22/9/11

- Only α-amino acid can be determined by Ninhydrin Assay :-

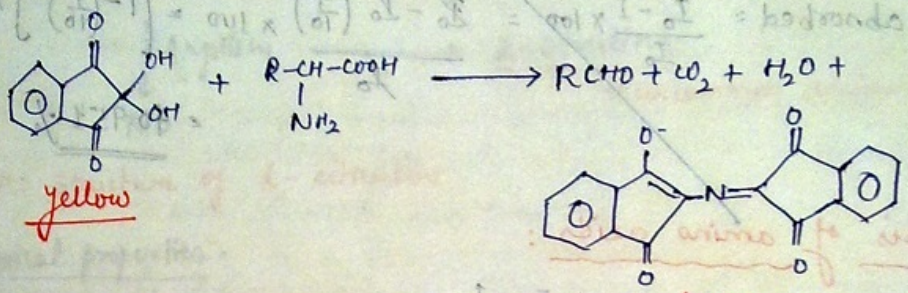
Solution I :- $SnCl_2.2H_2O$ dissolved in 0.2 M Citrate buffer, pH = 5.0

Solution II : Ninhydrin dissolved in 2-methoxy ethanol.

} 1:2 ratio → Ninhydrin reagent

Ninhydrin reagent → yellow in colour.

When it reacts with α-amino acid, purple colour complex is formed and the amino acid is converted into corresponding aldehyde.



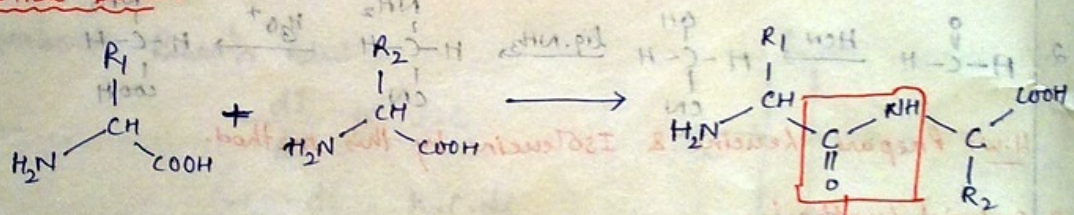
Yellow

$\lambda_{max} \doteq 570$

purple

To take the standard curve, we take a simple amino acid like glycine or alanine so that we avoid steric hindrance coming from larger amino acid.

- Peptide Bond :



peptide linkage.

If the number of amino acids linked = 2 → dipeptide

If the number of amino acids linked <= 100 → polypeptide

If the number of amino acids linked > 100 → PROTEIN.

PROTEINS

- Proteins : i) Simple – only amino acids linked together.

ii) Complex – Protein combines with some groups

iii) Derived – Synthesised protein.

- Proteins
  - Simple
    - Globular — e.g. BSA
    - Fibrous — e.g. Collagen
  - Complex — e.g. Lipoprotein
  - Derived

Bovine serum Albumin

- A process called Post Translational Modification :- i) Phosphorylation converts simple proteins to complex proteins. ii) Glycation iii) Deamidation.

H.W: What happens in these three processes.

- Based on the structure and function of proteins, they can be divided as

i) Structural protein :- Only have structure – No function in the body.
e.g. γ-crystalline present in eye lens

ii) Muscular protein :- Help in the development of muscles
e.g. Myosin

iii) Hormonal protein :- They are the hormones
e.g. Insulin

iv) Catalytic protein/ Functional protein :- May/may not have structure.
e.g. All enzymes are functional proteins.

The enzymes cleaving the proteins are called Protease.

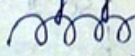
e.g. i) Pepsin – cleaves b/w hydrophobic amino acids & phenyl alanine, tryptophan and Tyrosine.
ii) Trypsin – cleaves only Lysine and Arginine linkage in the body
iii) Chymotrypsin
iv) Carboxy peptidase
v) Amino peptidase

H.W: Where are these enzymes present in the body and what linkage do they cleave.

- Papaya has large amount of pepsin in it.

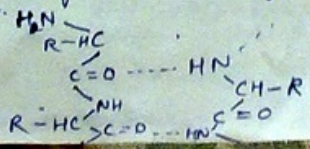
Structure of protein:

i) Primary :- Simple amino acid chain.

ii) Secondary :- Folding and coiling of primary structure.
i) α-Helix →  – each turns contains ≈ 3.6 amino acid
distance b/w 2 amino acids = 1.5 Å
ii) β-Sheet Coiling is stabilised by Hydrogen bonding.
iii) Random coil

- The molecular wt. of protein is expressed in Dalton (D)
Then 100 kD protein means if we need to prepare 1M solution then we have to add 100×10^3 g of the protein in 1000 ml of solvent.
- The average molecular wt. of protein = 110 D.

β-sheet: Two layers linked together by H-bonding
The distance b/w two molecules ≈ 3.5 Å



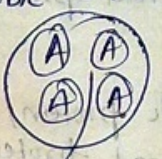
e.g. Myoglobin is pure α-helical protein. (95% α-helix, 5% - β-sheet)
Collagen is pure β-sheet protein
α-Casin (milk protein) is pure random coil protein.

- In α-helix structure, the coil form has less sterically hindered amino acid.

Tertiary structure :- Three dimensional structure of the protein.

Quaternary Structure :- If the protein is oligomeric protein then this structure comes into picture.

Many proteins (same) linked by Hydrophobic interaction.



A ≡ protein.

Hydrophobic interaction

Estimation of protein concentration :- (Standard curve taken by BSA)

i) Bradford method

→ More sensitive method.
Applied when conc. of protein < 10 μg/ml

→ Protein should have arginine, lysine and/or histidine

ii) Lowry method

→ Less sensitive.
Applied when conc. of protein > 10 μg/ml

→ Protein should have tryptophan, tyrosin and/or phenyl alanine

* Many proteins do not have tryptophan, tyrosin or phenyl alaine because they are suseptible to photolytic cleavage. But all proteins contain arginine, lysine or histidine. So bradford is widely used method.

Reagents:

Comasie G250 dissolved in methanol + 85% H_3PO_4

- Brandford reagent → Red wine colour

BSA + → Blue colour

λ_{max} = 595 nm

Reagents:

Sol^n I :- Alkaline sodium carbonate solution

Sol^n II : $CuSO_4$ solution + Sod. pot. tartarate sol^n ($CuSO_4 \cdot 5H_2O$)

Sol^n II : Sol^n I = 1 : 50 → Solution III

BSA + Sol^n III → (Incubate at Room temp. for 5-10 min) → *

Sol^n IV : Sodium Tungsten molybdate + phosphate

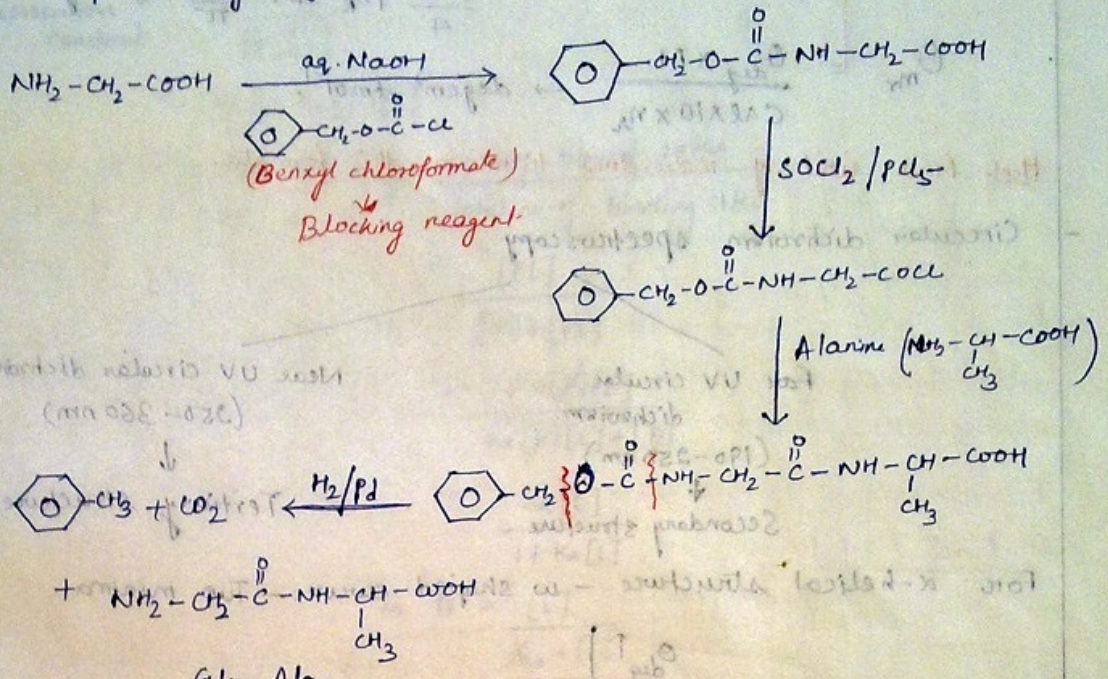
* + Sol^n IV → (Incubate at R.T for 10-30 min)

13/10/11

Preparation of Peptides :-

Preparation from n-terminal – C terminal.
This is done in solid phase.

e.g. Preparation of Gly-Ala.



Gly-Ala

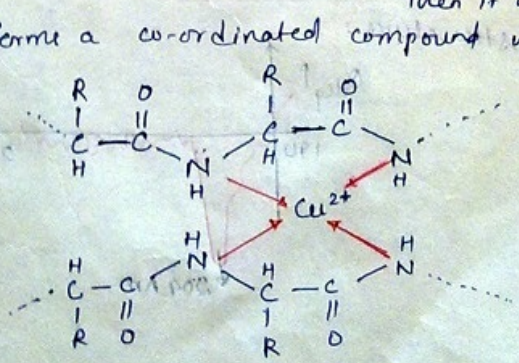
Blocking is required to produce the required peptide. Otherwise, the other amino acid might react at $-NH_2$ group of first amino acid.

→ How to identify b/w glycine and polyglycine ?

Biuret test :-

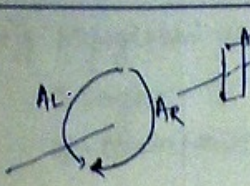
1 ml of glycine sol^n + 1 ml of 1% NaOH
↓ Wait for 2-5 minutes
Add few drops of $CuSO_4$ sol^n
↳ Colour changes to purple/red
then it is polyglycine

Because Cu^{2+} forms a co-ordinated compound with the nitrogen lone pair.



→ Differentiating b/w diff. secondary structure :-

α-helix, β-sheet and random coil structures can be differentiated by circular dichroism spectroscopy.



$|A_R - A_L|$

Ellipticity, $\theta = \frac{2.303}{4} |A_R - A_L| \rightarrow$ radian

$= \frac{2.303}{4} |A_R - A_L| \times \frac{180}{\pi} \rightarrow$ deg.

$$\theta_{mr} = \frac{\theta_{deg} \times M}{C \times l \times 10 \times n_r} \rightarrow deg\, cm^2\, dmol^{-1}.$$

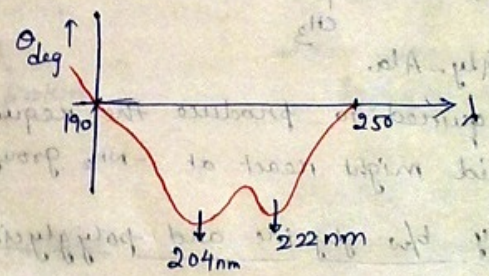
H.W: Find unit of individual terms in this formula.

- Circular dichroism spectroscopy

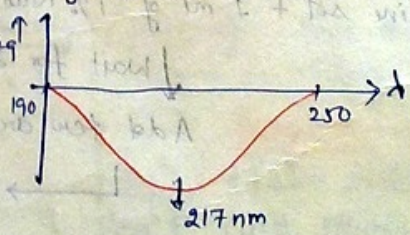
Far UV circular dichroism (190–250 nm) → Secondary structure

Near UV circular dichroism (250–360 nm) → Tertiary structure

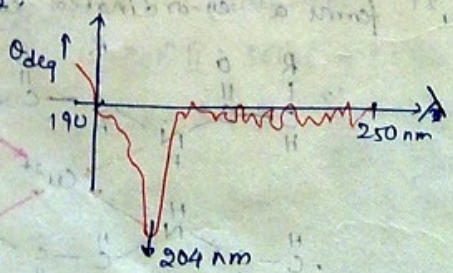
For α-helical structure – w shaped curve: – Two minima



For β-sheet structure – Only one minima



For random-coil structure:



- H.W: i) ANS
  ii) bi-ANS → What is name, What is structure
  iii) TNS → At what λ, the absorption is maximum. (λ^{ex}_{max})
  What is emission zone

Binding of ligands with the macromolecules:

Protein + ligand

$P + L \rightleftharpoons PL$

association constant $K_a = \frac{[PL]}{[P][L]}$

$\Rightarrow [PL] = K_a[P][L]$

if θ = fraction binding = $\frac{\text{no. of bound sites}}{\text{Total no. of binding sites}}$

$$= \frac{[PL]}{[P]+[PL]}$$

$$= \frac{K_a[P][L]}{K_a[P][L]+[P]}$$

$$= \frac{K_a[L]}{1+K_a[L]}$$

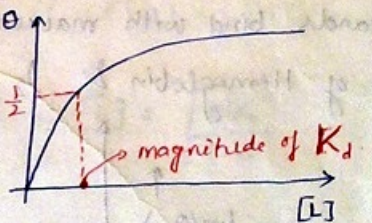
$$\Rightarrow \theta = \frac{[L]}{1/K_a + [L]}$$

$$\Rightarrow \boxed{\theta = \frac{[L]}{K_d+[L]}}$$

K_d = dissociation const.

When $\theta = \frac{1}{2} \Rightarrow \boxed{K_d = [L]}$

$\Rightarrow$



This is one-one binding as one ligand is binding to protein.

→ If n-ligands bind to the protein molecule then:

$$P + nL \rightleftharpoons PL_n$$

$$\Rightarrow K_a = \frac{[PL_n]}{[P][L]^n} \quad \Rightarrow \theta = \frac{[PL_n]}{[P]+[PL_n]}$$

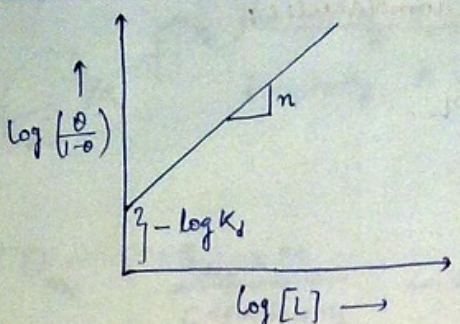
$$= \frac{K_a[P][L]^n}{[P]+K_a[P][L]^n}$$

$$\Rightarrow \boxed{\theta = \frac{[L]^n}{K_d+[L]^n}}$$

$$\Rightarrow 1-\theta = \frac{K_d}{K_d+[L]^n} \Rightarrow \frac{\theta}{1-\theta} = \frac{[L]^n}{K_d} \Rightarrow \boxed{\log\left(\frac{\theta}{1-\theta}\right) = n\log[L] - \log K_d}$$

n → Hill coefficient

Hill equation



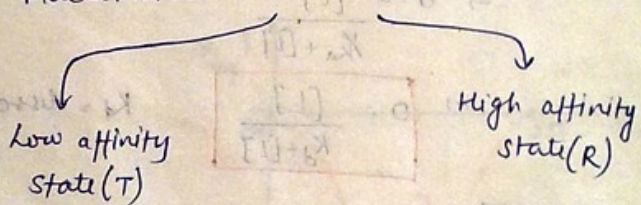
When $n=1$: non-cooperative binding
$n>1$: +ve cooperative binding
$n<1$: -ve cooperative binding

- When binding of a ligand to a ~~two~~ protein doesn't change the affinity of other binding sites to bind with ligands → non-cooperative binding.
- When binding of a ligand to a protein increases the affinity of other binding sites to bind with ligands → +ve cooperative binding.
- When binding of a ligand to a protein decreases the affinity of other binding sites to bind with ligands → -ve cooperative binding.

Two models are used to explain this :

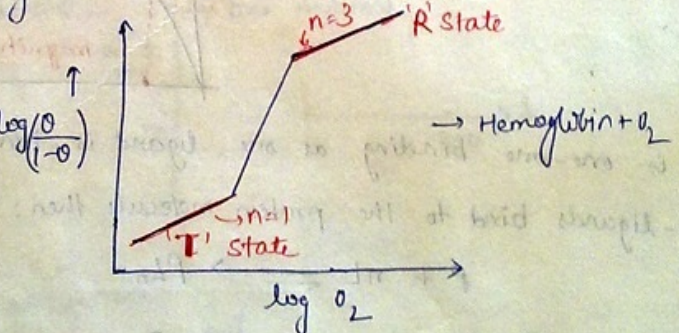
(i) MWC model :-

Macromolecules have two states



→ When ligands bind with macromolecules they go from 'T' state to 'R' state

eg. Binding of Hemoglobin & O_2.



→ But this model can't explain -ve cooperative binding, so the 2nd model came :

(ii) KNF model :-

→ KNF model is based on conformation change of the micro-environment of the protein.

→ When one ligand binds to the protein

a) Some of the binding sites from the core come to the surface and their conformation changes in such a way that, the affinity for ligand binding increases
⇒ positive binding cooperativity.

b) or some of the binding sites go from the surface to the core and the conformation changes in such a way that the affinity for binding sites to bind with ligands decreases.

⇒ negative binding cooperativity.

c) or the protein conformational change is not there

⇒ non-cooperative binding.

Q.1 If a protein binds to a ligand in 1:1 ratio and $K_d = 1\,\mu M$. Find the ligand concentration is (i) 0.2 (ii) 0.5 (iii) 0.8

Q.2 Calculate the same if the binding is 1:2 ratio.

Ans.1: $\theta = \frac{[L]}{K_d + [L]} \Rightarrow \theta K_d + \theta[L] = [L]$

$$\Rightarrow [L] = \frac{\theta K_d}{1-\theta}$$

(i) $\theta = 0.2 \Rightarrow [L] = \frac{0.2 \times 1}{0.8} = \frac{2}{8}\,\mu M = 0.25$

$$\Rightarrow [L] = 0.25\,\mu M //$$

(ii) $\theta = 0.5 \Rightarrow [L] = 1\,\mu M //$

(iii) $\theta = 0.8 \Rightarrow [L] = \frac{0.8}{0.2} \times 1$

$$\Rightarrow [L] = 4\,\mu M //$$

Ans.2: $\theta = \frac{[L]^2}{K_d + [L]^2} \Rightarrow [L]^2 = \frac{\theta K_d}{1-\theta} \Rightarrow [L] = \sqrt{\frac{\theta K_d}{1-\theta}}$

(i) $\theta = 0.2 \Rightarrow [L] = 0.5\,mM //$

(ii) $\theta = 0.5 \Rightarrow [L] = 1\,mM //$

(iii) $\theta = 0.8 \Rightarrow [L] = 2\,mM //$

Enzyme Kinetics:-

→ some enzymes act as catalyst and they do not participate in the reaction but in affect the rate of reaction.

→ In some reactions, enzymes participate in the reaction.

* In all reactions — enzyme conc. is very small compared to substrate concentration.

→ Enzyme + Substrate $\underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}}$ ES (Fast step)

	E	+	S		ES
t=0:	E_0		S_0		

$ES \xrightarrow{K_2} E + P$ (slow step)

Steady state approximation – The net accumulation of intermediate = 0

i.e. rate of formation of intermediate = rate of decomposition of intermediate

→ Here the intermediate is ES.

⇒ Rate of formation of $ES = K_1[E][S]$

And rate of decomposition of $ES = K_{-1}[ES] + K_2[ES]$

⇒ Acc. to steady state approx.

$$K_{-1}[ES] + K_2[ES] = K_1[E][S] \longrightarrow (1)$$

- $E + S \rightleftharpoons ES$

$t=0$:	E_0	S_0	0	$\longrightarrow [E_0] \ll [S_0] \Rightarrow [S] \simeq S_0$
$t=t$	$E_0 - ES$	S	ES	$\Rightarrow [E] = [E_0] - [ES]$

⇒ Equation (1) becomes:

$$K_{-1}[ES] + K_2[ES] = K_1\{[E_0] - [ES]\}[S_0]$$

$$= K_1[E_0][S_0] - K_1[ES][S_0]$$

$$\Rightarrow [ES]\{K_1[S_0] + K_2 + K_{-1}\} = K_1[E_0][S_0]$$

$$\Rightarrow [ES] = \frac{K_1[E_0][S_0]}{K_1[S_0] + K_2 + K_{-1}}$$

$$\Rightarrow [ES] = \frac{[E_0][S_0]}{[S_0] + \frac{K_2 + K_{-1}}{K_1}}$$

$$\Rightarrow \boxed{[ES] = \frac{[E_0][S_0]}{[S_0] + K_M}} \longrightarrow (2)$$

where $K_M = \frac{K_2 + K_{-1}}{K_1}$ → Michelis menten const.

⇒ Rate of the reaction, $r = K_2[ES]$

$$= \frac{K_2[E_0][S_0]}{[S_0] + K_m} \longrightarrow (3)$$

Case-1: When $[S_0] \gg K_m$

$$\Rightarrow r = \frac{K_2[E_0][S_0]}{[S_0]} \Rightarrow \boxed{r = K_2[E_0]} \rightarrow r_{max}$$

→ Pseudo first order reaction

Case-2 : When $K_m \gg [S_0]$

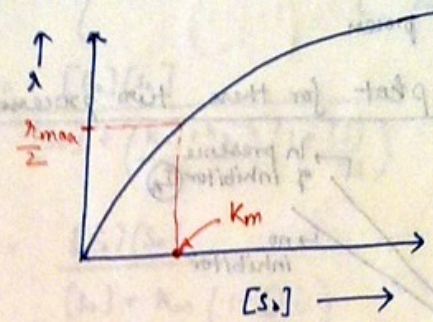
$$\Rightarrow r = \frac{K_2[E_0][S_0]}{K_m}$$

$$\Rightarrow \boxed{r = K'[E_0][S_0]} \rightarrow \text{Second order reaction; where } K' = \frac{K_2}{K_m}$$

$\Rightarrow$ Equation (3) becomes:

$$\boxed{r = \frac{r_{max}[S_0]}{[S_0] + K_m}}$$

$\Rightarrow$



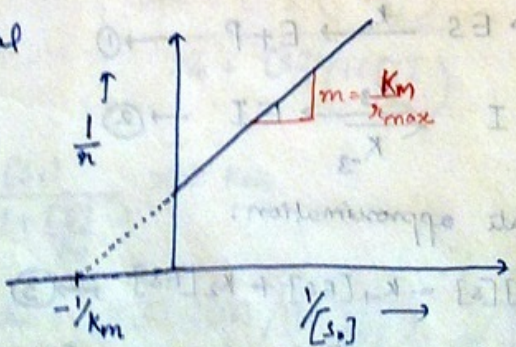
When $r = \frac{r_{max}}{2} \Rightarrow K_m = [S_0]$.

So when rate = $\frac{r_{max}}{2}$, the magnitude of K_m = initial substrate conc.

Taking the reciprocal of r

$$\Rightarrow \frac{1}{r} = \frac{[S_0] + K_m}{r_{max}[S_0]} \Rightarrow \boxed{\frac{1}{r} = \frac{1}{r_{max}} + \frac{K_m}{r_{max}} \cdot \frac{1}{[S_0]}}$$

Double reciprocal



when $\frac{1}{r} = 0$

$$\Rightarrow \frac{-1}{r_{max}} = \frac{K_m}{r_{max}} \cdot \frac{1}{[S_0]}$$

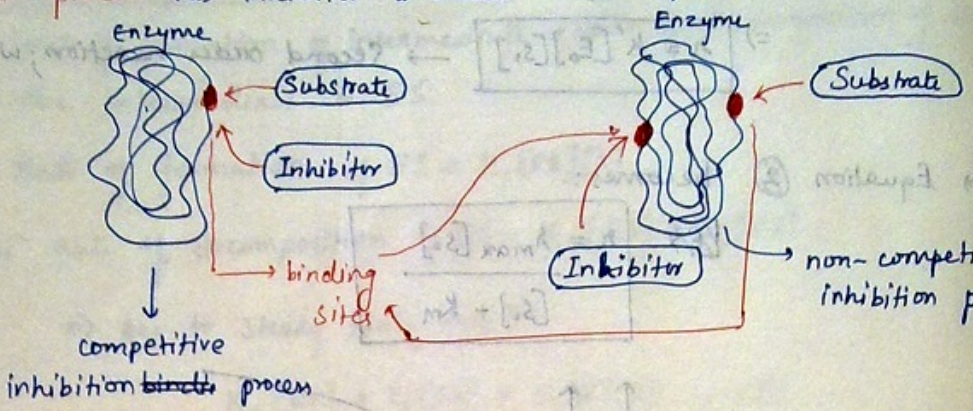
$$\Rightarrow \frac{1}{[S_0]} = \frac{-1}{K_m}$$

This plot is called double reciprocal plot. It is very imp. to determine the presence of inhibitor.

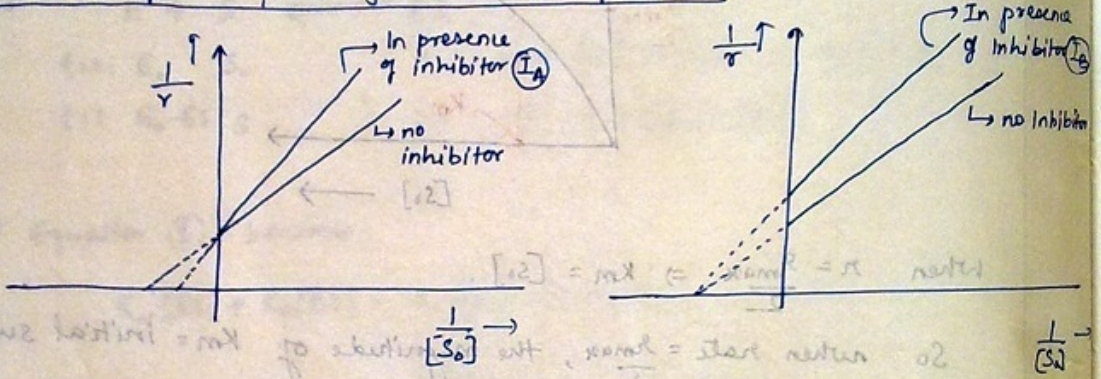
Inhibitor:

If inhibitor binding sites is equivalent to the substrate binding site then there is competition b/w substrate and inhibitor to bind with the enzyme. Then this is called competitive inhibition process. This inhibitor is called competitive inhibitor.

- Some inhibitors bind in diff. binding sites of the enzyme than that of the the substrate. Hence there is no competition. This is called non-competitive inhibition process. This inhibitor is called non-competitive inhibitor.



Double-reciprocal plot for these two processes:



$\Rightarrow$ I_A = competitive inhibitor

I_B = non-competitive inhibitor

Rate expression for competitive inhibition process:

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

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI \quad \rightarrow (2)$$

Applying steady state approximation:

$$k_1[E][S] = k_{-1}[ES] + k_2[ES] \quad \rightarrow (3)$$

At $t = 0$: $[E] = [E_0]$; $[S_0] = [S]$ and $[S_0] >>> [E_0]$

At $t = $ ~~∞~~: $[S] \simeq [S_0]$; $[E] = [E]$

$$= [E_0] - [ES] - [EI]$$

For the reaction (2): $K_d = \frac{k_3}{k_{13}} = \frac{[E][I]}{[EI]}$ (or) $[EI] = \frac{[E][I]}{K_d}$

$$\Rightarrow [E] = [E_0] - [ES] - \frac{[E][I]}{K_1}$$

⇒ Equation (2)

$$\Rightarrow [E]\left(1+\frac{[I]}{K_d}\right) = [E_0]-[ES]$$

⇒ Equation (3)

$$\Rightarrow \frac{K_1([E_0]-[ES])[S_0]}{\left(1+\frac{[I]}{K_d}\right)} = K_{-1}[ES]+K_2[ES]$$

$$\Rightarrow K_1[E_0][S_0]-K_1[S_0][ES] = (K_{-1}+K_2)\left(1+\frac{[I]}{K_d}\right)[ES]$$

$$\Rightarrow K_1[E_0][S_0] = \left\{K_1[S_0]+(K_{-1}+K_2)\left[1+\frac{[I]}{K_d}\right]\right\}[ES]$$

$$\Rightarrow [ES] = \frac{[E_0][S_0]}{[S_0]+\left(\frac{K_{-1}+K_2}{K_1}\right)\left(1+\frac{[I]}{K_d}\right)}$$

$$\Rightarrow [ES] = \frac{[E_0][S_0]}{[S_0]+K_m\left(1+\frac{[I]}{K_d}\right)}$$

Rate of the reaction,

$$r = K_2[ES]$$

$$= \frac{K_2[E_0][S_0]}{[S_0]+K_m\left(1+\frac{[I]}{K_d}\right)}$$

$$\Rightarrow r = \frac{K_2[E_0][S_0]\Big/\left(1+\frac{[I]}{K_d}\right)}{K_m+[S_0]\Big/\left(1+\frac{[I]}{K_d}\right)}$$

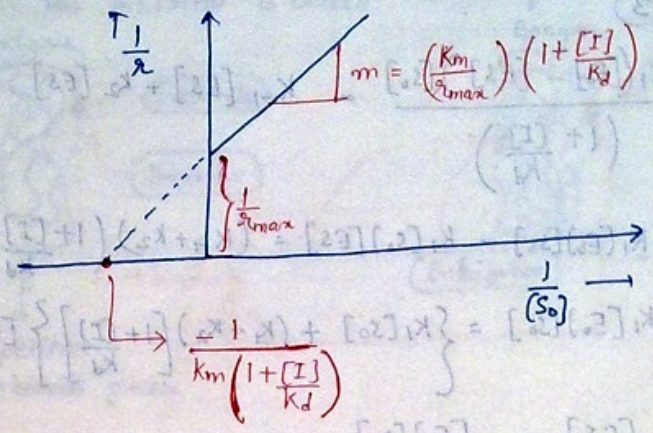
when $\frac{[S_0]}{1+\frac{[I]}{K_d}} >>> K_m$

$$\Rightarrow r_1 = \frac{K_2[E_0][S_0]\Big/\cancel{\left(1+\frac{[I]}{K_d}\right)}}{[S_0]\Big/\cancel{\left(1+\frac{[I]}{K_d}\right)}} \Rightarrow \boxed{r_{max} = K_2[E_0]}$$

⇒ rate, $r = \frac{r_{max}[S_0]}{[S_0]+K_m\left(1+\frac{[I]}{K_d}\right)}$

$$\Rightarrow \frac{1}{r} = \frac{[S_0]+K_m\left(1+\frac{[I]}{K_d}\right)}{r_{max}[S_0]}$$

$\Rightarrow \frac{1}{v} = \frac{1}{v_{max}} + \left(\frac{K_m}{v_{max}}\right)\left(\frac{1}{[S_0]}\right)\left(1+\frac{[I]}{K_d}\right)$ → Double reciprocal plot in presence of competitive inhibitor.



3/11/17

Antigens and Antibodies

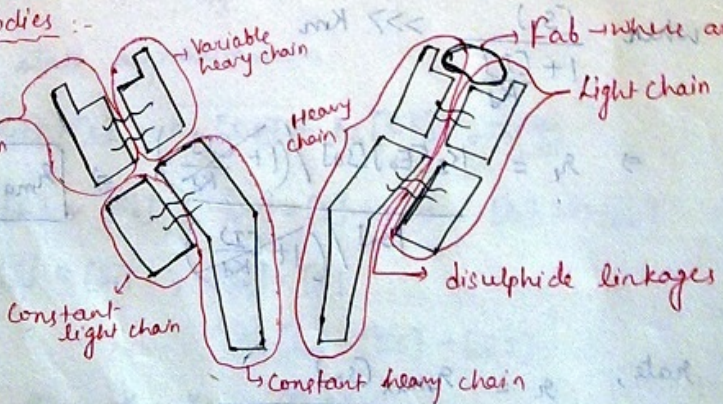
- Antibodies are also called – Immunoglobulin
  - Antibodies are globular proteins.
  - Antibodies trap the specific antigens.

→ Antigens – can be macromolecules or small molecules. These are foreign molecules. They enter the body and trigger the immune system.

→ Antigens are also called Immunogen / Immunogenic molecules.

- We divide the antigens according to their molecular weights:
  a) Low risk low immunogenic (Mol.wt < 10 KDa)
  b) Moderate risk moderate immunogenic (Mol.wt > 10 KDa & Mol.wt < 100 KDa)
  c) High risk potent immunogenic (Mol.wt > 100 KDa)

Structure of Antibodies :-



Avg. mol. weight of antibodies is 150 kDa.

→ There are 5 types of heavy chains: a) α d) γ
b) δ
c) ∈ e) μ

- Common antibodies present in the bodies: IgA, IgD, IgE, IgG and IgM.
  → Igm is Multimeric antibodies.
  → IgA has α heavy chain.
  IgD " δ " "
  IgE " ϵ " "
  IgG " γ " "
  IgM " μ " "
  → Light chain can be divided in two types: i) K (70%)
  ii) λ (30%).