Biochemistry lecture 2: enzymes 2 of 3

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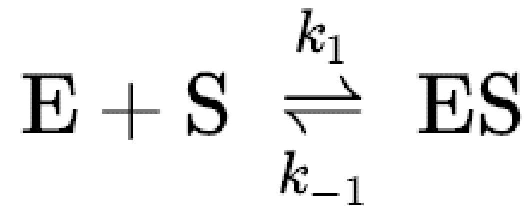
Enzymes II	1.Effect of substrate concentration on rate of enzymatic reaction 2. Understanding enzyme kinetics 3. Michaelis-Menten equation 4. What are Km and V max values? 5.Enzyme activation and inhibition 6.Irriversible and reversible inhibitors 7.Kinetics of reversible inhibitors
Enzymes III	1. What are isozymes? 2. Application of isozymes in diagnosis 3.Control of enzyme activity a. Allosteric regulation b. Covalent modification

Factors affecting the rate of enzymatic reaction

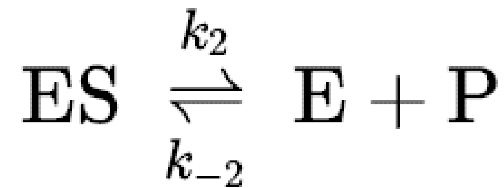
1. Enzyme concentration
2. Substrate concentration
3. Product concentration
4. Temperature
5. Hydrogen ion concentration (pH)
6. Presence of activators
7. Presence of inhibitors
8. Presence of repressor or derepressor.
9. Covalent modification

MICHAELIS–MENTEN THEORY

- In 1913, Michaelis and Menten put forward the Enzyme–Substrate complex theory.
- In this model, the enzyme (E) reversibly combines with its substrate (S) to form an ES complex that subsequently yields product (P), regenerating the free enzyme.



$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES}$$



$$\mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

MICHAELIS–MENTEN THEORY

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$$

- S is the substrate.
- E is the enzyme.
- ES is the enzyme–substrate complex
- P is the product.
- k1, k−1, and k2 (or, kcat) are rate constants.
  - **kcat** is the turnover number and this describes **how many substrate molecules** are transformed into products **per unit time** by a single enzyme.

MICHAELIS–MENTEN EQUATION

- The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:

Vo = initial reaction velocity

Vmax = maximal velocity

Km = Michaelis constant

[S] = substrate concentration

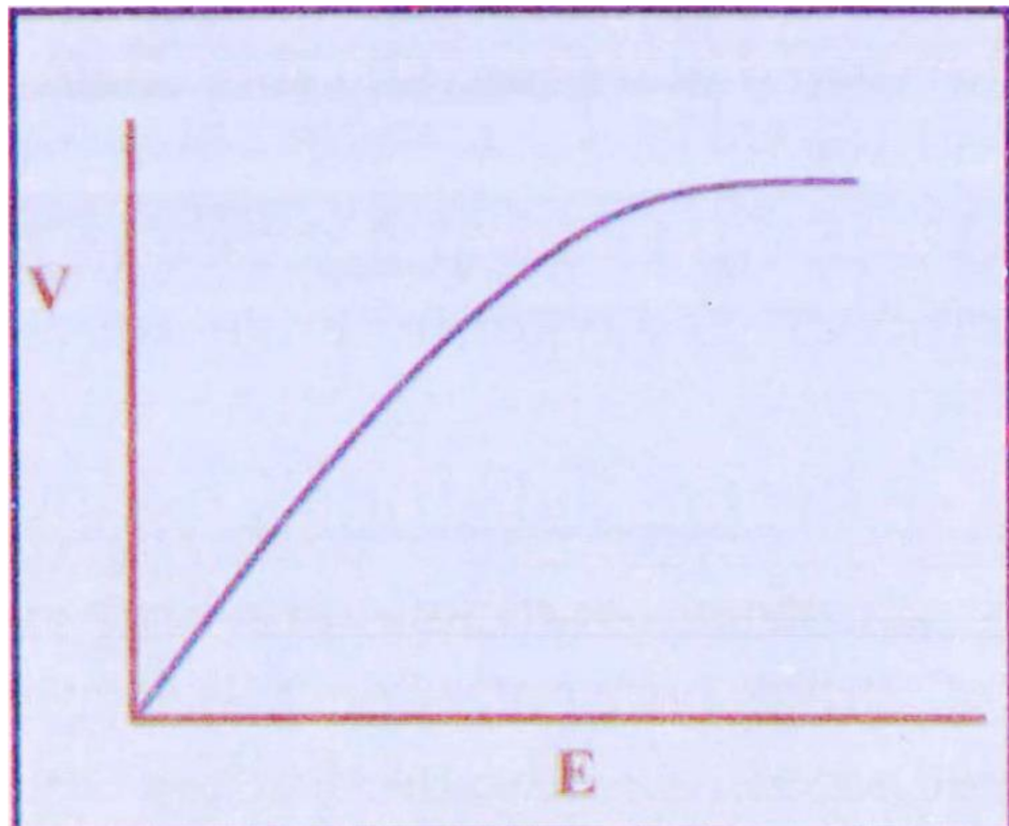
$$V_0 = \frac{V_{\mathrm{max}}[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}$$

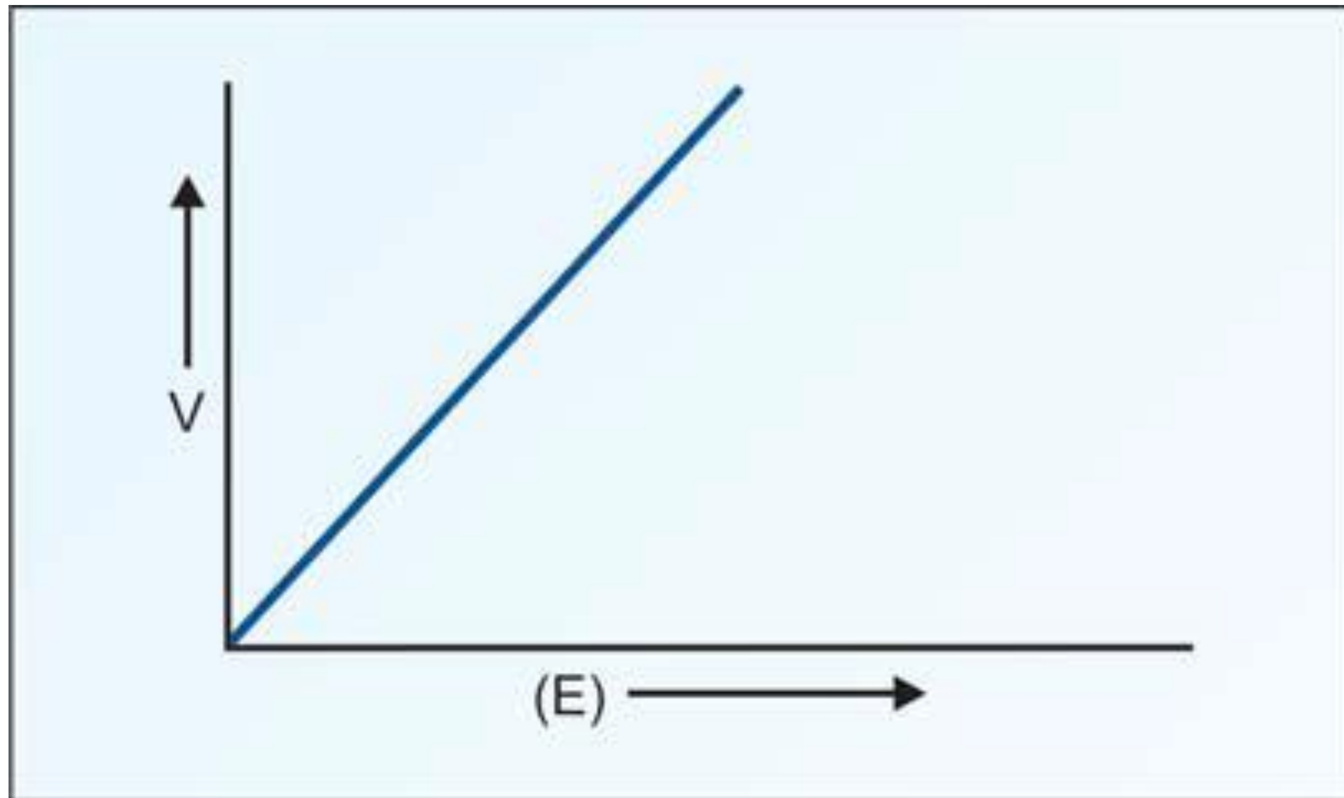
Effect of enzyme concentration

- **Rate of a reaction or velocity** (V) is **directly proportional** to the **enzyme concentration**, when sufficient substrate is present.

- This is true up to a point when a further increase in the enzyme concentration is not accompanied by an increase in the velocity of the reaction
  - At this point the **substrate is said to be the limiting factor**

- This property is made use of in determining the level of particular enzyme in plasma, serum or tissues
  - Known volume of serum is incubated with substrate for a fixed time
  - Then reaction is stopped, and product is quantitated
  - Since the **product formed will be proportional to the enzyme concentration**, the latter could be assayed.


V
E


V
(E)

Effect of substrate concentration

- The velocity of the reaction ↑ as the **substrate concentration** ↑ up to point where the enzyme is saturated.

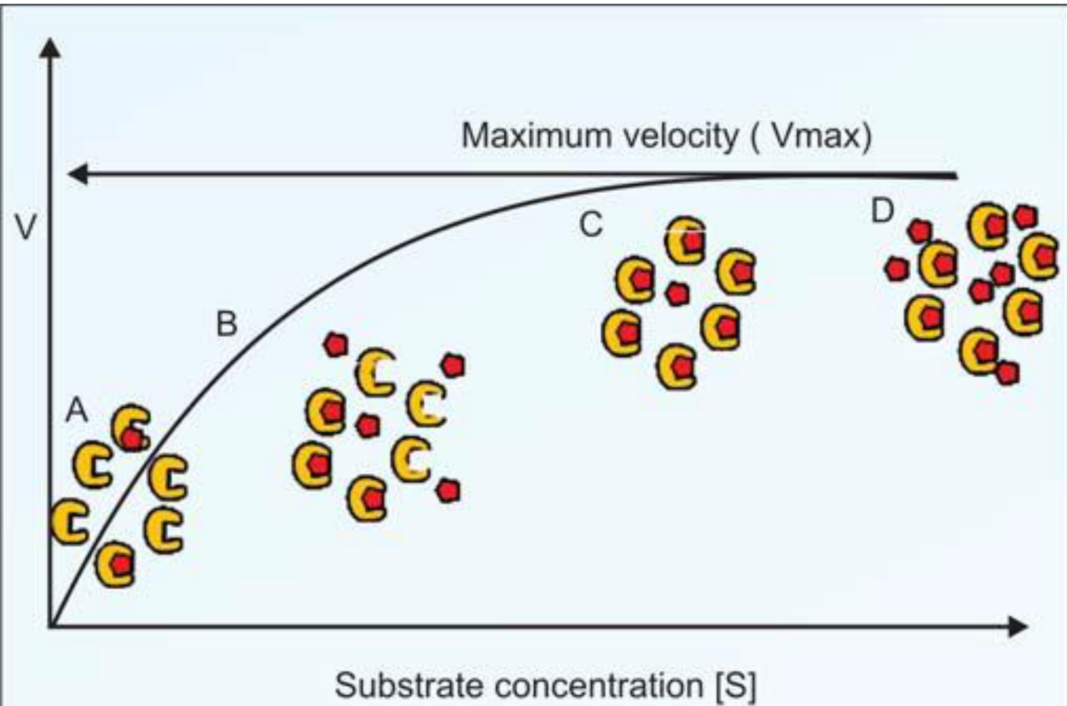
  If the enzyme is saturated with substrate—meaning that as soon as a product molecule is released, a new substrate is bound—then the reaction will reach a maximum rate, or velocity.

- The Vi increases to a maximum value Vmax.

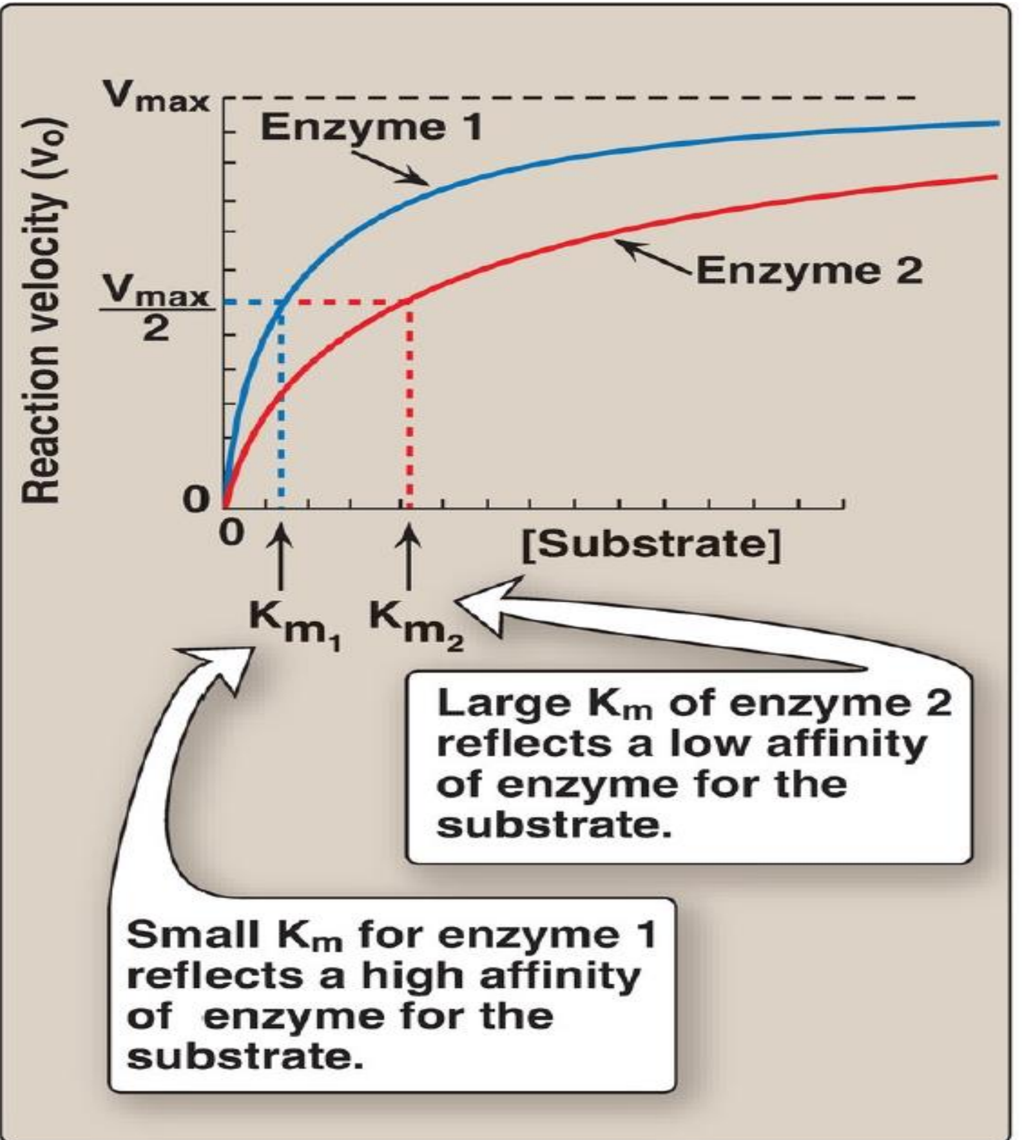
- The substrate concentration that produces half the maximal velocity is termed **Michaelis constant or Km.**

- **Km** is a **substrate concentration** and is the amount of substrate it takes for an **enzyme** to reach Vmax/2

- When [S] is approximately equal to Km, Vi is very responsive to changes in [S], and the enzyme is working at half-maximal velocity



Enzyme molecules are shown as half-circles. Substrate molecules are red dots. (A) Substrate molecules are low; so only a few enzyme molecules are working and velocity is less. (B) At half-maximal velocity (Km), 50% enzyme molecules are bound with substrate. (C) As a lot of substrate molecules are available, all enzyme molecules are bound. (D) Further increase in the substrate will not increase the velocity further.



Michaelis Menten Constant

- Describes the behavior of enzymes as substrate concentration is changed.
- **Km** denotes the affinity of enzyme for substrate
- **The lesser the numerical value of Km, the affinity of the enzyme for the substrate is more**

Michaelis Menten Constant

- Km is independent of enzyme concentration
    - If enzyme concentration is doubled, the Vmax will be doubled
    - But the Km will remain exactly same

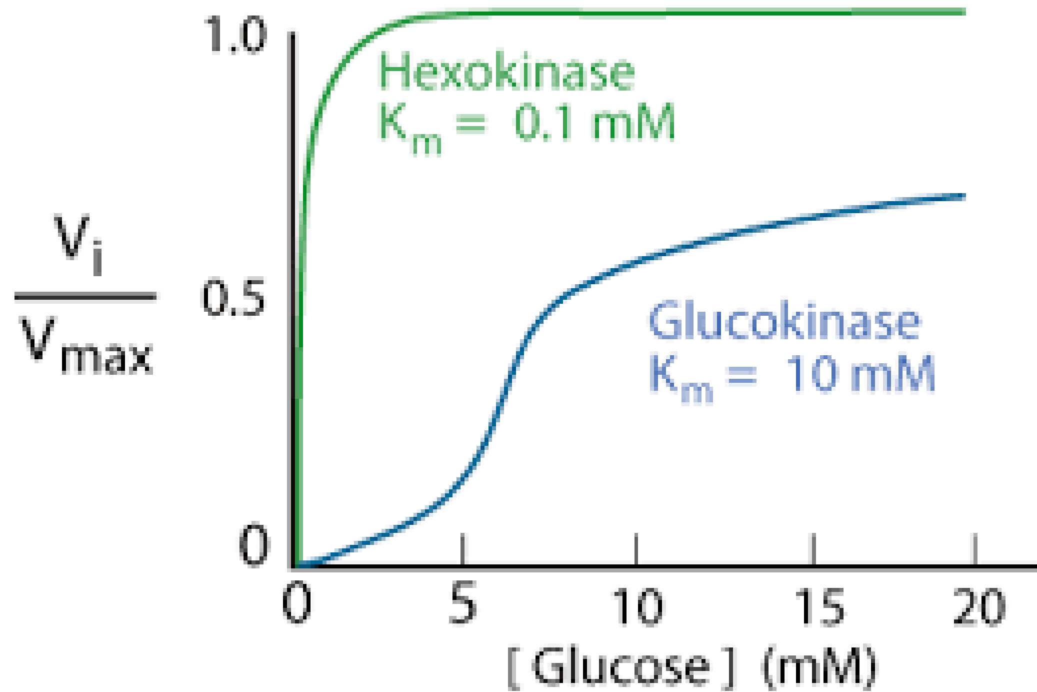
- According to Michaelis Menten Constant:
    - the enzyme– substrate complex is a reversible reaction
    - the breakdown of the complex to enzyme + product is irreversible.

Salient Features of Km

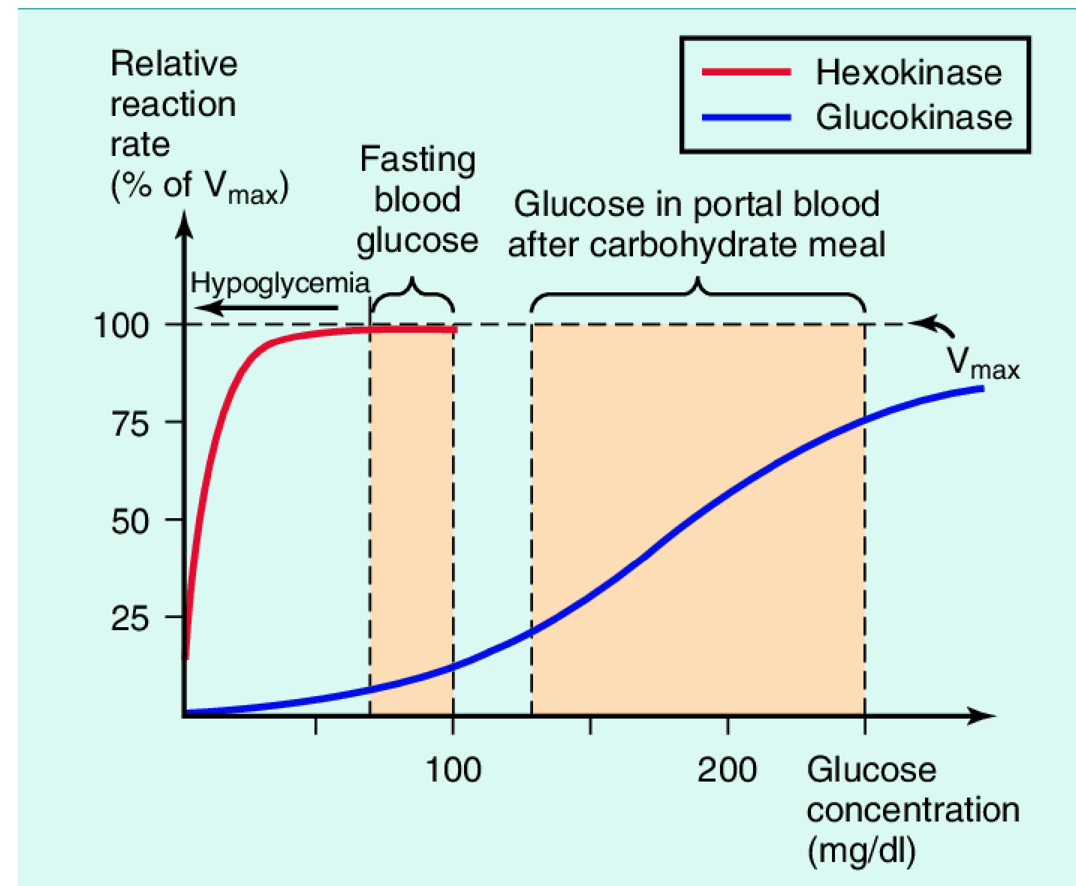
- **Km value is substrate concentration** (expressed in moles/L) **at half-maximal velocity**

- **It denotes that 50% of enzyme molecules are bound with substrate molecules at that particular substrate concentration**

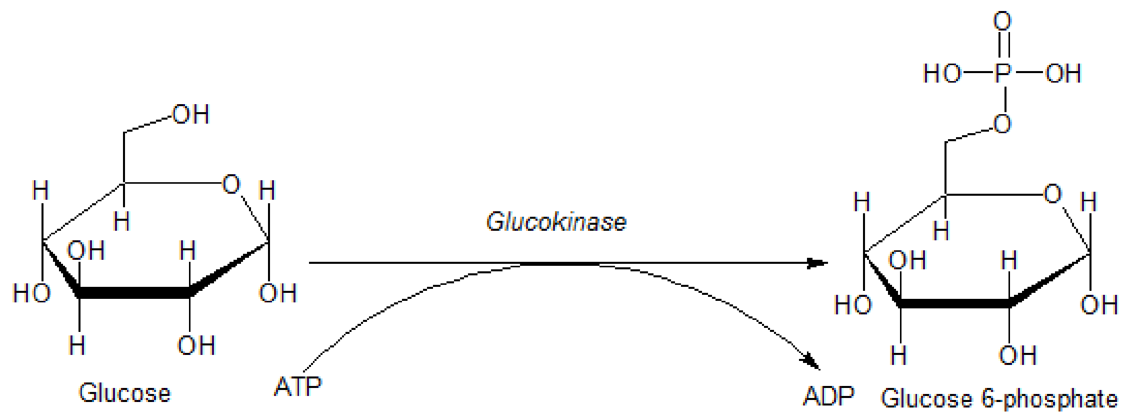
- **Km is the Signature of the Enzyme**
  - Km value is thus a constant for an enzyme
  - It is the **characteristic feature of a particular enzyme <u>for a specific substrate</u>**

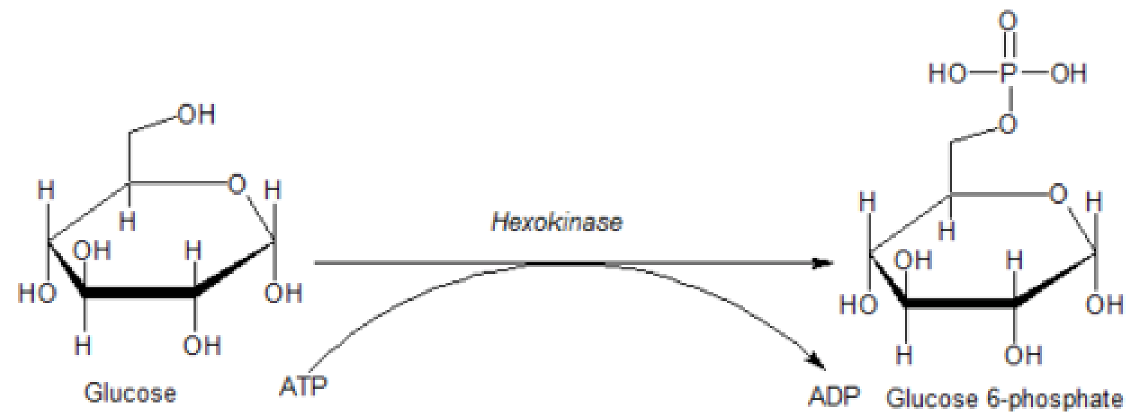


1.0
0.5
0
V_i / V_{max}
Hexokinase
K_m = 0.1 mM
Glucokinase
K_m = 10 mM
0
5
10
15
20
[Glucose] (mM)



Relative reaction rate (% of V_{max})
Hexokinase
Glucokinase
Fasting blood glucose
Glucose in portal blood after carbohydrate meal
Hypoglycemia
100
75
50
25
V_{max}
100
200
Glucose concentration (mg/dl)


Glucose
ATP
Glucokinase
ADP
Glucose 6-phosphate


Glucose
ATP
Hexokinase
ADP
Glucose 6-phosphate

Effect of temperature

- The velocity of enzyme reaction **increases** when temperature of the medium is increased → reaches a maximum and then falls **(Bell shaped curve)**

- **Optimum temperature:** Temperature at which maximum amount of the substrate is converted to the product per unit time

- As temperature is increased, more molecules get activation energy, or molecules are at increased rate of motion

  → their collision probabilities are increased and so the **reaction velocity is enhanced**

Effect of temperature

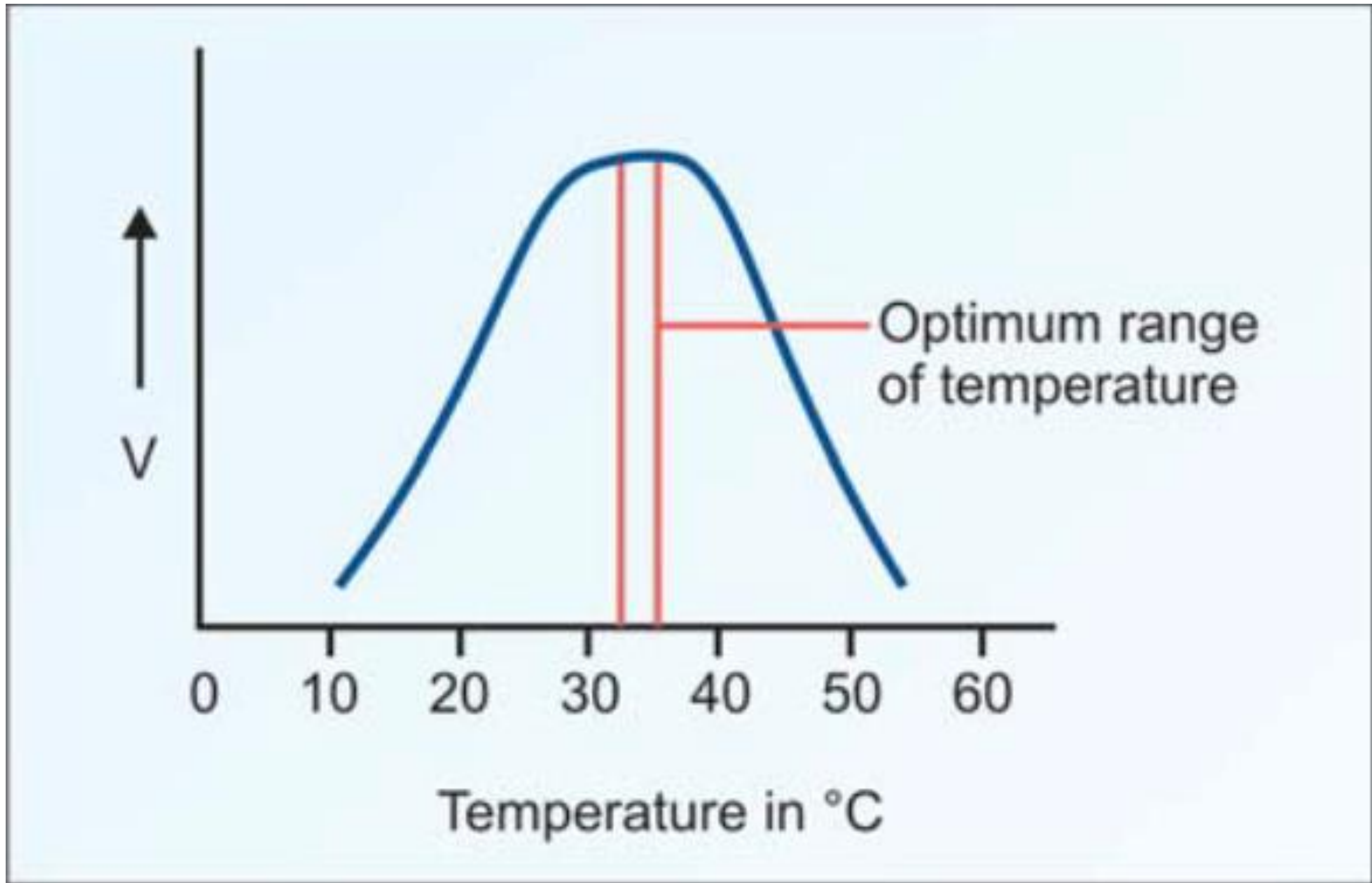
- But when **temperature is more than 50°C,** heat **denaturation** and consequent loss of tertiary structure of protein occurs:

  Activity of the enzyme is decreased

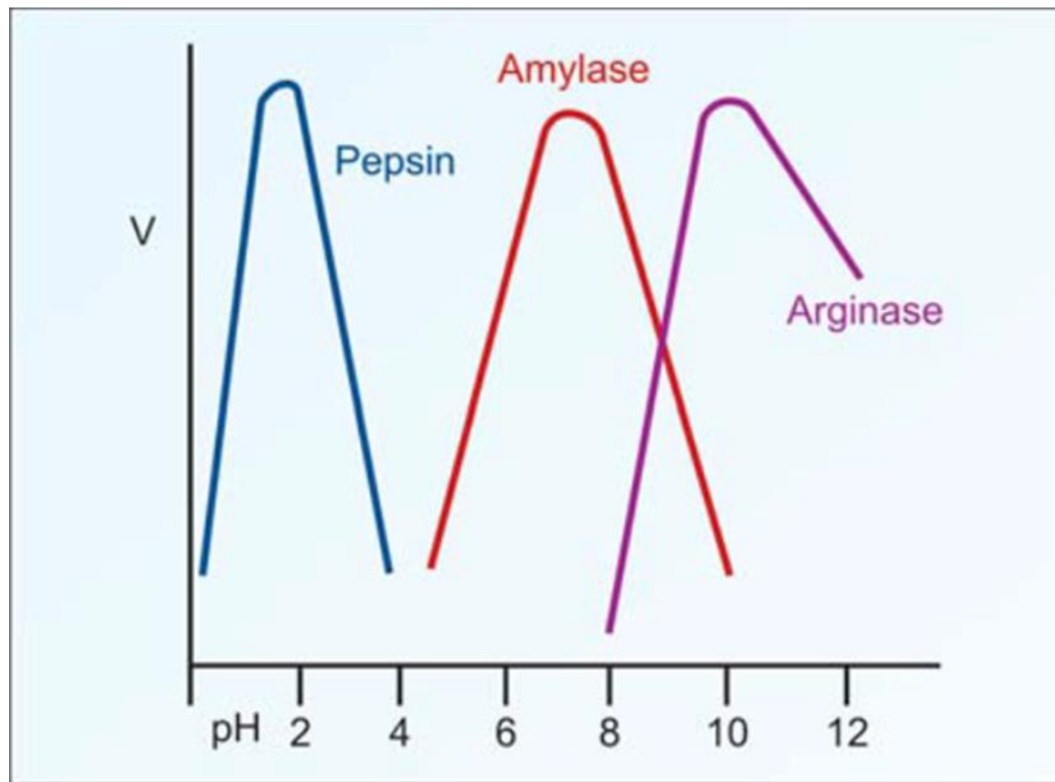
- Most **human** enzymes have the optimum temperature around 37°C

- **Plants:** optimum temperature around 50°C

- Certain **bacteria** living in hot springs will have enzymes with optimum temperature near 100°C


V
Optimum range
of temperature
0
10
20
30
40
50
60
Temperature in °C

Effect of pH

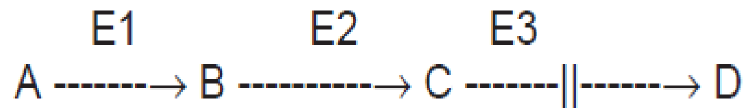


- Each enzyme has an optimum pH at which it shows maximal activity
- Activity decreases as we go away from the optimum pH
- Activity virtually stops about 2 units of pH above or below this pH

Effect of pH

- Slight changes in pH causes marked changes in enzyme activity due to **alteration of the charges on the substrate and on the catalytic site of the enzyme**
- Extreme changes of pH cause denaturation and irreversible inhibition of enzyme action
- Usually, enzymes have the optimum pH between 6 and 8
- Some important exceptions are:
    - Pepsin (with optimum pH 1-2)
    - Alkaline phosphatase (optimum pH 9-10)
    - Acid phosphatase (optimum pH 4-5)

Effect of Concentration of Products

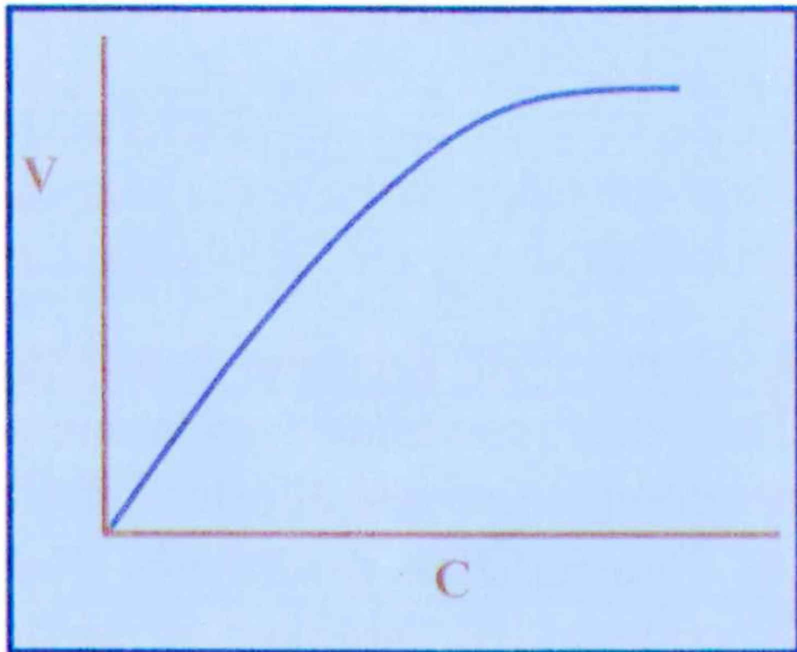


If E3 enzyme is absent, C will accumulate, which in turn, will inhibit E2. Consequently, in course of time, the whole pathway is blocked.

- In a reversible reaction ($S \leftrightarrow P$), when equilibrium is reached:
  - The reaction rate is slowed down

- When product concentration is $\uparrow$, the reaction is **slowed** or **stopped** $\rightarrow$ **feedback inhibition**

Effect of cofactor concentration



- If the enzyme requires a cofactor (coenzyme or activator) for its activity:
  - The velocity of the **reaction will be directly proportional to the concentration of the cofactor**
- This is true till a certain point
- After this point, any increase in cofactor concentration will **not** increase the velocity of the reaction:
  - The **enzyme concentration is the limiting factor**

Enzyme activation

- Some enzymes show higher activity in presence of inorganic ions
  - chloride ions activate salivary amylase
  - calcium activate lipases

- Another type of activation is the conversion of an inactive pro-enzyme or **zymogen** to the active enzyme
    - By splitting a single peptide bond & removal of a small polypeptide from trypsinogen → active trypsin is formed
    - This results in unmasking of active center

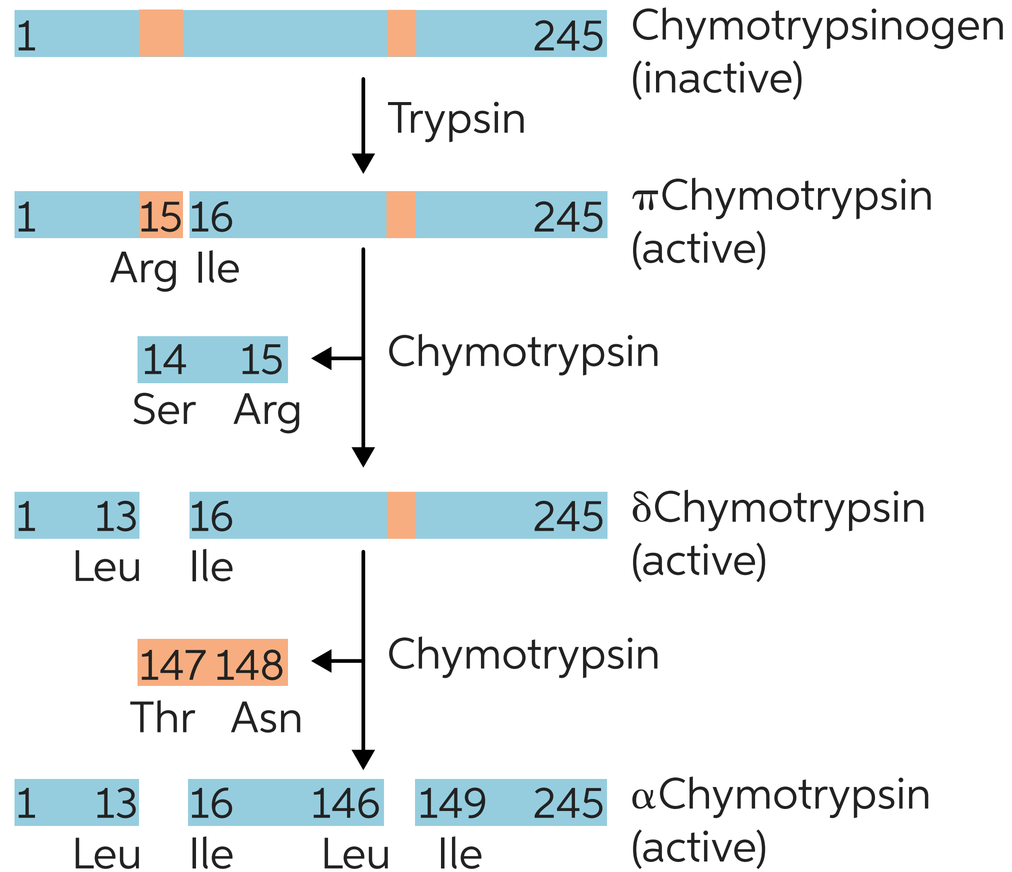
Enzyme activation

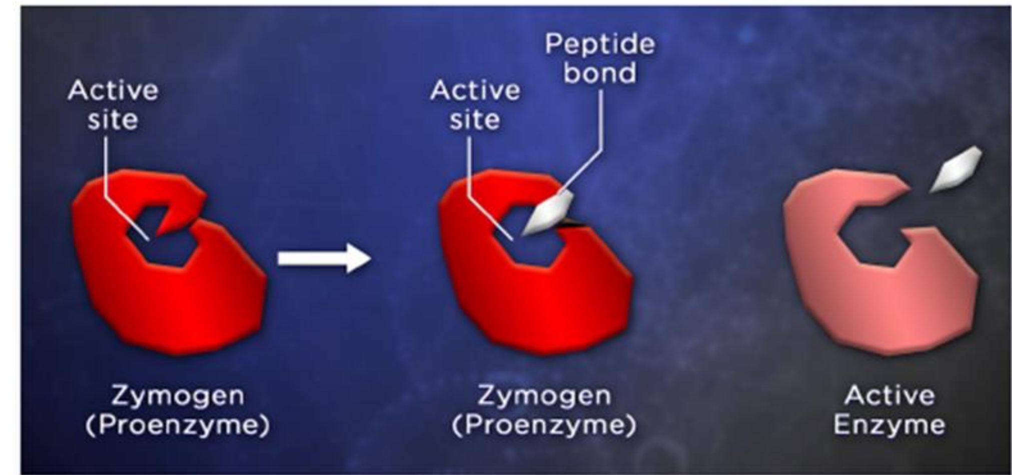
- Trypsin activates **chymotrypsinogen**, to form active chymotrypsin and two peptides (A and B peptides)

- All the gastrointestinal enzymes are synthesized in the form of pro-enzymes, and only after secretion into the alimentary canal, they are activated. This **prevents autolysis of cellular structural proteins.**

- **Coagulation factors** are seen in blood as zymogen form.

+ Activation of Chymotrypsin





Enzyme inhibition

Two main types:

1. Reversible
   - **Competitive inhibition**

2. Irreversible
   - **Non-competitive inhibition**

Competitive inhibition (reversible)

- A competitive inhibitor is **structurally similar to that of substrate**:

  It competes with substrate to bind reversibly at active or catalytic site

- The degree of inhibition depends on the **ratio** of the concentration of the inhibitor:substrate and not on the **absolute** concentration

- The inhibition also depends on the relative affinity of the substrate and the inhibitor to the enzyme

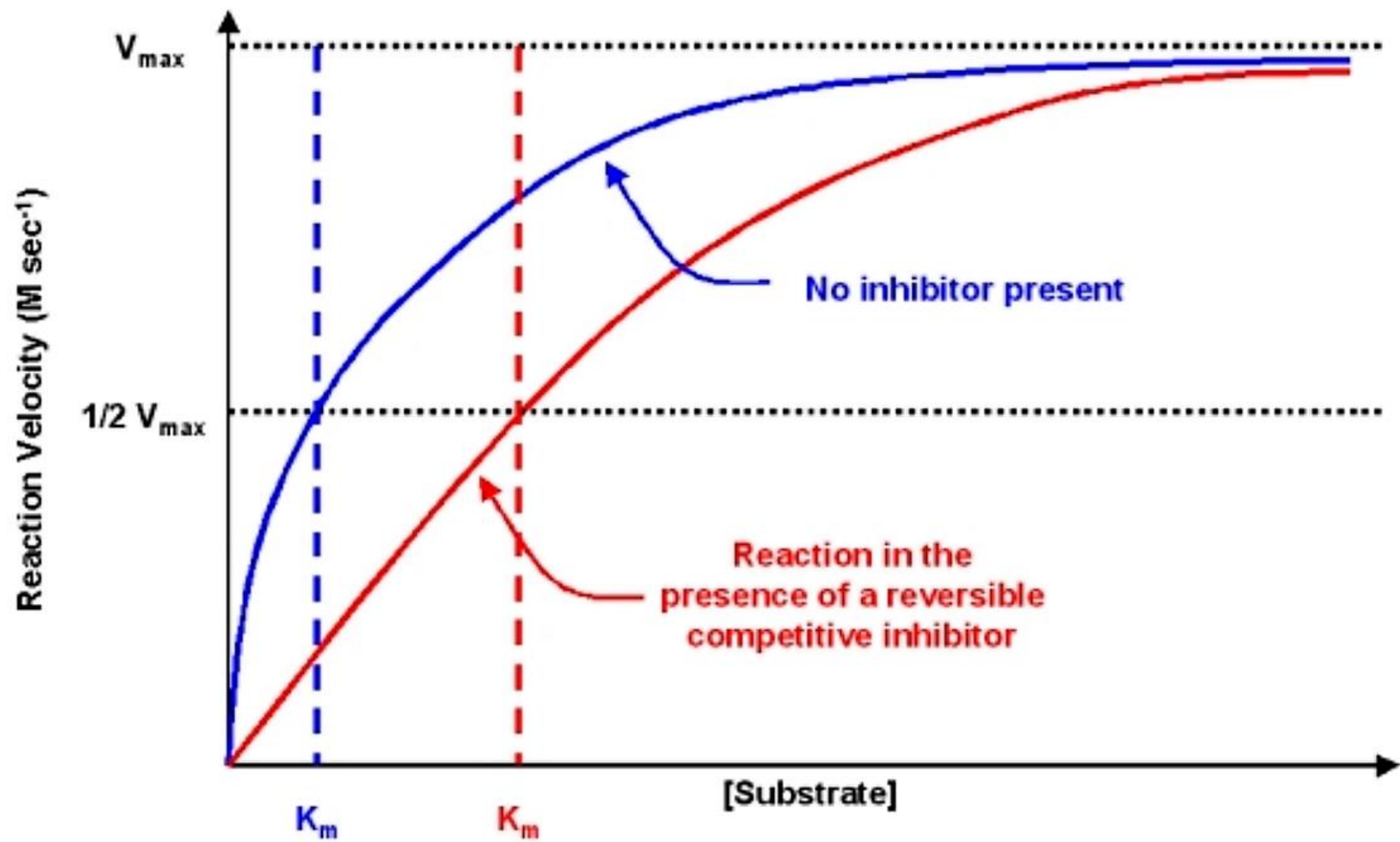
Competitive inhibition (reversible)

No Effect on V max:

- Effect of a competitive inhibitor is reversed by ↑ [S]
- At a sufficiently high [S] concentration, the reaction velocity reaches the Vmax observed in absence of inhibitor

Increase of Km:

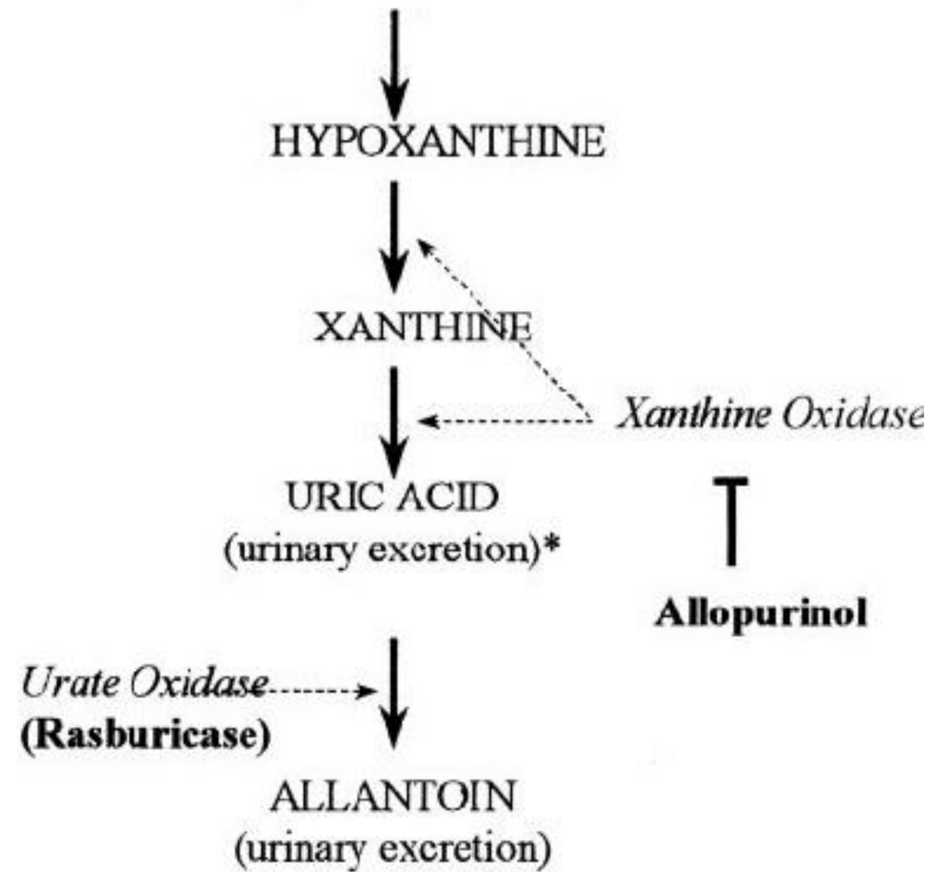
- A competitive inhibitor increases the apparent Km for a given [S]
- This means that, in the presence of a competitive inhibitor, more [S] is needed to achieve ½ Vmax

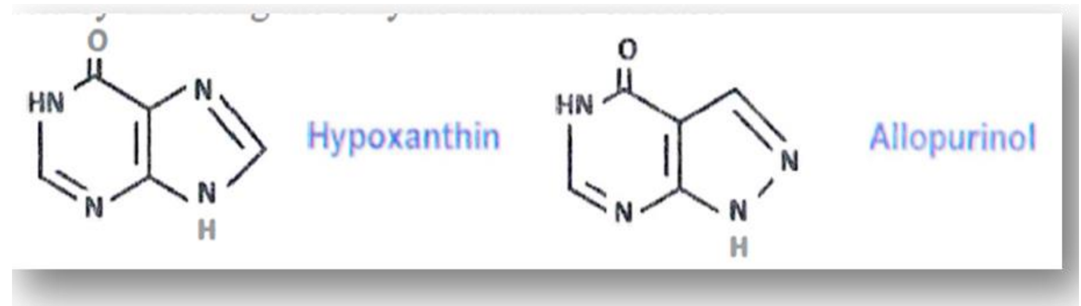


V_{max}
1/2 V_{max}
Reaction Velocity (M sec⁻¹)
No inhibitor present
Reaction in the
presence of a reversible
competitive inhibitor
[Substrate]
K_m
K_m

Table 5.5. Clinically useful Competitive Inhibitors

Drug	Enzyme inhibited	Clinical use	Refer chapter
1. Allopurinol	xanthine oxidase	gout	39
2. Dicoumarol	vit.K-epoxide-reductase	anti-coagulant	33
3. Penicillin	transpeptidase	bacteria	2
4. Sulphonamide	pteroid synthetase	bacteria	34
5. Trimethoprim	FH2-reductase	bacteria	34
6. Pyrimethamine	do	malaria	34
7. Methotrexate	do	cancer	51
8. 6-mercapto-purine	adenylosuccinate synthetase	cancer	51
9. 5-fluorouracil	thymidylate synthase	cancer	51
10. Azaserine	phosphoribosyl-amidotransferase	cancer	51
11. Cytosine arabinoside	DNA polymerase	cancer	51
12. Acyclovir	DNAP of virus	antiviral	42
13. Neostigmine	ACh-esterase	myesthenia	23
14. Alpha-methyl dopa	dopa-decarboxylase	hypertension	17
15. Lovastatin reductase	HMGCoA-lowering	cholesterol	12
16. Oseltamiver (Tamiflu)	Neuraminidase	Influenza	


PURINE CATABOLISM
HYPOXANTHINE
XANTHINE
Xanthine Oxidase
URIC ACID
(urinary excretion)*
Allopurinol
Urate Oxidase
(Rasburicase)
ALLANTOIN
(urinary excretion)
* A normal endpoint of purine metabolism in humans


Hypoxanthin
Allopurinol

Non-competitive inhibitor

- **No competition occurs between substrate and inhibitor** to bind at active site of enzyme

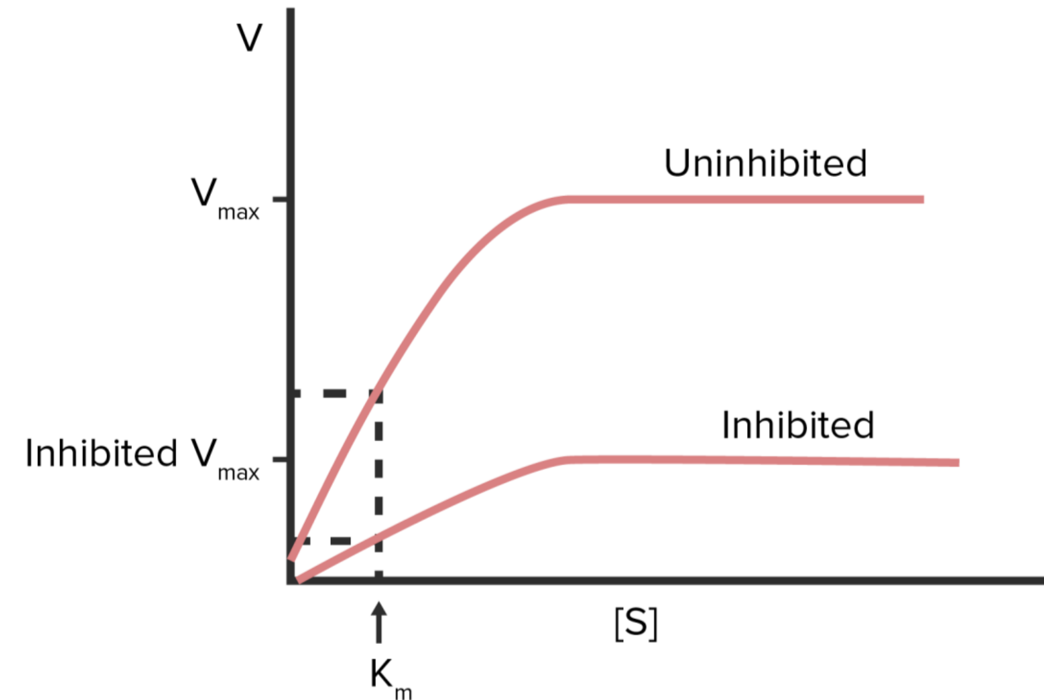
- Inhibitor is **not structurally related to substrate**
    - Inhibitor binds to a site **different than the active site** of enzyme

- The inhibitor can bind either the free enzyme (***non-competitive***) or the enzyme substrate (ES complex; ***un-competitive***)

- Increase in the substrate concentration generally **does not relieve this inhibition.**

Non-competitive inhibitor

- **Effect on Vmax:**
  - Non-competitive inhibition cannot be overcome by increasing the concentration of substrate
  - → non-competitive inhibitors decrease the Vmax
- **Effect on Km:**
  - **Non**-competitive inhibitors do not interfere with the binding of substrate to enzyme
  - the enzyme shows the same Km in the presence or absence of the non-competitive inhibitor
    - Remaining enzyme has same affinity for substrate



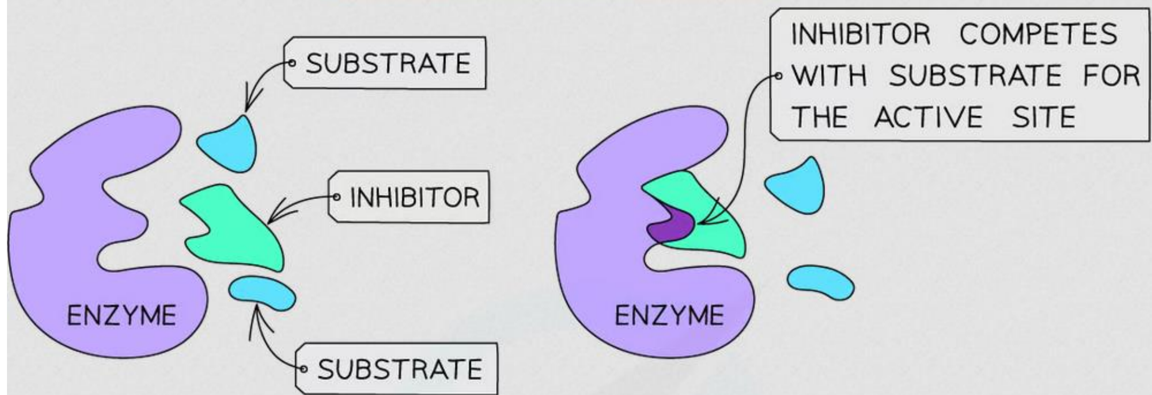
Examples of non-competitive inhibitors

- **Cyanide** and **carbon monoxide** inhibits cytochrome oxidase
- **Fluoride** will remove magnesium and manganese ions and so will inhibit the enzyme, enolase, and consequently the glycolysis
- **Iodoacetate** would inhibit enzymes having-SH group in their active centers
- **BAL** (British Anti Lewisite; dimercaprol) is used as an antidote for heavy metal poisoning
  - The heavy metals act as enzyme poisons by reacting with the SH group
  - BAL has several SH groups with which the heavy metal ions can react and thereby their poisonous effects are reduced.

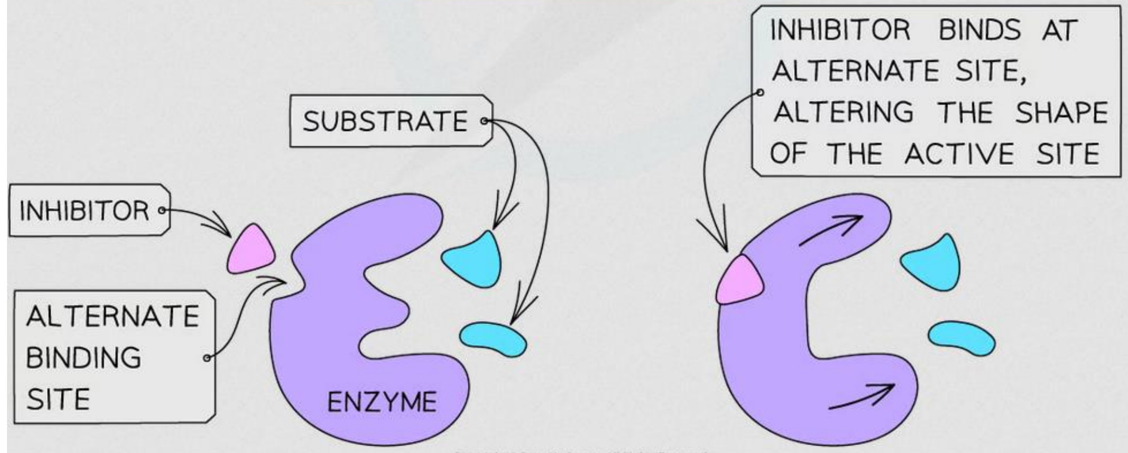
Table 5.6. Comparison of two types of inhibition

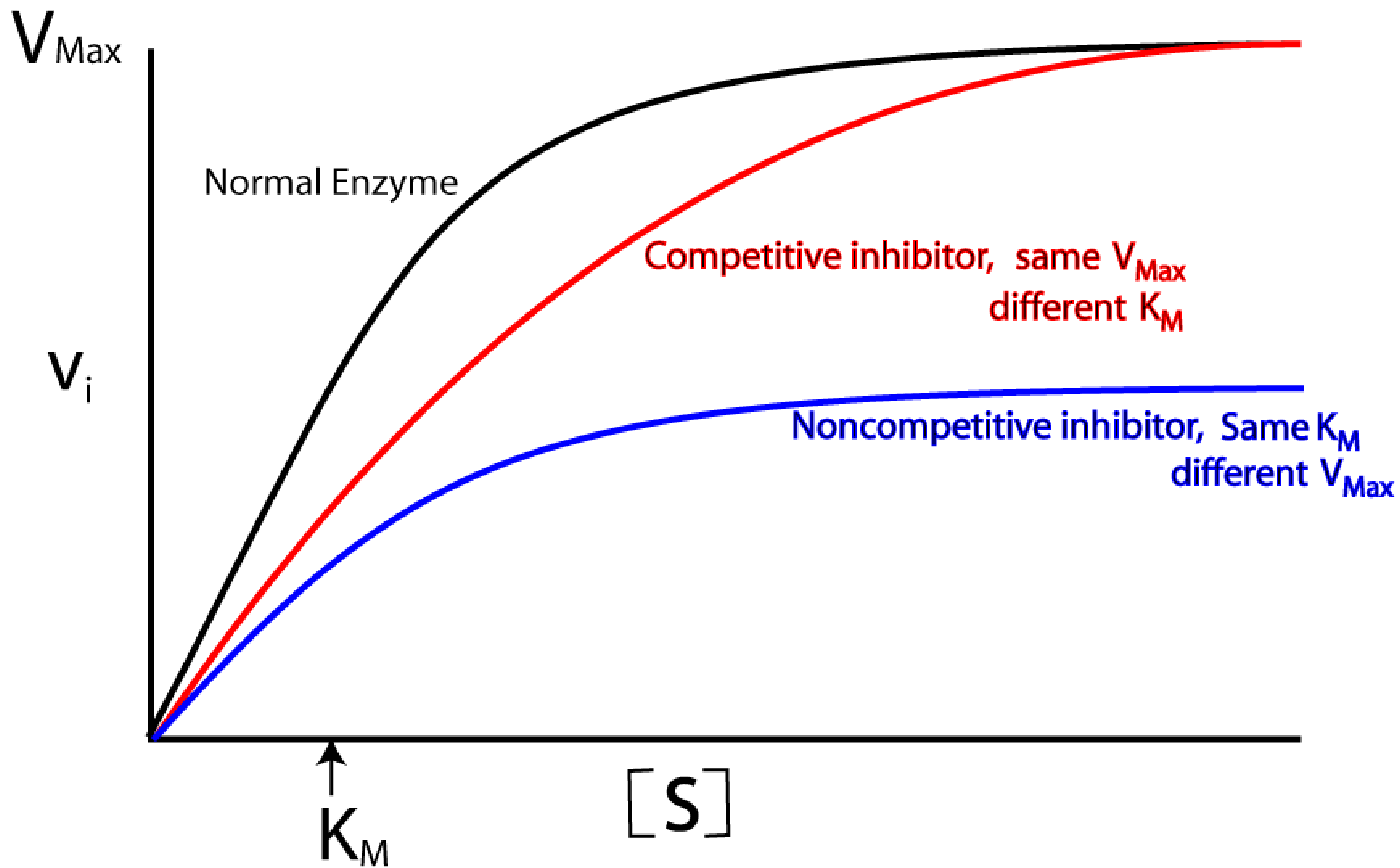
	Competitive inhibition	**Non-competitive inhibition**
Acting on	Active site	May or may not
Structure of inhibitor	Substrate analog	Unrelated molecule
Inhibition is	Reversible	Generally irreversible
Excess substrate	Inhibition relieved	No effect
Km	Increased	No change
Vmax	No change	Decreased
Significance	Drug action	Toxicological

COMPETITIVE INHIBITION



NON COMPETITIVE INHIBITION





V_{Max}
Normal Enzyme
Competitive inhibitor, same V_{Max}
different K_M
V_i
Noncompetitive inhibitor, Same K_M
different V_{Max}
K_M
[S]

Allosteric regulation

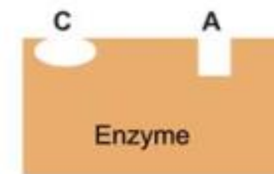
- Allosteric enzymes are enzymes that have an **additional** binding site for effector molecules other than the active site.

- The binding brings about conformational changes, thereby changing its catalytic properties. The effector molecule can be an inhibitor or activator.

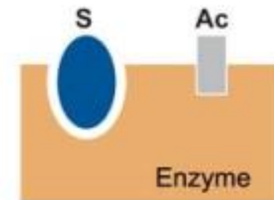
- An allosteric site is a region of an enzyme that allows activator or inhibitor molecules to bind to the enzyme and either activate or inhibit enzyme activity.

Allosteric regulation - Control of enzyme activity

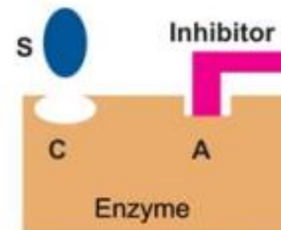
- Allosteric enzyme has one catalytic site where the substrate binds and another **separate allosteric site** where the modifier binds (*allo* = other)



Enzyme has separate catalytic (C) and allosteric (A) sites



When activator (Ac) is fixed, the catalytic site assumes correct three dimensional structure, so that substrate (S) can now bind



When inhibitor is attached to the allosteric site (A), the catalytic site (C) do not assume the correct shape, so that substrate (S) cannot fit correctly

Allosteric regulation - Control of enzyme activity

- Allosteric and substrate binding sites may or may not be physically adjacent

- The binding of the regulatory molecule can either:
  - Enhance the activity of the enzyme (allosteric activation) → **positive modifier**, or
  - Inhibit the activity of the enzyme (allosteric inhibition) → **negative modifier**

Allosteric regulation - Control of enzyme activity

- The inhibitor/ activator is **not** a substrate analog
- When an inhibitor binds to the allosteric site, the configuration of catalytic site is modified such that substrate cannot bind properly
- **Km** is usually increased & **Vmax** is reduced when inhibitor binds
- It is partially reversible, when excess substrate is added
- The effect of allosteric modifier is maximum at or near substrate concentration equivalent to Km

Examples of allosteric enzymes

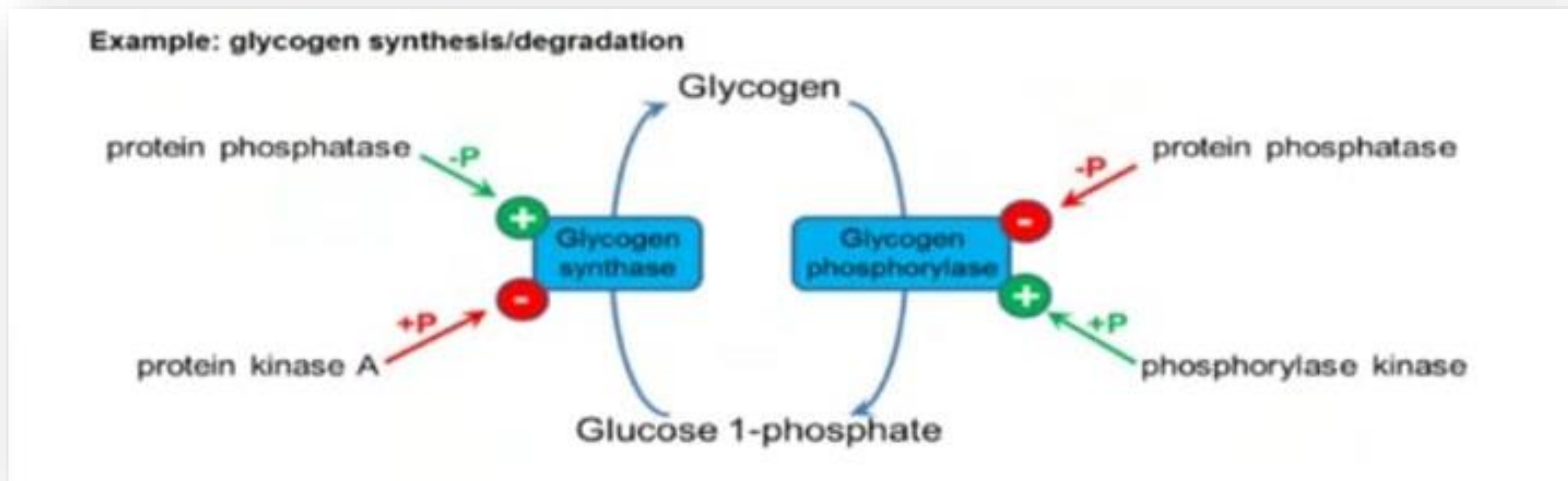
Enzyme	allosteric inhibitor	allosteric activator
1. ALA synthase	heme	
2. Aspartate trans-carbamoylase	CTP	ATP
3. HMGCoA-reductase	Cholesterol	
4. Phospho-fructo kinase	ATP, citrate	AMP, F-2,6-P
5. Pyruvate carboxylase	ADP	AcetylCoA
6. Acetyl CoA-carboxylase	AcylCoA	Citrate
7. Citrate synthase	ATP	
8. Carbamoyl phos-phate synthetase I	NAG	
9. Carbamoyl phos-phate synthetase II	UTP	

Covalent modification - Control of enzyme activity

- The activity of enzymes may be **increased** or **decreased** by covalent modification
- Either addition of a group to the enzyme protein by a covalent bond; or removal of a group by cleaving a covalent bond
- **Zymogen activation** by partial proteolysis is an example of covalent activation
  - Addition or removal of a particular group brings about covalent modification of enzyme protein. This is a reversible reaction.
- Commonest type of covalent modification is the reversible **protein phosphorylation** and **ADP ribosylation**.

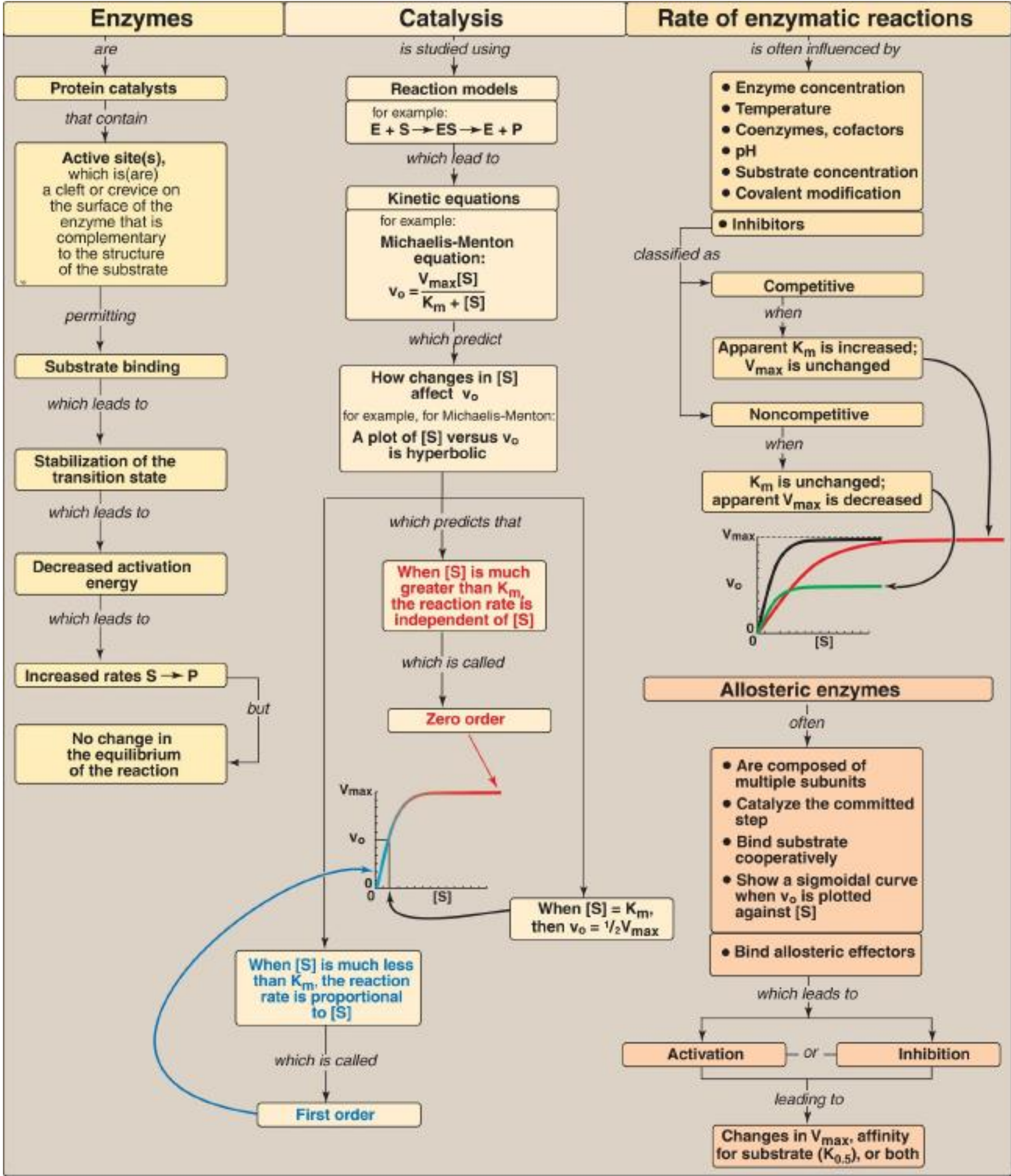
Examples of covalent modification

Enzyme	Phosphorylated enzyme
Acetyl-CoA carboxylase	Inactive
Glycogen synthase	Inactive
Pyruvate dehydrogenase	Inactive
HMG-CoA reductase	Inactive
Pyruvate kinase	Inactive
PFK2	Inactive
Glycogen phosphorylase	Active
Citrate lyase	Active
Phosphorylase b kinase	Active
HMG-CoA reductase kinase	Active
Fructose-2,6-bisphosphatase	Active



Insulin (well fed state): works via phosphatase → activates synthase and inactivates phosphoylase

Glucagon (fasting): works via kinase → activates phosphoylase and inactivates synthase



Enzymes
Catalysis
Rate of enzymatic reactions
are
Protein catalysts
that contain
Active site(s), which is(are) a cleft or crevice on the surface of the enzyme that is complementary to the structure of the substrate
permitting
Substrate binding
which leads to
Stabilization of the transition state
which leads to
Decreased activation energy
which leads to
Increased rates S → P
but
No change in the equilibrium of the reaction
is studied using
Reaction models
for example:
E + S → ES → E + P
which lead to
Kinetic equations
for example:
Michaelis-Menton equation:
$v_o = \frac{V_{max}[S]}{K_m + [S]}$
which predict
How changes in [S] affect v_o
for example, for Michaelis-Menton:
A plot of [S] versus v_o is hyperbolic
which predicts that
When [S] is much greater than K_m, the reaction rate is independent of [S]
which is called
Zero order
V_{max}
v_o
0
[S]
When [S] = K_m, then v_o = ½V_{max}
When [S] is much less than K_m, the reaction rate is proportional to [S]
which is called
First order
is often influenced by
• Enzyme concentration
• Temperature
• Coenzymes, cofactors
• pH
• Substrate concentration
• Covalent modification
• Inhibitors
classified as
Competitive
when
Apparent K_m is increased; V_{max} is unchanged
Noncompetitive
when
K_m is unchanged; apparent V_{max} is decreased
V_{max}
v_o
0
[S]
Allosteric enzymes
often
• Are composed of multiple subunits
• Catalyze the committed step
• Bind substrate cooperatively
• Show a sigmoidal curve when v_o is plotted against [S]
• Bind allosteric effectors
which leads to
Activation
or
Inhibition
leading to
Changes in V_{max}, affinity for substrate ($K_{0.5}$), or both