equation representing the conservation condition for the enzyme.

- The rate equations for the above-described bisubstrate mechanisms in the absence of products are given below in double reciprocal form.

For ordered Bi-Bi reactions:

$$\frac{1}{V} = \frac{1}{Vm} + \frac{K_M^A}{Vm[A]} + \frac{K_M^B}{Vm[B]} + \frac{K_S^A K_M^B}{Vm[A][B]}$$

Y = a + bX1 + cX2 + dX3

Y = 1/V, a = 1/Vm, b = K_M^A/Vm, c = K_M^B/Vm, d = $K_S^A K_M^B$/Vm, X1 = 1/[A], X2 = 1/[B], and X3 = 1/([A][B])

For random Bi Bi reactions :

$$\frac{1}{V} = \frac{1}{Vm} + \frac{K_S^A K_M^B}{VmK_S^B[A]} + \frac{K_M^B}{Vm[B]} + \frac{K_S^A K_M^B}{Vm[A][B]}$$

Y = a + bX1 + cX2 + dX3

Y = 1/V, a = 1/Vm, b = $K_S^A K_M^B/(VmK_S^B)$, c = K_M^B/Vm, d = $K_S^A K_M^B/Vm$, X1 = 1/[A], X2 = 1/[B], and X3 = 1/([A][B])

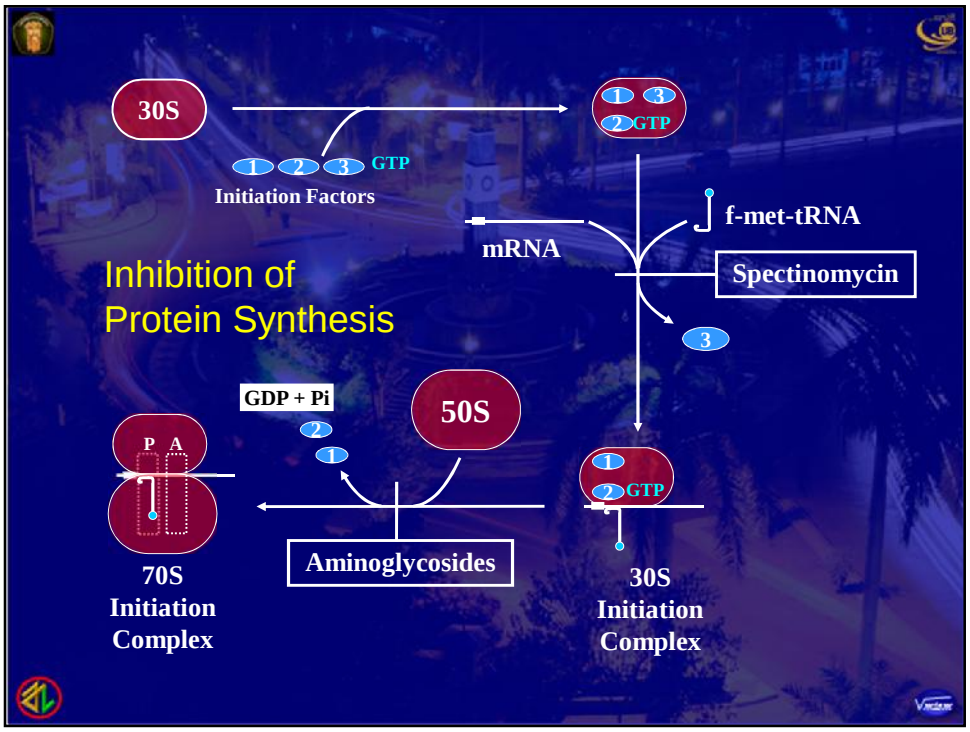
For ping pong Bi Bi reactions :

$$\frac{1}{V} = \frac{1}{Vm} + \frac{K_M^A}{Vm[A]} + \frac{K_M^B}{Vm[B]}$$

2. REACTION INHIBITION

- An important number of compounds have the ability to combine with certain enzymes in either a reversible or irreversible manner, and thereby
  1. block the enzyme, but do not usually destroy it
  2. reduce the rate of enzymic reactions
  3. work specifically in general, and at low concentrations

- Such compounds are called INHIBITORS and include;
  - drugs,
  - antibiotics,
  - poisons,
  - anti metabolites
  - products of enzymic reactions.



- **Two general classes of inhibitors are recognized ;**
  1. **Irreversible**
  2. **Reversible**

2.1 Irreversible

- An irreversible inhibitor forms a covalent bond with a specific function, usually an amino acid residue, which may, in some manner, be associated with the catalytic activity of the enzyme.
  - Many examples of enzyme inhibitors covalently bind not at the active site, but physically block the active site

- The inhibitor cannot be released by dilution or dialysis; kinetically, the concentration and hence the velocity of active enzyme is lowered in proportion to the concentration of the inhibitor and thus the effect is that of noncompetitive inhibition.

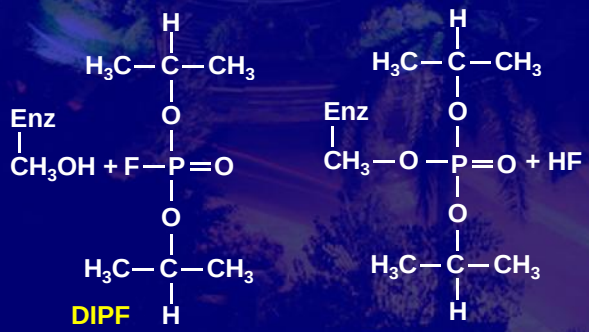
$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P$$

$$E + I \xrightarrow{k_I} EI$$

IRREVERSIBLE INHIBITORS

- Examples of irreversible inhibitors include;
  - DIFP (diisopropyl fluorophosphate) which reacts irreversibly with serine proteases, chymotrypsin
  - Iodoacetate which reacts with essential sulfhydryl group of an enzyme such as triose phosphate dehydrogenase:

$$E\text{-}SH + ICH_2COOH \rightarrow E\text{-}SCH_2COOH + HI$$

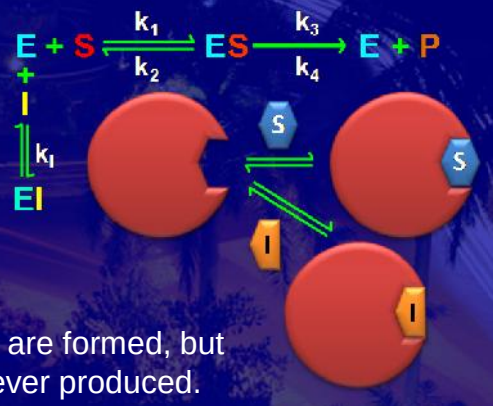


2.2 Reversible Inhibition

- As the term implies, this type of inhibition involves equilibrium between the enzyme and the inhibitor, the equilibrium constant (Ki) being a measure of the affinity of the inhibitor for the enzyme.
- Three distinct types of reversible inhibition are known;
  - Competitive inhibition,
  - Uncompetitive inhibition
  - Noncompetitive inhibition

2.2.1 Competitive Inhibition

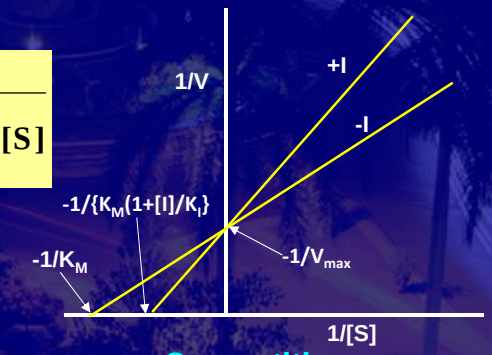
- Compounds that may or may not be structurally related to the natural substrate combine reversibly with the enzyme at or near the active site
- The inhibitor and the substrate therefore compete for the same site according to the reaction as shown on the right side.



- ES and EI complexes are formed, but EIS complexes are never produced.

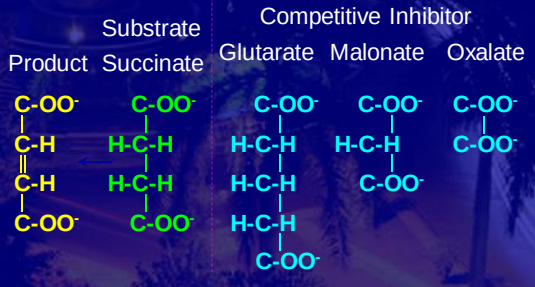
- One can conclude that high concentrations of substrate will overcome the inhibition by causing the reaction sequence to swing to the right. The velocity of reaction can be calculated by the following equation
- The rate of reaction can be calculated from the following equation.

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



Competitive

- Among other enzymes that may undergo competitive inhibition is succinic dehydrogenase, which readily oxidizes succinic acid to fumaric acid.
- If increasing concentrations of malonic acid, which closely resembles succinic acid in structure, are added, however, succinic dehydrogenase activity falls markedly
- This inhibition can now be reversed by increasing in turn the concentration of the substrate succinic acid.



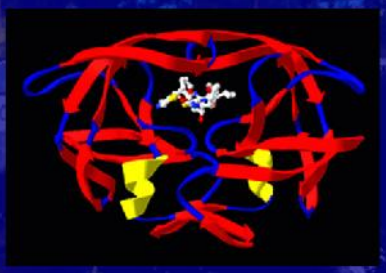
Succinate Dehydrogenase

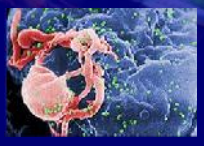
Competitive Inhibition

2.2.2 Uncompetitive Inhibition

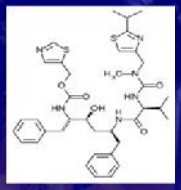
- Compounds that combine only with the ES complex, not with the free enzyme, are called uncompetitive inhibitors. The inhibition is not overcome by high substrate concentrations.

- *HIV protease* in a complex with the protease inhibitor *ritonavir*
- The structure of the protease is shown by the red, blue and yellow ribbons. The inhibitor is shown as the smaller ball-and-stick structure near the centre. Created from PDB





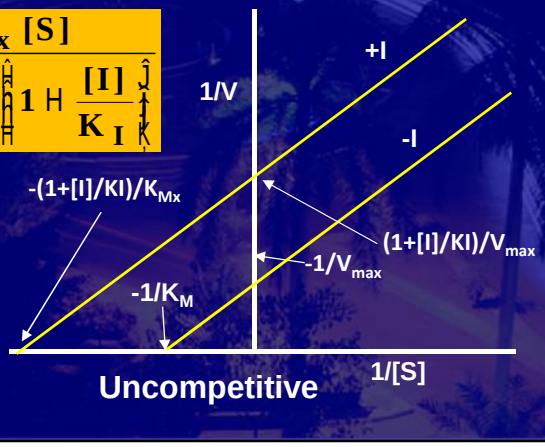
Peptide-based protease inhibitor *ritonavir*



Human immunodeficiency virus
Scanning electron micrograph of HIV-1 (in green) budding from cultured lymphocyte. Multiple round bumps on cell surface represent sites of assembly and budding of virions

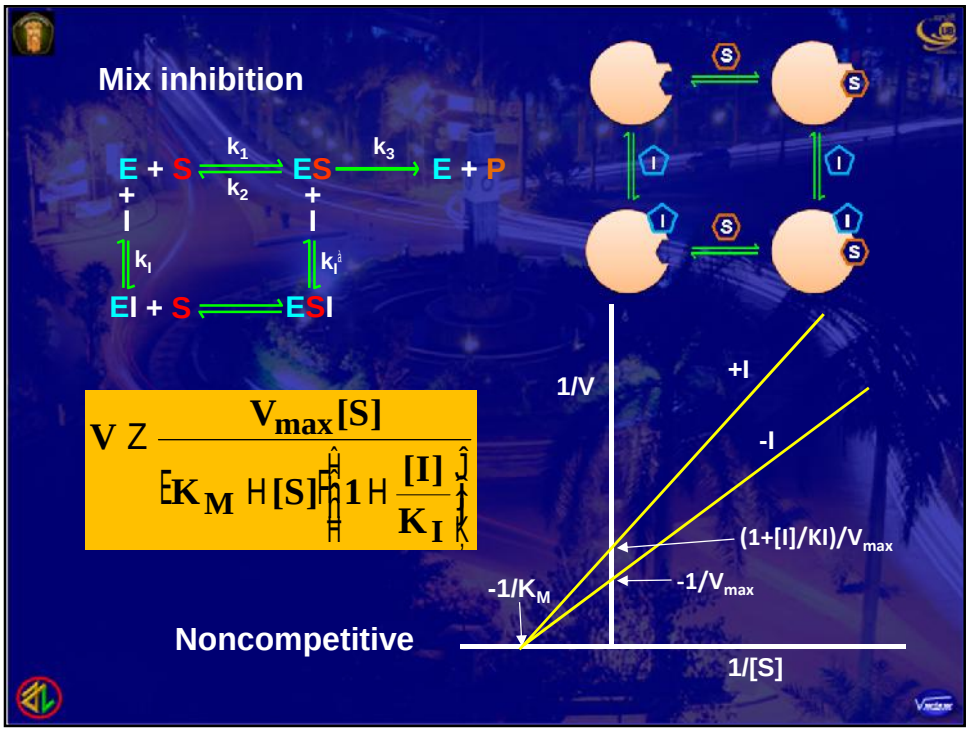
- K_M value is consistently smaller than the K_M value of the uninhibited reaction which implies that S is more effectively bound to the enzyme in the presence of the inhibitor.
- The equation used to calculate the velocity of the noncompetitive inhibition is as follows

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



2.2.3 Noncompetitive Inhibition

- Compounds that reversibly bind with either the enzyme or the enzyme substrate complex are designated as noncompetitive inhibitors
- Noncompetitive inhibition therefore differs from competitive inhibition in that the inhibitor can combine with ES, and S can combine with EI to form in both instances EIS.
- This type of inhibition is not completely reversed by high substrate concentration since the closed sequence will occur regardless of the substrate concentration
- Since the inhibitor binding site is not identical to nor does it modify the active site directly, the K_M is not altered.



2.3 Feedback Inhibition (Allosteric Effectors)

- The activity of some enzymes is controlled by certain molecules binding to a specific regulatory (or **allosteric**) site on the enzyme, distinct from the active site.
- Different molecules can either inhibit or activate the enzyme, allowing sophisticated control of the rate. Only a few enzymes can do this, and they are often at the start of a long biochemical pathway.
- They are generally **activated by the substrate** of the pathway and **inhibited by the product** of the pathway, thus only turning the pathway on when it is needed.

The switch: Allosteric inhibition

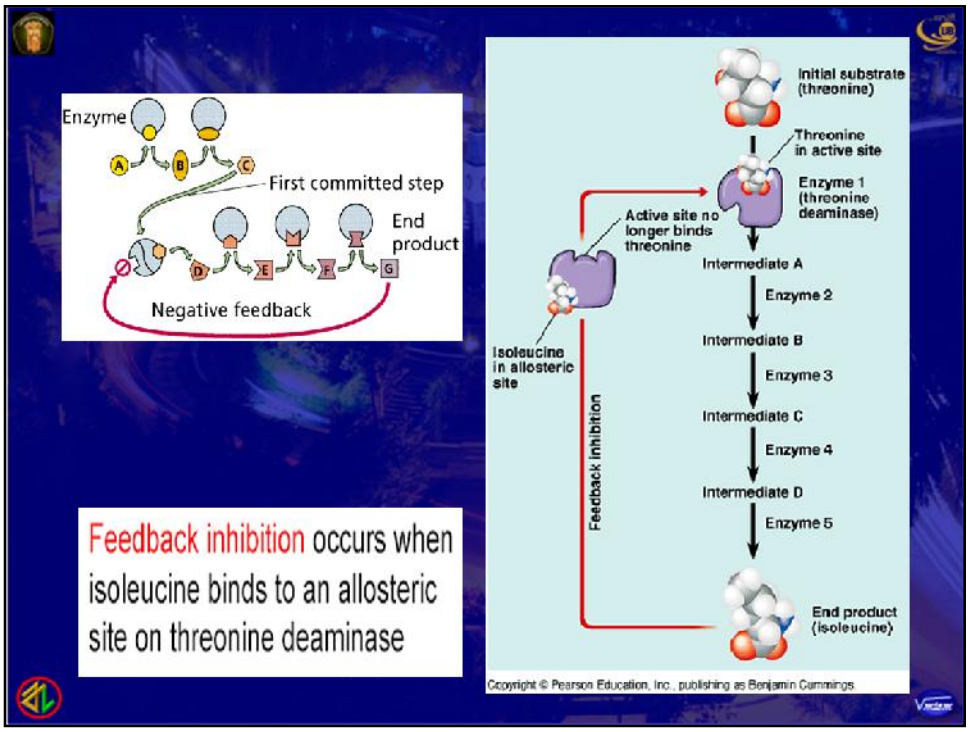
Allosteric means **"other site"**

Switching off

- These enzymes have **two receptor sites**
- One site fits the substrate like other enzymes
- The other site fits an inhibitor molecule

Substrate cannot fit into the **active site**

Inhibitor fits into **allosteric site**


Enzyme
A
B
C
First committed step
End product
D
E
F
G
Negative feedback
Feedback inhibition occurs when isoleucine binds to an allosteric site on threonine deaminase
Initial substrate (threonine)
Threonine in active site
Enzyme 1 (threonine deaminase)
Active site no longer binds threonine
Intermediate A
Enzyme 2
Intermediate B
Enzyme 3
Intermediate C
Enzyme 4
Intermediate D
Enzyme 5
Isoleucine in allosteric site
Feedback inhibition
End product (isoleucine)
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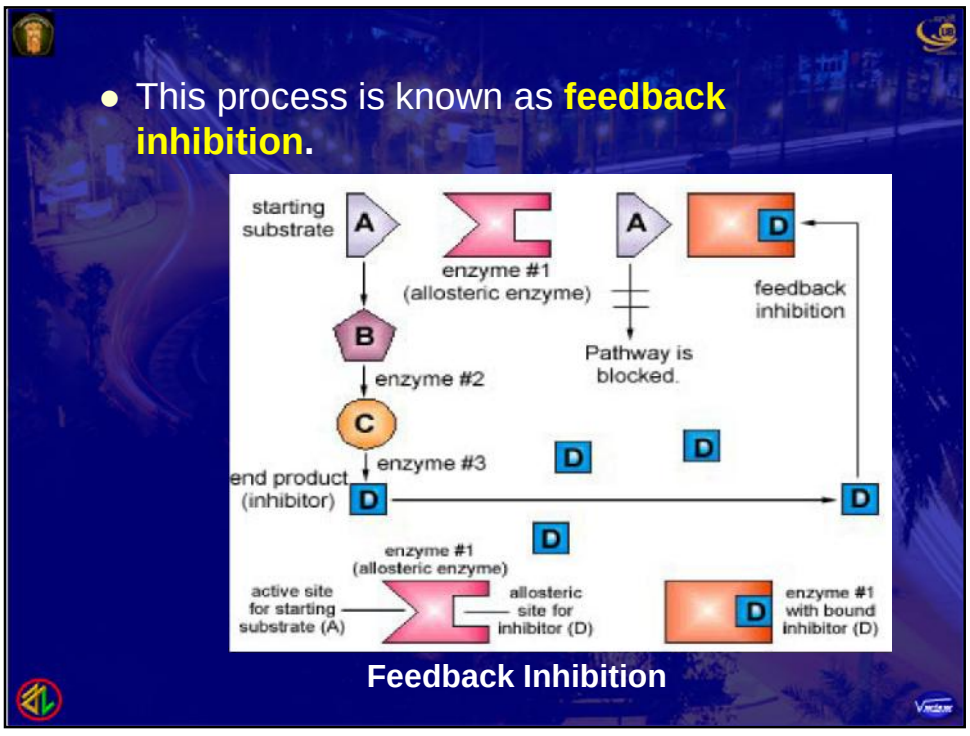

This process is known as feedback inhibition.
starting substrate
A
enzyme #1 (allosteric enzyme)
A
D
feedback inhibition
B
Pathway is blocked.
enzyme #2
C
enzyme #3
D
D
end product (inhibitor)
D
D
D
enzyme #1 (allosteric enzyme)
active site for starting substrate (A)
allosteric site for inhibitor (D)
D
enzyme #1 with bound inhibitor (D)
Feedback Inhibition

HOW TO SOLVE THE EQUATIONS

Type of Inhibition	Equation	V_{max}	K_m
None	$v = \frac{V_{max}[S]}{K_m + [S]}$	—	—
Competitive	$v = \frac{V_{max}[S]}{K_m\left(1 + \frac{I}{K_i}\right) + [S]}$	No change	Increased
Noncompetitive	$v = \frac{V_{max}[S]}{(K_m + S)\left(1 + \frac{I}{K_i}\right)}$	Decreased	No change
Uncompetitive	$v = \frac{V_{max}[S]}{K_m + [S]\left(1 + \frac{I}{K_i}\right)}$	Decreased	Decreased

1. Competitive inhibitor

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

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

- y =1/V; x = 1/[s]
- a = 1/Vmax
- b = $K_M(1+[I]/K_I)/V_{max}$

2. Uncompetitive

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

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

- y =1/V; x = 1/[s]
- a = (1+[I]/K_I)/Vmax
- b = K_M/Vmax

3. Noncompetitive Inhibition

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

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

- y =1/V; x = 1/[s]
- a = (1+[I]/K_I)/Vmax
- b = K_M(1+[I]/K_I)/Vmax

QUIZ (10 min)

1. How is enzyme specificity achieved ?
2. Calculate Vmax & KM from the following data, and does the reaction obey Michaelis-Menten kinetics ?

[DNA] mol total nucleotides/L	Free nucleotides in solution, V (pmol/L)	
	0 min	10 min
1.0×10^{-5}	0.05	5.1
1.0×10^{-6}	0.04	4.5
1.0×10^{-7}	0.06	3.2
1.0×10^{-8}	0.04	1.4
1.0×10^{-9}	0.04	0.23

ANSWERS

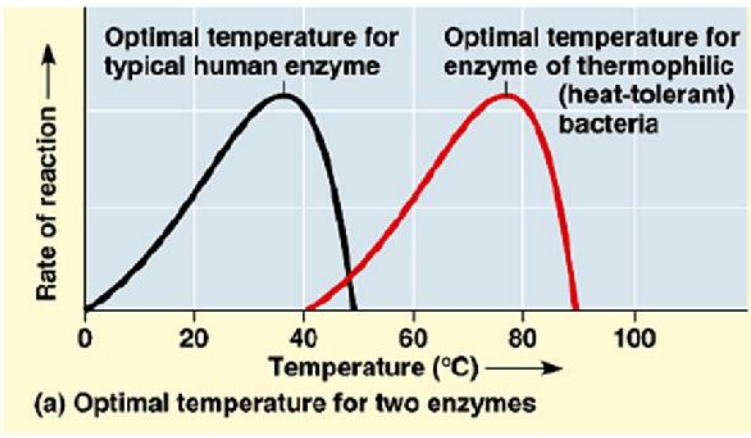
1. The enzyme specificity is achieved through the characteristic of active site
2. Vmax = 4.36695
   KM = 2.2E-08
   R^2 = 0.999864, so the reaction obeys Michaelis-Menten kinetics

SOAL

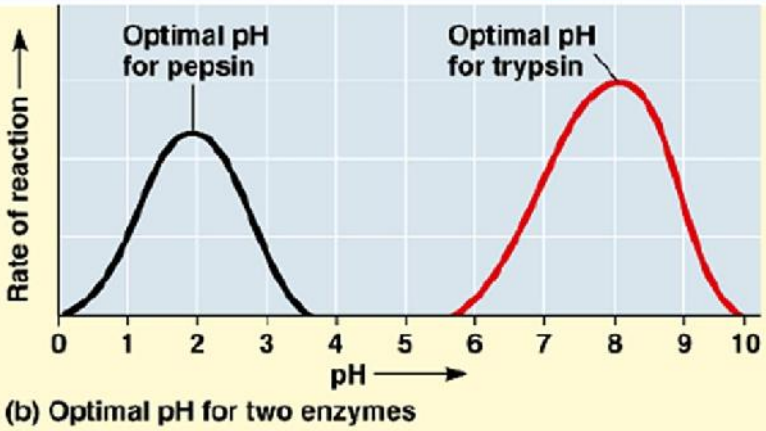
- Diketahui suatu reaksi enzimatis tanpa dan dengan inhibitor dengan [I] = $2{,}2.10^4$M.
- Hitunglah KM dan Vmax tanpa dan dengan I serta K_I

[S]	V(-I)	V(+I)
$1*10^{-4}$	28	17
$1.5*10^{-4}$	36	23
$2.0*10^{-4}$	43	29
$5*10^{-4}$	65	50
$7.5*10^{-4}$	74	61

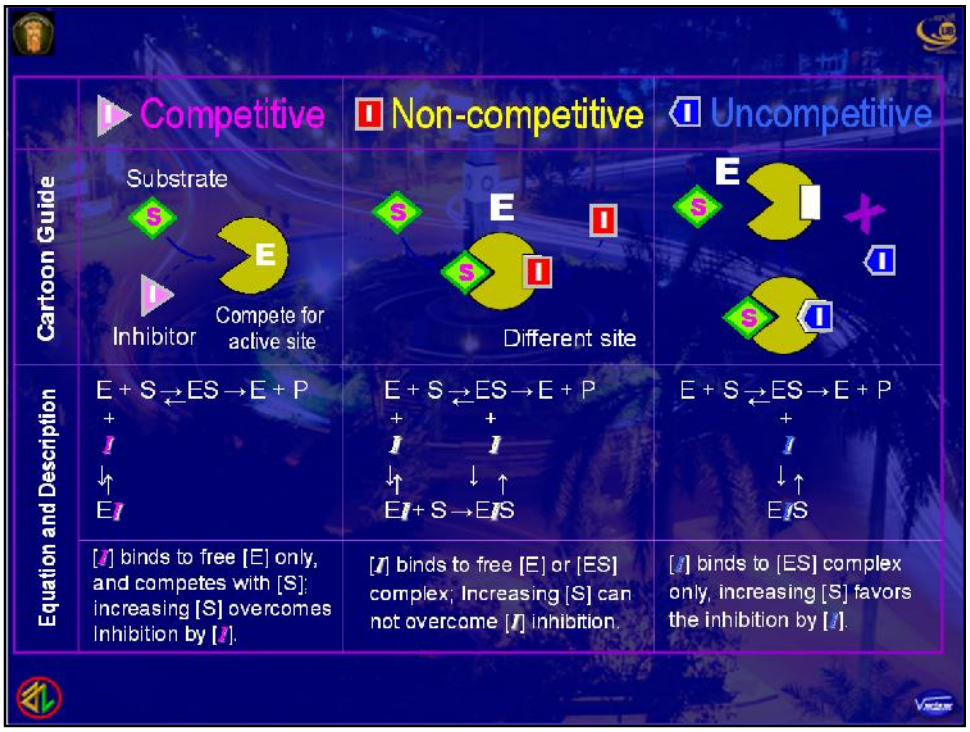
Figure 6.16 Environmental factors affecting enzyme activity

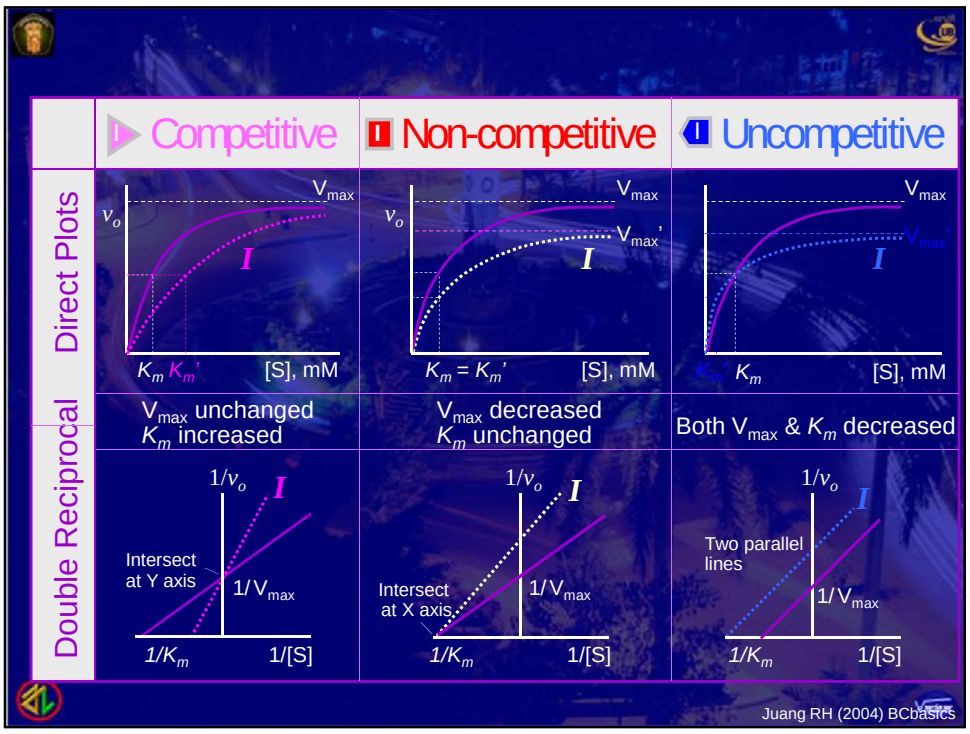


(a) Optimal temperature for two enzymes



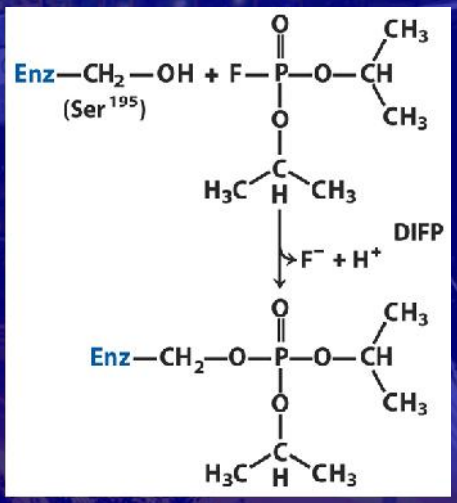
(b) Optimal pH for two enzymes


Competitive
Non-competitive
Uncompetitive
Cartoon Guide
Substrate
Inhibitor
Compete for active site
Different site
Equation and Description
E + S ⇄ ES → E + P
+ I
⇅
EI
+ S → ESI
[I] binds to free [E] only, and competes with [S]: increasing [S] overcomes inhibition by [I].
[I] binds to free [E] or [ES] complex; Increasing [S] can not overcome [I] inhibition.
[I] binds to [ES] complex only, increasing [S] favors the inhibition by [I].


Competitive
Non-competitive
Uncompetitive
Direct Plots
Double Reciprocal
Vmax
Vmax'
vo
I
Km Km'
Km = Km'
Km' Km
[S], mM
Vmax unchanged
Km increased
Vmax decreased
Km unchanged
Both Vmax & Km decreased
1/vo
Intersect at Y axis
1/Vmax
1/Km
1/[S]
Intersect at X axis
Two parallel lines
Juang RH (2004) BCbasics

Example of a suicide inhibitor

- Diisopropylfluorophosphate (DIFP) forms a covalent bond with an active -site residue (Ser) of the enzyme chymotrypsin.
- Every molecule that reacts is inactivated irreversibly.
- Here, a key active site Ser is bound irreversibly to the inhibitor, preventing it from doing its "normal" job.



Irreversible inhibitor

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9/28/05

The effect of enzyme inhibition

- **Irreversible inhibitors:** Combine with the functional groups of the amino acids in the active site, irreversibly

Examples: nerve gases and pesticides, containing organophosphorus, combine with serine residues in the enzyme acetylcholine esterase