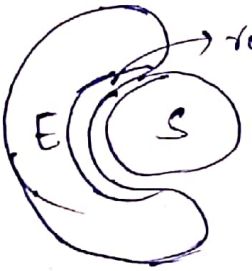
Enzyme Technology

- enzymes → protein catalysts.
  - alter the rate of the reaction w/o undergoing a permanent change in structure.
- reduced activation energy & $\Delta G_{reaction}$
  - stays the same → kinetic
  - → thermodynamic



→ residues

* active site is lined with residues.
  - imp. properties
    1. charge (partial, dipoles, helix dipoles)
    2. pKa
    3. hydrophobicity
    4. flexibility
    5. reactivity

- chymotrypsin : 2 Gly & 1 Ser
- trypsin : 2 Gly & 1 Asp.
  - → -ve residue
- elastase

→ CLASSIFICATION

- simple enzymes : composed of whole protein. eg. ribonuclease
- complex enzyme : protein + small org. molecule
  - protein → apoenzyme
  - small org. molecule → prosthetic group / coenzyme
  - holoenzyme
  - bound to [illegible] by covalent

* coenzyme : when binding b/w apoenzyme & organic molecule is non-covalent.

- on the basis of the reactions they'll catalyse :

① Oxidoreductases : acts on chemical groupings to add or remove hydrogen atoms.

② Transferases : transfer functional groups b/w donor & acceptor.

* Kinases : specialised transferases that regulate metabolism by transferring phosphate from ATP to other molecules.

③ Hydrolases : add water across a bond, hydrolysing it.

④ Lyases : add water, ammonia or CO_2 across double bonds or remove these to form double bonds.

⑤ Isomerases : carry out many kinds of isomerisations (shifts chemical groups)

⑥ Ligases : reactions in which two chemical groups are joined with the use of energy from ATP.

→ ΔG & $\Delta G^{\ddagger}$
- ΔG → thermodynamic quantity / free energy change of system
- $\Delta G^{\ddagger}$ → free energy of activation → related to rate / kinetics of the system.

$E + S \longrightarrow E + P$

- E → enzyme / catalyst (stays unchanged)
- S → substrate
- ES → enzyme substrate complex, [ES]

$\Delta G^{\ddagger}$ uncatalyzed rxn 107 kJ (say)

$\Delta G^{\ddagger}$ catalyzed rxn 46 kJ

- Arrhenius expression to find rate change.

~~$k_{uncat} = A e^{-}$~~

$$k = A e^{-\Delta G^{\ddagger}/RT}$$

$$\frac{k_{cat}}{k_{uncat}} = \sim 5 \times 10^{10}$$

⇒ Enzymes:

① acc. rxns by ↓ $\Delta G^{\ddagger}$

② do so by binding the transition state of the rxn better than the substrate.

③ exhibit

- catalytic efficiency:

~~Turnover~~ Turnover no. → maxm no. of moles of substrate that can be converted to product per mole of catalytic site per sec.

- effect of temperature:
not too low, not too high but an intermediate one
- effect of pH: depends on the amino acid composition of the enzyme

* pepsin ≈ 2 pH
trypsin ≈ 7 pH
} optimum activity

→ Enzyme kinetics

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

Velocity, v (rate at which products are formed) $= k_2 [ES]$

$$v = \frac{d[P]}{dt}$$

$$\frac{d[ES]}{dt} = k_1 [E][S] - k_{-1}[ES] - k_2 [E][P]$$

$$= 0 \quad (\because \text{steady state})$$

$$\frac{k_1 [E][S]}{k_{-1} + k_2} = [ES]$$

We know, $[S], E_T = [E] + [ES]$

$$[E] = (E_T - [ES])$$

$$(k_{-1} + k_2)[ES] = k_1 (E_T - [ES])[S]$$

$$(k_{-1} + k_2 + k_1 [S])[ES] = k_1 E_T [S]$$

$$v = k_2 [ES]$$

v_{max}, (maxm velocity enzyme can attain) $= k_2 E_T$

$(k_{-1} + k_1[S])[ES] + v = k_1 E_T [S]$

multiply both sides by k_2

$\therefore k_m = \frac{k_{-1} + k_2}{k_1}$

↓

michelis constant

$v = \frac{v_{max}[S]}{k_m + [S]}$

$k_2(k_{-1} + k_1[S])[ES] + k_2 v = k_1 k_2 E_T [S]$

$(k_{-1} + k_1[S])v + k_2 v = k_1 v_{max}[S]$

$v(k_{-1} + k_1[S] + k_2) = k_1 v_{max}[S]$

$\frac{v(k_{-1} + k_2) + k_1[S]v - v_{max}[S]}{k_1}$

$v k_m + v[S] = v_{max}[S]$

$v = \frac{v_{max}[S]}{k_m + [S]}$

if $v = v_{max}/2$

$v_{max}/2 = \frac{v_{max}[S]}{k_m + [S]} \Rightarrow k_m = [S]$

⇒ THIS IS THE ~~MICHELIS~~ MICHAELIS - MENTIN KINETICS. Its features are:

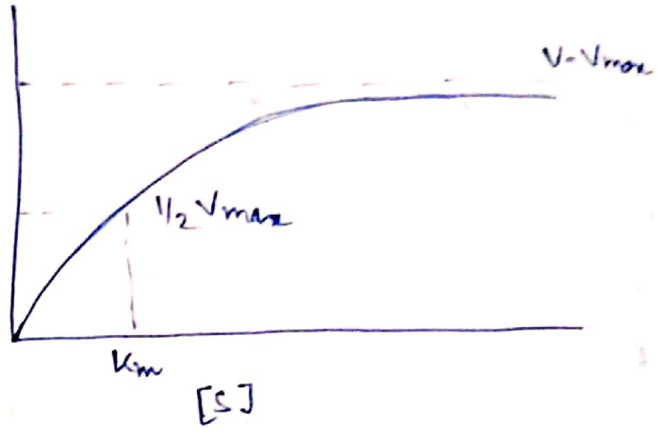
① assumed formation of ES complex

② assumes ES complex is in rapid eqbm with free enzymes.

③ Breakdown of ES to form products assumed to be slower than

(i) formation of ES &

(ii) breakdown of ES to re-form E & S.



① low [S], $V \propto [S]$ → first order

② high [S], V independent of [S] → zero order.

- Vitamins: group of organic compounds needed in small quantities in the diet for normal activity of tissues
- many vitamins act as cofactors, coenzymes or prosthetic groups for enzymes
- derived by diet, can't be generated by mammalian cells.
- thiamine / Vitamin B_1 → first discovered vitamin.
- not all are amines or N-containing compounds.

- **Water soluble vitamins:**

Thiamin (B_1), Riboflavin (B_2), Niacin (B_3), Pantothenic acid (B_5), Pyridoxal (B_6), Biotin (B_7), Cobalmin (B_{12}), folic acid & ascorbic acid (C).

- **Lipid soluble vitamins:**

Vitamin A, D, E & K.

- **Vitamin loss:**

A → sensitive to oxygen & light

D → usually little loss

E → sensitive to oxidation when heated or with alkali.

K → very sensitive to acids, alkali, light & oxidizing agents

C → very sensitive to oxidation, esp. when heated in contact with metals.

B complex → water solubility results in loss in cooking water.

Riboflavin → sensitive to light.

- **cofactors:** low MW component essential for protein function.

Apoenzyme + coenzyme → Holoenzyme

* prosthetic groups

(i) organic / inorganic eg. Heme groups

(ii) coenzymes.

* COFACTORS

- essential ions
  - activator ions (loosely bound)
  - metal ions (metallo-enzymes) (tightly bound)
- coenzymes
  - co-substrates (loosely bound)
  - prosthetic groups (tightly bound)

* all water-soluble vitamins except C are converted to cofactors

* only vitamin K of lipid-soluble vitamins is conv. to cofactors.

* may also act as carriers of specific functional groups such as methyl groups & acyl groups.

eg. # (1) Oxygen binding (hemoglobin)

C–O binding → irrev. at physiological T

S&I^n – covalent attachment to metal bound protein.

(2) chemical capture of light

photon energy changes covalent structure / denature.

S&I^n – reversible ionisation of cofactor

(3) Protein / nucleic acid radicals reactive → denaturation.

poly-aromatic / conjugated co-factors / enzymes like NAD, FAD etc.

→ classes of coenzymes:

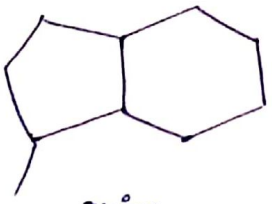
① cosubstrates: altered during rxn, regenerated by another enzyme

② prosthetic groups: remain bound to the enzyme during rxn & may be covalently or tightly bound to enzyme.

③ metabolite coenzymes - synthesized from common metabolites.

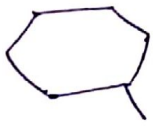
④ vitamin - derived coenzymes - can't be synthesized by mammals.

* Note: base families



purine

(A & G)



pyrimidine.

(C, T, U)

Purine & pyrimidine base + pentose sugar = nucleotide

base + sugar + phosphate group = nucleotide

ribose → OH at 2'

deoxyribose → H at 2'

→ ATP (Adenosine Triphosphate)

AMP → adenosine monophosphate

dAMP → deoxyadenosine monophosphate

ADP → adenosine diphosphate (5)

ATP → adenosine triphosphate.

- Source of immediately usable energy for cell.
- Versatile reactant that can donate phosphoryl group
  - (i) phosphoryl group
  - (ii) pyrophosphoryl group.
  - (iii) adenylyl group (AMP)
  - (iv) adenosyl group
- required in transport work, mechanical work & chemical work.

→ coenzyme	vitamin	role
① ATP	-	energy & PO_3^- transfer
② NAD(P)	Niacin	Redox
③ ~~coenzyme~~ FAD/FMN	riboflavin (B_2)	Redox
④ coenzyme A	Pantothenic acid (B_2)	Acyl transfer
⑤ TPP	Thiamine (B_1)	Transfers 2C
⑥ PLP	Pyridoxine (B_6)	amino acid transferases
⑦ lipoamide	-	acyl transfer
⑧ ubiquinone	-	e^- carrier

→ Thiamine (B_1)

* heterocyclic components
* pyrimidine
* thiazole

- active form of ~~thiamine~~ → thiamine pyrophosphate.
- Involved in ~~oxidative~~ aldehyde transfer
- lack causes Beri Beri

Thiamine (vitamin B_1) + TPP-~~synthetase~~

$$\text{Thiamine} + ATP \longrightarrow AMP + TPP$$