6/12/2022

ENZYMES

- Biological catalysts
- Highly specialised proteins
- word meaning: in yeast

Classification by International Union of Biochemistry and Molecular Biology [IUBMB]

The enzymes are divided into 7 major classes.

- Each enzyme is characterised by a code no - E.C number - comprising of 4 digits, separated by pts

IUBMB Classification (1964)

E.C no: (enzyme class no:)

① class ② subgp

② Subclass ④ serial no: of the particular enzyme.

Class

① Oxidoreductases
② Transferases
③ Hydrolases (split)
④ Lyases (splitting)
⑤ Isomerases
⑥ Ligases (joining)
⑦ Translocases

Imp

TTO HILL

CLASS - I

OXIDOREDUCTASES

- Enzymes involved in oxidation - reduction rn
- A reduced + Oxidised B → Oxidised A + Reduced B

- Lactate + NAD $\xrightarrow{\text{Lactate dehydrogenase}}$ Pyruvate + NADH + H^+

eg: Lactate dehydrogenase
Alcohol dehydrogenase
cytochrome oxidase

TRANSFERASES

- Catalyse the transfer of a gp. other than hydrogen, from one substrate to another

$$A-R + B \longrightarrow A-B + R.$$

- Transmethylases
- Transaldolases & Transketolase
- Transacylases
- Transaminases

- Kinases (Transfer of phosphate gp)
- Transfer phosphoryl gp. from ATP or another nucleotide triphosphate

Hexose + ATP $\xrightarrow{\text{Hexokinase}}$ Hexose - 6 - PO_4 + ADP

CLASS-3

HYDROLASES

- Hydrolyse bonds by + H_2O

$$Urea + H_2O \xrightarrow{urease} CO_2 + NH_3$$

eg: Lipases, Phosphatases, choline, esterase, Pepsin, trypsin, chymotrypsin

CLASS-4

LYASES

- Remove gps from substrates or break bonds by mechanisms other than hydrolysis.

Fructose-1,6-bisphosphate $\xrightarrow{aldolase}$ Dihydroxy acetone phosphate + Glyceraldehyde-3-phosphate

CLASS-5

ISOMERASES

- catalyse geometric or structural changes within a single molecule.

SUBCLASSES:

- Recemases & Epimerase

① * epimerases

uridine diphosphate

UDP Glucose ⟷ UDP Galactose

② * cis-trans isomerase

Retinine isomerase

11-Trans-retinene ⟷ 11-cis-retinene

- Interconvern of aldoses & ketoses

Glucose 6-PO_4 $\xrightarrow[\text{isomerase}]{\text{Glucose phosphate}}$ Fructose 6 PO_4

LIGASES (class-6)

- Link 2 substrates together
- ATP dependent condensation of 2 molecules
- Synthesize a new molecule & are called Synthetase.

eg: Glutamine synthetase, Acetyl CoA carboxylase

Synthases	Synthetases
• Catalyse biosynthetic rxn	• Catalyse biosynthetic rxn
• They are not ligases	• Ligases
• Not ATP-dependent	• ATP dependent
eg: Glycogen synthases	eg: Glutamine synthetase

CLASS-7

TRANSLOCASES

- They catalyse the translocation of ions and molecules across membrane

eg: ATP-ADP translocase

COENZYMES

- Enzymes can be simple proteins or complex enzymes containing a non-protein part.
- Protein part → Apoenzyme
- Non-protein part → coenzyme

} holo enzyme

- coenzyme → ↓ molecular wt, dialysable, organic substance, without which the enzyme cannot exhibit any fn, not easily removable

coenzymes

Grp 1
- involves transfer of hydrogen or e^-

eg: NAD, FAD, FMN

Grp 2
- Involves transfer of gp other than H_2.

eg: amino gp by pyridoxal phosphate (PLP), hydroxyethyl gp by TPP

~~Nicotinamide~~

Vitamin related coenzymes ∴ Imp.

Prosthetic gp

- Non protein, tightly integrated to the enzyme structure by covalent forces.
- Metals are most common prosthetic gp.
- Enzymes which are tightly bound to metal is metalloenzymes

Metalloenzymes

Zn - Carbonic an

Fe - catalase & Peroxidase

Mg - Hexokinase

- Non protein, inorganic molecule bound to apoenzyme
- Enzymes requiring the presence of a certain

Cofactors

coenzymes	Metal ions
• organic.	• Inorganic
• FAD, NAD, TPP	• Mg ions, Zn ions etc

9/12/22 Mechanism of enzyme acn

Active site

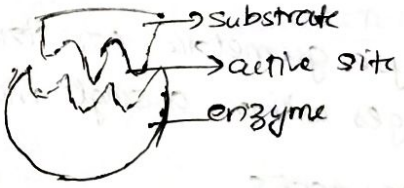
→ Specific & ↑ catalytic efficiency

→ Substrate ⟷ Enzyme
↓
non-covalent bonds

Active site
- substrate binding site
- catalytic site

→ a.ā / gps participating in catalysis → catalytic residues / gp.

→ During binding, gps realign themselves to provide a unique str. orienta, so as to provide exact fitting



chymotrypsin	• His, Asp, Ser
Trypsin	• Serine, His
Thrombin	• Serine, His.
carbonic anhydrase	• Cysteine

Enzymes (cont.)

K Theory of rxn

→ chemical rxn
→ molecules should collide
→ come within bonding dist
→ should overcome the energy barrier

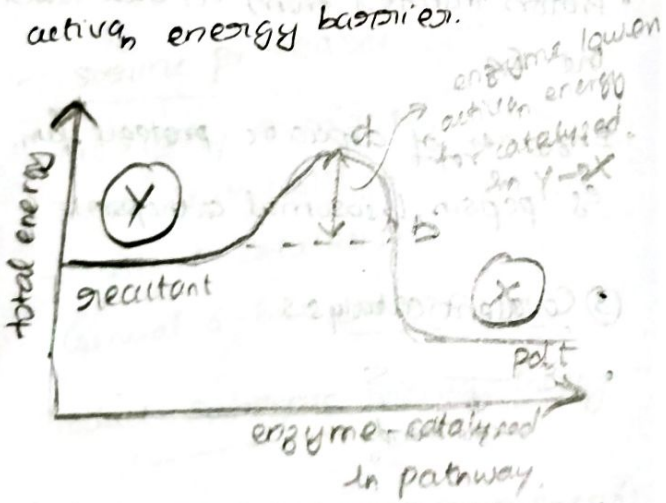
Mechanism of action

• Explained by diff. theories

① Lowering of E_A
② Fischer's Template theory
③ Michaelis-Menten theory
④ Koshland's Induced Fit theory

① Lowering of E_A

(i) Enzymes work by lowering the activation energy barriers.



• Ground state ⟷ transition state
energy diff. is E_A.

• ↑ E_A → ↓ rxn rate (without enzyme)

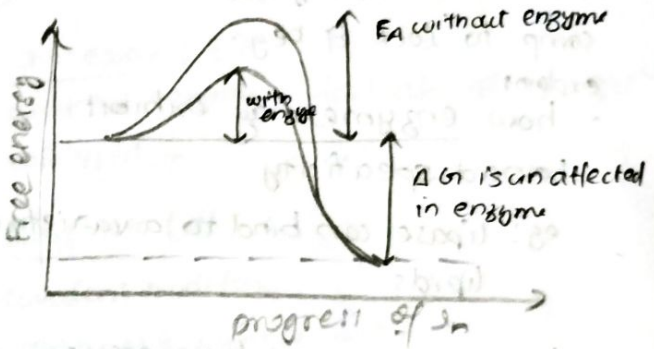
• Enzymes ↓ E_A by forming E-S complex

• E-S complex formation is reversible ⇒ weak non-covalent bonds.

• E + S ⟶ E-S
small release of free energy.

• Total energy released by E-S complex is binding energy

• B.E ↓ the E_A.



Fischer's Template theory

• Lock & Key model
• By Emil Fischer in 1894
• Failed to explain the dynamic changes that accompany catalysis.

Michaelis-Menten theory

• E + S ⟷ E-S complex ⟶ E + P
• By Michaelis & Menten in 1913.
• Explained mode of action of enzyme on the basis of E_A.
• " In an uncatalysed rxn, no: of substrate mol. in transition state is low so rate ↓. & vice versa for catalysed rxn.

KOSHLAND's Induced Fit theory

- By Daniel Koshland in 1959.
- Binding of substrate induce conf. change in enzyme binding site.

Lock & Key
↓ v/s
Induced fit.

- IFT E-S interac has d adv. comp to lock & key.
  explains
  - how enzymes may exhibit broad specificity
    eg: lipase can bind to a variety of lipids.
  - how enzyme catalysis occurs.
    [conf. change stresses bonds in subs. ↑ reactivity]

Basic mechanisms of Catalysis

To ↓ E_A

1. Substrate strain
2. ā - base catalysis
3. covalent catalysis
4. Metal-ion catalysis
5. catalysis by proximity
6. Product substrate orientation theory

① Substrate strain

- Binding of substrate to site → enzyme induce strain → ↑ energy level. → strain stretches targeted bond → vulnerable to cleavage.
- Mimics transin state inter.
- enzymes catalysing lytic rn which involves covalent bond breakage

Lysozyme - substrate strain & ā base catalysis

② Acid - base catalysis.

- Active site of some enzyme contain ionizable fnal gp of amino acyl side chain that can contribute to catalysis by acting as ā or base
- Proton transfer from ā/base lower the E_A
- Enzymes of aspartic protease fam.
  eg: pepsin, lysosomal cathepsin.

③ Covalent catalysis

- E — S
  ↓
  cov. bond.
- common among enzymes catalysing gp. transfer rn.
- serine protease (eg:)

④ Metal-ion catalysis

- Helps in orientation of S
- Stabilize charged reaction states.
- Mediate oxidation-reduction.

- Cu^{2+} or Zn^{2+} can stabilize the transition state. The metal binds to the charged intermediate
- Likely to be found in 1/3 of all enzymes

eg: Fe, Mo, Zn, Mg

- carbonic anhydrase - Zn^{2+}

⑤ Catalysis by proximity.

- Mol. should come in the bonding dist, collin freq ↑, rate of rxn ↑

The Serine proteases

- proximity & orientation
- Transition state stabilization
- covalent catalysis.
- General acid-base catalysis.

⑥ Product-substrate orientation theory

- E - 3D str. to keep S in specific orientation.
- eg: hexokinase

Summary

- Active site
- Diff. theories of mechanism of action
- Methods to ↓ EA
  - substrate strain
  - acid-base
  - covalent
  - metal-ion
  - P-S orientation theory

13/12/22 Tuesday

Factors influencing enzyme activity

Short essay (8)

What are enzymes, factors affecting vel. of enzyme catalysed rxn.

Short note (4)

- covalent modification
- Michaelis menten equation
- Proenzymes

Define optimum pH & one eg

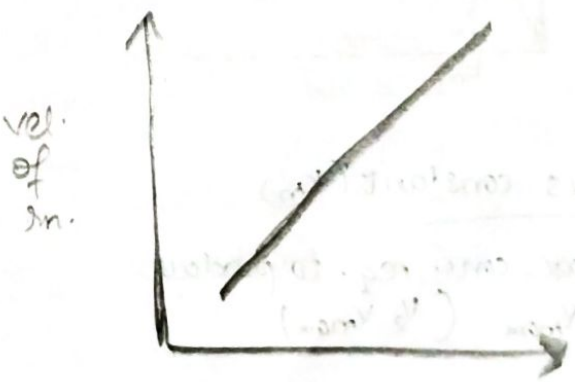
Precise quick (1)

- Significance of K_m value
- Zymogen activation

Enzyme conc.

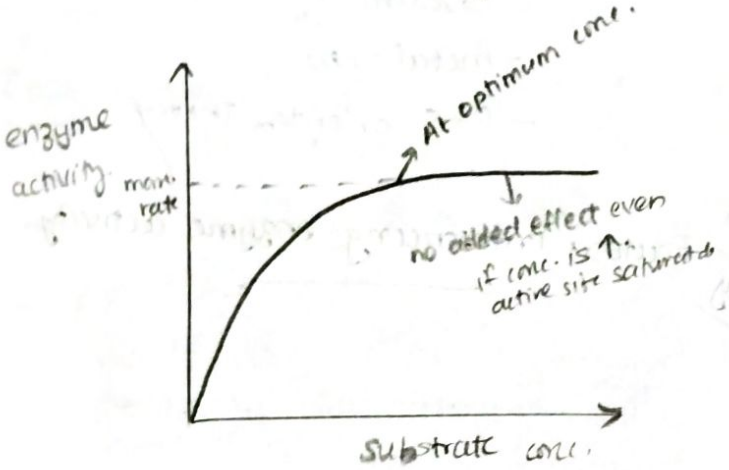
- Rate of a rxn is directly proportional to enzyme conc. when sufficient substrate is present

ie, vel. of rxn ∝ enzyme conc



Substrate conc.

- If enzyme conc. is fixed, for a typical enzyme, and the substrate conc. is ↑, the vel is also correspondingly ↑ in the initial phase, but later no change in the vel.



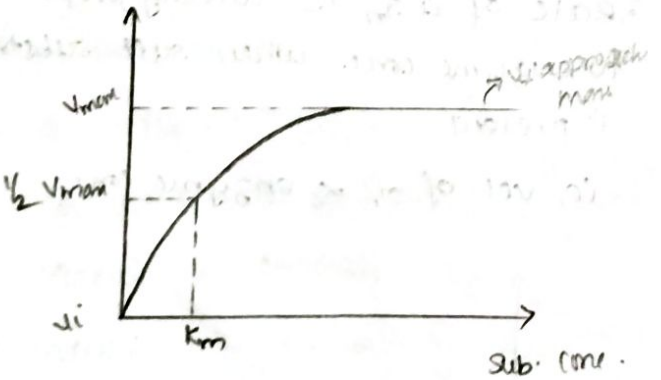
V_i → initial vel

V_{max} → maximal vel. / max rate attainable in the presence of excess substrate

Michaelis-menten equation.

(relation blw V_i & $[S]$)

(Same graph as above) → Michaelis-menten graph



Michaelis constant (K_m)

- Substrate conc. req. to produce half - V_{max} ($\frac{1}{2} V_{max}$)
- Ind. of enzyme conc
- Unique for each enzyme-substrate pair

Michaelis-menten eqn

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E-S \xrightarrow{k_3} E + P$$

$$K_m = \frac{k_2 + k_3}{k_1}$$

$$-\frac{dS}{dt} = \frac{dP}{dt} = \text{vel} = \frac{V_{max} \times [S]}{K_m + [S]}$$

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

V_0 - initial vel

moles/time

low $[S]$, $V \propto [S]$ - first order

hig $[S]$, V ind. of $[S]$ - zero order

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

⇒ If $K_m = [S]$

$$V_0 = \frac{V_{max}[S]}{[S] + [S]} = \frac{V_{max}}{2}$$

⇒ If $K_m \approx 0$

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

} 0 order

K_m

- const. for an enzyme
- Denotes affinity of enzyme to substrate
- K_m helps to understand the natural substrate of an enzyme

$K_m \downarrow \Rightarrow$ affinity ↑↑

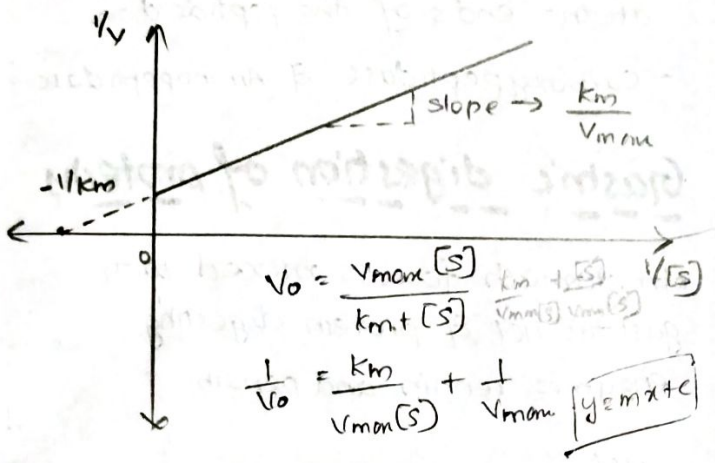
- Natural substrate ⇒ ↓ low K_m value

Lineweaver - burk or double reciprocal plot

- A double - reciprocal plot of enzyme kinetics is generated by plotting $1/V$ as a fn of $1/[S]$
- The slope is the K_m / V_{max}.

$$\frac{1}{V} / \frac{1}{[S]} = \frac{K_m}{V_{max}}$$

- Intercept on vertical axis is $1/V_{max}$
- Intercept on horizontal axis is $-1/K_m$.

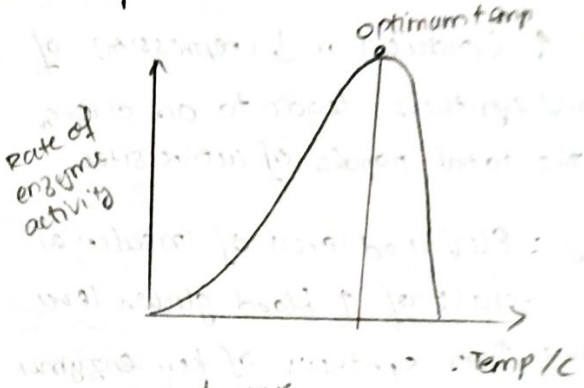


Product concentration.

- Products formed as a result of enzymatic rn accumulate & lower the rn rate by occupying the active site of enzyme
- In case of reversible rn, the rn is reversed

Temperature

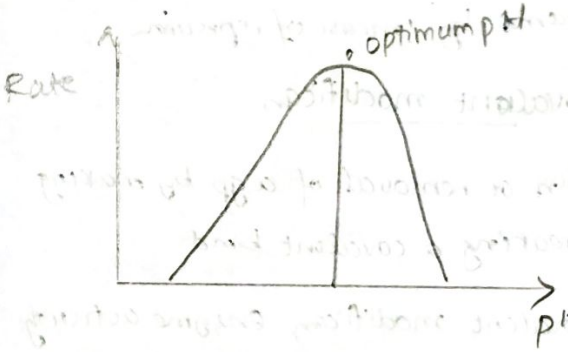
- ↑ temp, ↑ rate of rn, until peak vel. is reached
- ↑ K.E
- ↑ collision freq.
- With ↑ temp, rn vel. is ↓ due to denaturation of enzymes
- Optimum temp: temp @ which max amount of substrate is conv. to pdt / unit time
- Depends on normal temp. of cells in which it resides
- Most human enzyme has optimum Temp = 37°C



bell shaped curve

pH or H^+ ion conc.

- Imp. in all enzyme catalyzed rn
- Optimum pH of most enzymes 6-8.
- At ↑ or ↓ pH - enzyme



Effect of Inhibitors

- Any subst. which can diminish the vel. of an enzyme catalysed rn.
  - competitive inhibition
  - non competetive "
  - un competetive "
  - Succidic "
  - Allostonic "
  - Feedback "

Induction and Repression

- cells can regulate the amt of enzyme act.
- usually at by altering the rate of enzyme synthesis
- The ↑ (induction) or ↓ (repression) of enzyme synthesis leads to an alteration in the total population of active sites
- Eg: Elevated levels of insulin as a result of ↑ blood glucose level cause ↑ in synthesis of key enzymes involved in glucose metabolism
- This is called induction of enzyme glucokinase by insulin
- The key enzyme of heme synthesis, ALA synthase, is autoregulated by the heme by means of repression

Covalent modification

- Addition or removal of a gp by making or breaking a covalent bond.
- By covalent modification, enzyme activity is either ↑ or ↓
- Commonest type of covalent modification is reversible protein phosphorylation

Common methods of reversible

- Phosphorylation/dephosphorylation
- Methylation
- Adenylation
- ADP ribosylation
- Acetylation

common

19/12/22 ~~Factors~~ affecting

Partial Proteolysis.

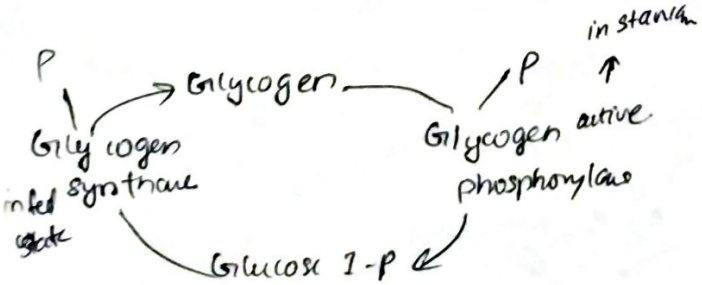
- certain enzymes are synthesised & secreted as inactive precursors — proenzyme/zymogens.
- selective proteolysis make them catalytically active
- Eg: Gastro-intestinal enzymes, lysosomal proteolytic enzymes, coagulam factors.

Enzymes active in phosphorylated state

- Usually enzymes are in Phosphorylated state when body is fasted. confusing
- Under the influence of Glucagon
1. Glycogen phosphorylase
2. HMG CoA reductase kinase
3. Key enzymes of Gluconeogenesis

Enzymes active in dephosphorylated state

- Usually enzymes are in dephosphorylated state when the body is in well fed state
- Under the influence of Insulin
1. Glycogen synthesis.
2. Key enzymes of Glycolysis
3. HMG CoA reductase



Properties of Enzymes. Short note

(1) Specificity of enzymes

1. Absolute - one enzyme acts only on one substrate (eg: urease decomposes only urea; arginase split only arginine)
2. Relative - one enzyme acts on different substrates which have the same bond type (eg: pepsin splits diff. protein)
3. Stereospecificity - some enzymes can catalyze the transformation only substrates which are in certain geometrical configuram, cis - or trans. -

ENZYME INHIBITION.

- Enzymes are subject to both reversible/ irreversible inhibition
- Most imp - pharmaceutical agents known
- Help in appropriate control of all rxns in the body

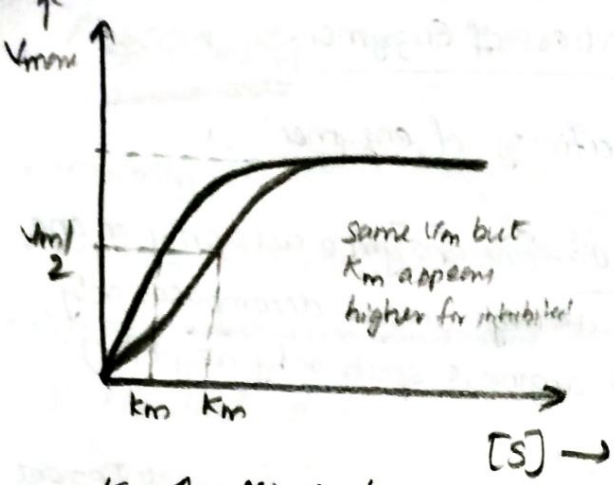
Types competetive inhibn.

~~e com~~

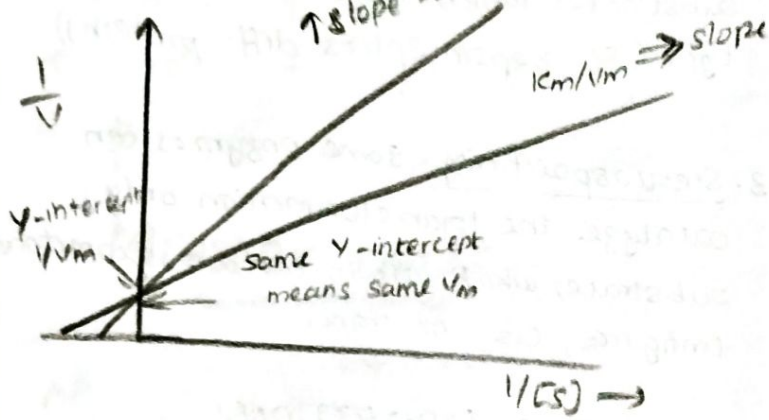
- Competetive inhibitor: A substance that compete directly with a normal substrate for an enzyme substrate binding site
- Inhibitors will be structural analogue of substrate

$$E + S \longrightarrow ES \longrightarrow E + P$$
$$E + I \longrightarrow E-I$$

- Reversible
- Excess substrate abolishes inhibn
- Km - ↑
- Vmax - no change



Km ↑ affinity ↓



eg:

Sulphonamides - as antibacterial drugs.
(affect purine synthesis)

Methotrexate - as anticancer drug.
↳ x dihydrofolate reductase is inhibited

Ethanol - antidote in methanol poisoning.

Di'coumarol - as anticoagulant

Sulphonamides

Inhibit bacterial cell folic ā synthesis.

HMG Co-A

Statins. ⊖ → HMG Co-A reductase

Mevalonic ā

↓

↓

Cholesterol

- Allopurinol inhibit xanthine oxidase & prevent uric ā synthesis } gout

Methanol

↓ Alcohol dehydrogenase (ADH) ← Fomepizole or ethanol

Formaldehyde

↓ Aldehyde dehydrogenase (ALDH)

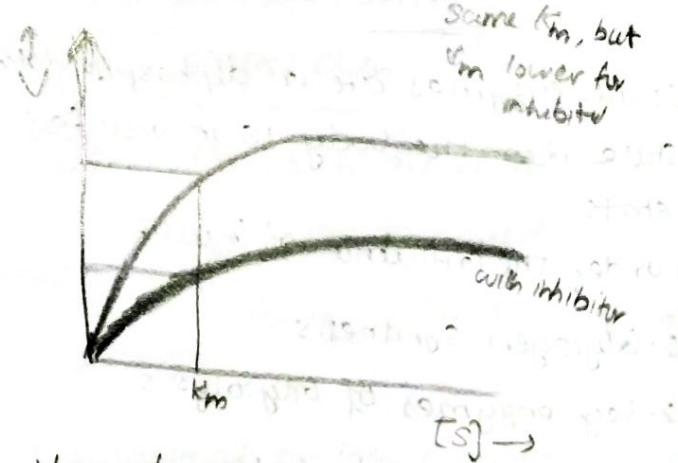
Formic ā

↓ Folic ā (vit Bc)

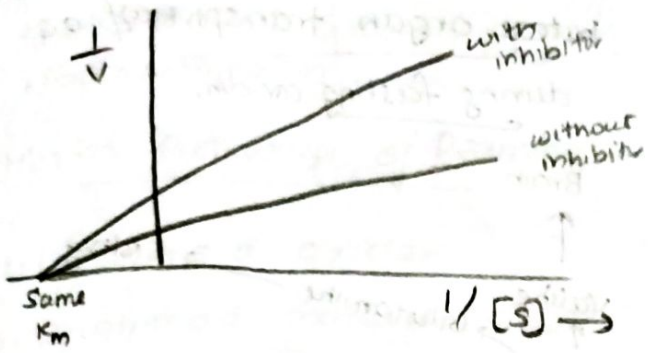
$CO_2 + H_2O$

Non-competitive inhibn.

- Inhibitor have no structural resemblance to substrate
- Inhibitor bind enzyme at sites distinct from substrate binding site
- Increase in substrate conc. does not relieve inhibn



Vmax ↓ Km same



22/12/22

Irreversible non-competetive inhibin

eg: cyanide
↳ affect cytochrome a_3 (respiran)

* Fluoride coated tubes are used for collecting blood samples for checking diabetes.

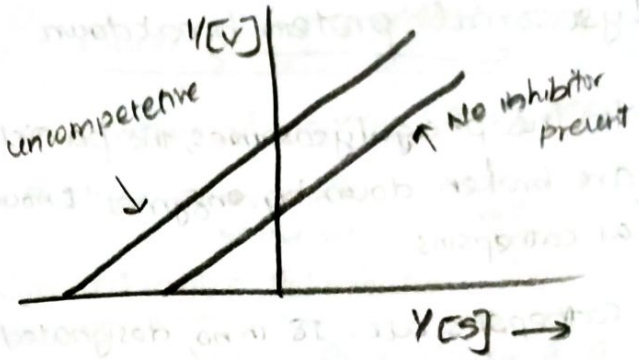
2-Phosphoglycerate
↕ enolase, Fluoride
Phosphoenol pyruvate

Uncompetetive inhibition

- Inhibitors need not resemble the substrate/doesn't have any affinity for free enzyme.
- Inhibitors binds to E-S complex
- K_m & V_{max} are ↓
- Eg: Inhibin of placental alkaline phosphatase by phenylalanine.

K_m ↓ affinity ↑

Allosteric inhibin ·X·
Sigmoid curve in SA

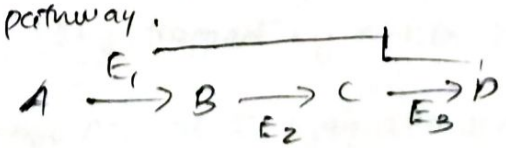


Suicidal inhibin.
[Mechanism based inactivan] ·X·

- Spcl cls of irreversible inhibin
- Inhibitors are relatively unreactive until they bind with the active site of specific enzyme
- Undergoes the 1st few chemical steps of normal enzymatic rxn, get converted into a very reactive comp. that combines irreversibly with the enzyme
- Play significant role in rational drug design.
- eg: Allopurinol
↓ xanthine oxidase
Alloxanthine → toxic to uric acid formn (suicidal inhibit)
- Treatment of Trypanosomiasis by DFMO (difluoromethyl ornithine)

Feedback inhibin.

- Also → end-pdt inhibin.
- The activity of the enzyme is inhibited by the final pdt of the pathway.



- eg: AMP inhibits 1st step in purine synthesis

2nd continue of Enzyme

Enzyme Regulan

- Regulan of enzyme quality / intrinsic catalytic efficiency
- Regulan of enzyme quantity.

Regulation of enzyme quality:

- Allosteric regulan
- Covalent modifican
- compartmentan
- Feed back regulan

Regulan of enzyme quantity:

- control of Enzyme synthesis (by induction/Repression)
- control of Enzyme Degradan

Allosteric regulan

- Allosteric enzymes are those whose activity at the active site may be modulated by the presence of effector at an allosteric site.
- Allosteric enzyme has:
  - catalytic site where the substrate binds
  - Allosteric site (Allo = other, steric = space) where the modifier molecules bind

Allosteric regulan.

Allo. activan → Allo. Inhibin.

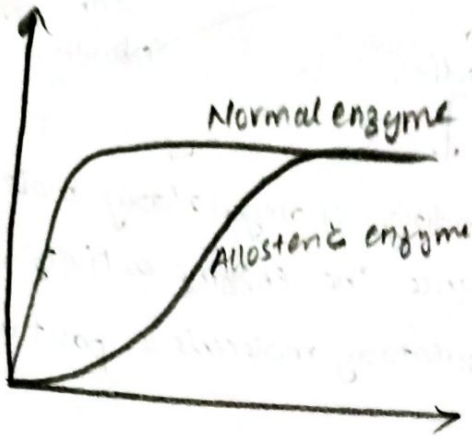
- Binding of regulatory molecule enhance the enzyme activity.
- Regulatory molecule - positive modifier

Inhibin

- Binding of regulatory mol. inhibit enzyme enzyme activity
- Regulatory molecule - negative modifier
- Partially reversible if excess substrate is added

Features of Allosteric regulan

- The modifier need not be a structural analogue of the substrate
- partially reversible when excess substrate is added.
- Effect of allosteric modifier is max. at or near substrate conc. equivalent to K_m.
- Most allosteric enzymes possess quaternary structure & made up of subunits.
- Does not follow Michaelis - Menten hyperbolic kinetics, gives Sigmoid Kinetics
- Sigmoid shape of the curve is due to cooperative binding
- Cooperative Binding means binding of substrate to one site ↑ sei the affinity of binding of the substrate to substrate binding site in other subunits
- Multiunit enz - shows +ve or -ve cooperation



- Allosteric inhibitors are metabolically most imp. when the substrate conc. falls to a low level
- when [S] goes ↑↑ allosteric inhibn ↑

Allosteric regulation

- Allosteric enzymes occupy key regulatory posin in metabolic pathways called key enzymes or rate limiting enzymes.

06/01/2023

- Allosteric activators
  - ↳ favourable conformational change
- Allosteric inhibitors
  - ↳ unfavourable conformational change
- K series enz - Only Km ↑ without change in Vmax
- V series - only Vmax ↓ without change in Km.

In Glycolysis

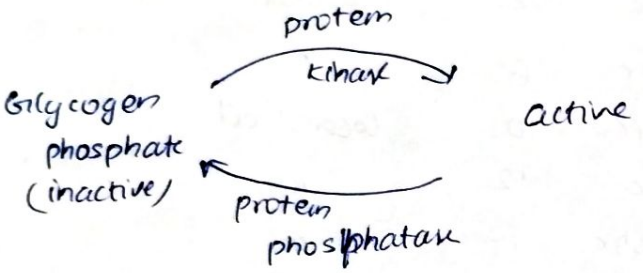
Allosteric activator
- ↳ Phospho-fructo kinase
- ↳ fructose-2,6-bisphosphate

Allosteric inhibitor
- ↳ ATP
- ↳ citrate

Enzymology (cont.)

Phosphorylation/Dephosphorylation [By covalent modification]

- Most common type
- Phosphorylation catalysed by protein kinases
- Dephosphorylation catalysed by protein phosphatases
- They act on more than 1 substrate



- Mechanism is ↑ versatile/selective
- Affect catalytic efficiency, affinity for substrate, location within the cell, regulation by allosteric ligands etc.
- Eg:

* covalent modification of enzymes → dephosphorylated form
  - Glycogen synthase ↔ enemies
  - Glycogen phosphorylase.

* Hormonal regulation.

Starvation

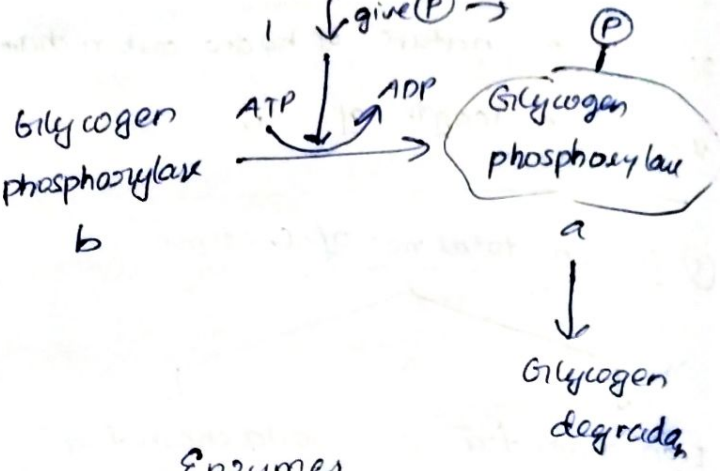
Glycogen phosphorylase → active
↳ phosphorylated state

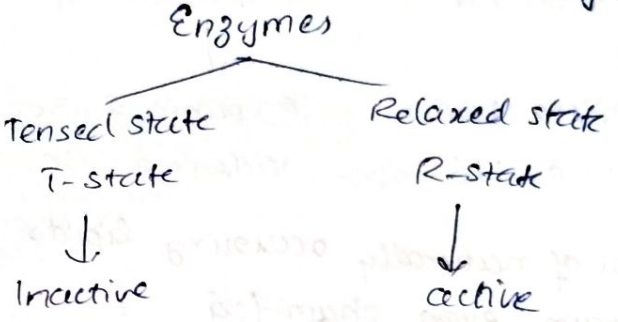
Glycogen synthase → active
↳ dephosphorylated state
} well fed state

phosphorylase a → active
" b → inactive

phosphorylase kinase a - active
" b - inactive

phosphate gp is provided by
↳ phosphorylase kinase
↓ give Ⓟ →





Glycogen synthase

Phosphorylated form: inactive
[Glycogen synthase b]

De-phosphorylated form: active
[glycogen synthase a]

phosphorylation by: protein kinases.
- Ca^{2+}/calmodulin dependent (in skeletal muscle)
- CAMP dependent.

Hormone (H) + Receptor (R)
↓
Hormone-Receptor complex (HR)
↓
HR binds on Gs protein
↓
Adenylcyclase activated
ATP → cyclic AMP → 5'AMP (Phosphodiesterase)
↓
Inactive protein kinase → Active protein kinase
↓
Enzyme → Phosphorylated enzyme
ATP, ADP

control of glycogenesis & glycogenolysis by cAMP dependent protein kinase

epinephrine (liver, m)
Glucagon (liver)
↓
Activation of adenyl cyclase
↓ +O

ATP → cAMP → 5'AMP
↓
cAMP dep. protein kinase
↓
· Phosphorylase kinase a.
Glycogen synthase b.

Result: Activation of glycogenolysis
: Inactivation of glycogenesis.

Enzyme active in phosphorylated state

- Usually enzyme are in phosphorylated state when body is fasting
- Under the influence of Glucagon

1. Glycogen phosphorylase
2. HMG CoA reductase kinase
3. Key enzymes of Gluconeogenesis.

Enzyme active in dephosphorylated state

- usually enzyme are in dephosphorylated state. when body is in well fed state
- Under the influence of insulin

1. Glycogen synthase
2. Key enzymes of Glycolysis.
- Acetyl CoA carboxylase
- Pyruvate dehydrogenase
- HMG CoA reductase

Feed back regulation

eg: control of hepatic cholestrol synthesis by dietary cholestrol.

- End pat of pathway feed back & control their own synthesis diff. from feed back inhibition
- Usually act at the level of gene expression

Compartmentation

- Eg: F.A Synthesis - cytoplasm
F.A oxidation - mitochondria.
- Protein & carbohydrate degrading enzymes reside inside lysosomes
- Anabolic & catabolic pathways occur in diff. cellular organelles / compartments
eg: Heme synthesis & Urea cycle