LECTURE 14

Enzymes

What are catalyst?

- Participate in reaction
- Not consumed or any permanent change
- Required in very small quantities.
- Lowers the activation energy
- Enhance the rate of reactions
- Do not change the equilibrium.
- Do not influence free energy change.

$K_{eq} = \frac{(P)}{(S)}$

$\Delta G^{\circ} = -RT \ln K_{eq}$

Enzymes/Biocatalyst:

99 % < 1 %

- Proteins or RNA — Organic
- Substrate specific, stereo-specific
- Enhance rate of reaction by forming weak interactions with substrates, Reversible
- Work at optimum pH and temperature ✓

Active site of enzyme

The active site is a 3-D cleft formed by R-groups of amino acids that come from different parts of the amino acid sequence.

The active site takes up a relatively small part of the total volume of an enzyme.

~ minimum 100 aa polypeptide or more

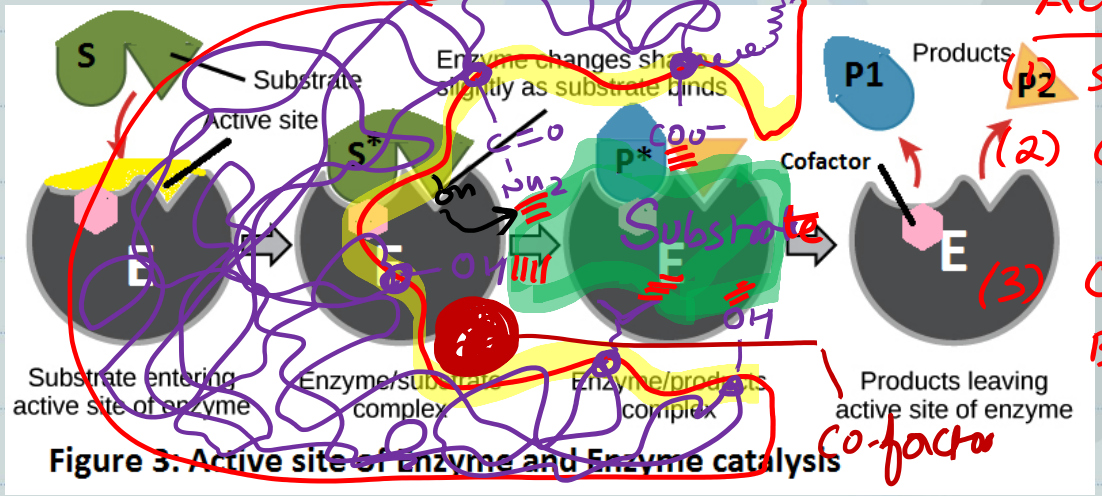


Figure 3: Active site of Enzyme and Enzyme catalysis

Co-factor

Active site
(1) substrate binding site
(2) catalytic site
S ~ S*
Co-factor
Binding site

Enzyme Specificity

→ Non-covalent Interactions between enzyme and substrate

• Specific

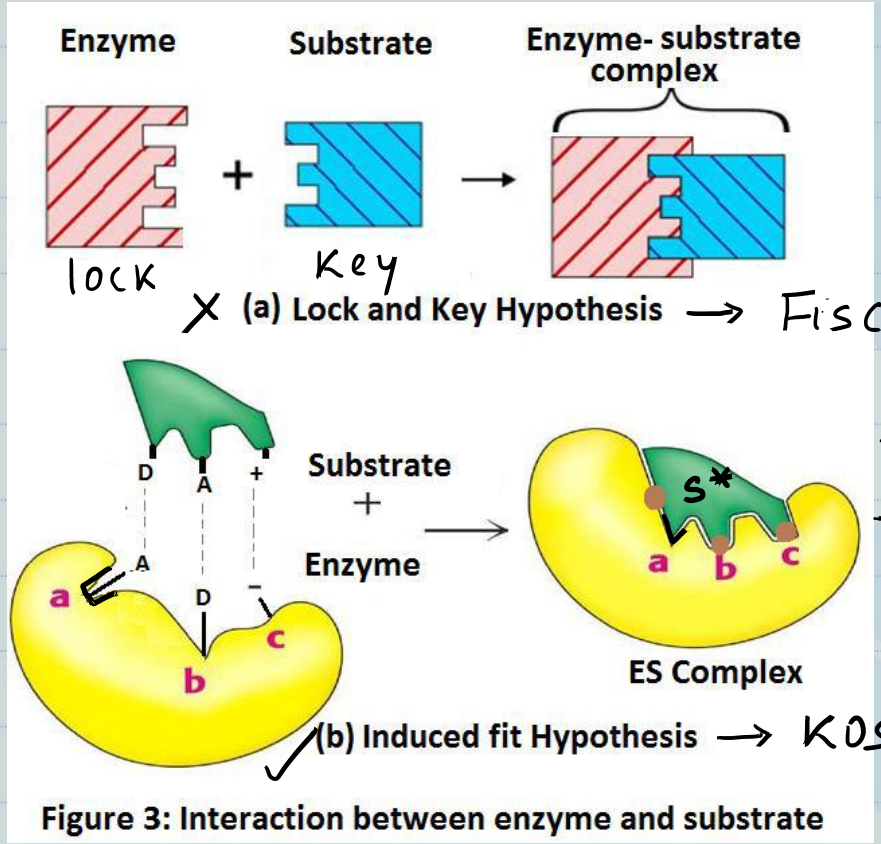


Figure 3: Interaction between enzyme and substrate

→ Enzyme & substrate must have structural compatibility

X → Fischer

→ Molecular compatibility

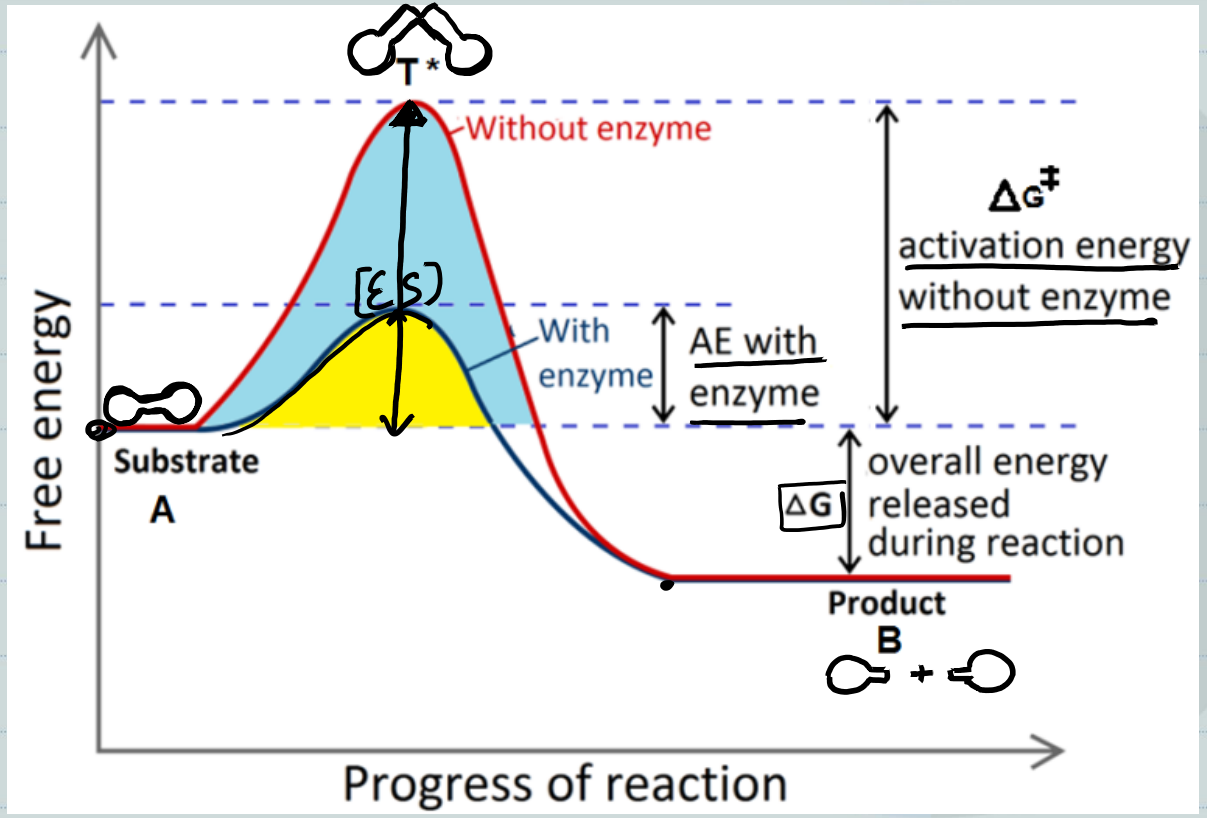
→ Non-covalent interaction

↓

Specific

✓ → Koshland

Free energy of activation : Energy required to attain transition state





- Enzyme lower A.E
- Provide alternative pathway

ΔG = -ve

Enzyme leads to

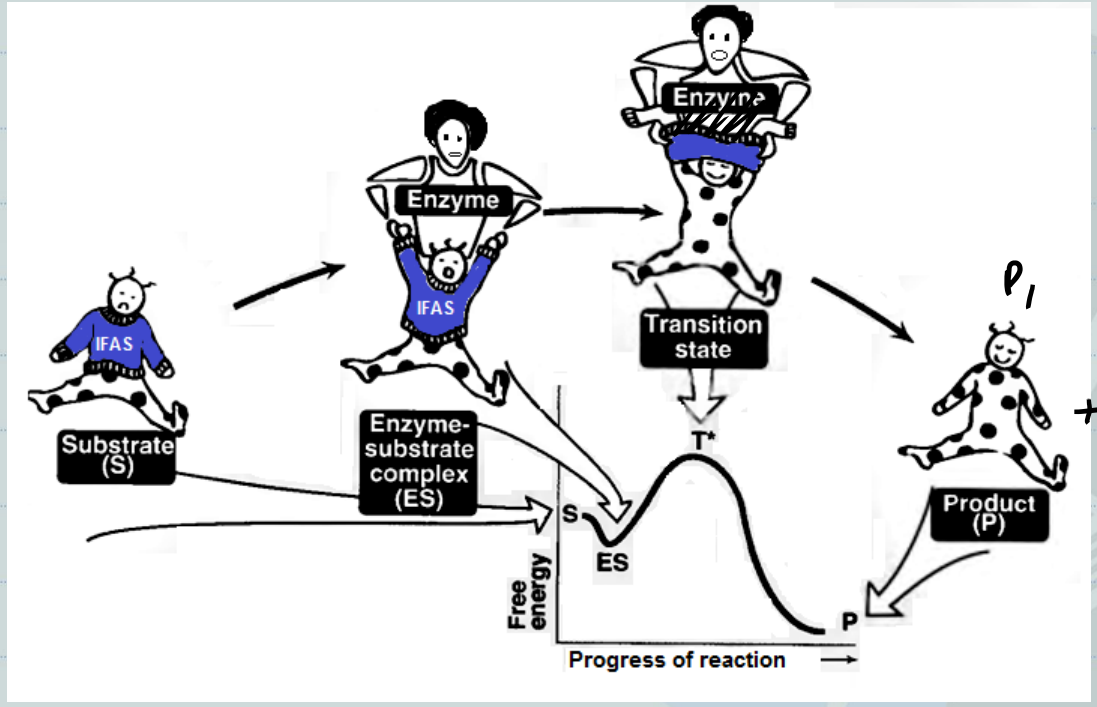
Transition-state stabilization: of substrate

multiple non-covalent interaction

↑ Binding energy = ↑

Activation energy = ↓

Rate of Rxn = ↑



P_1

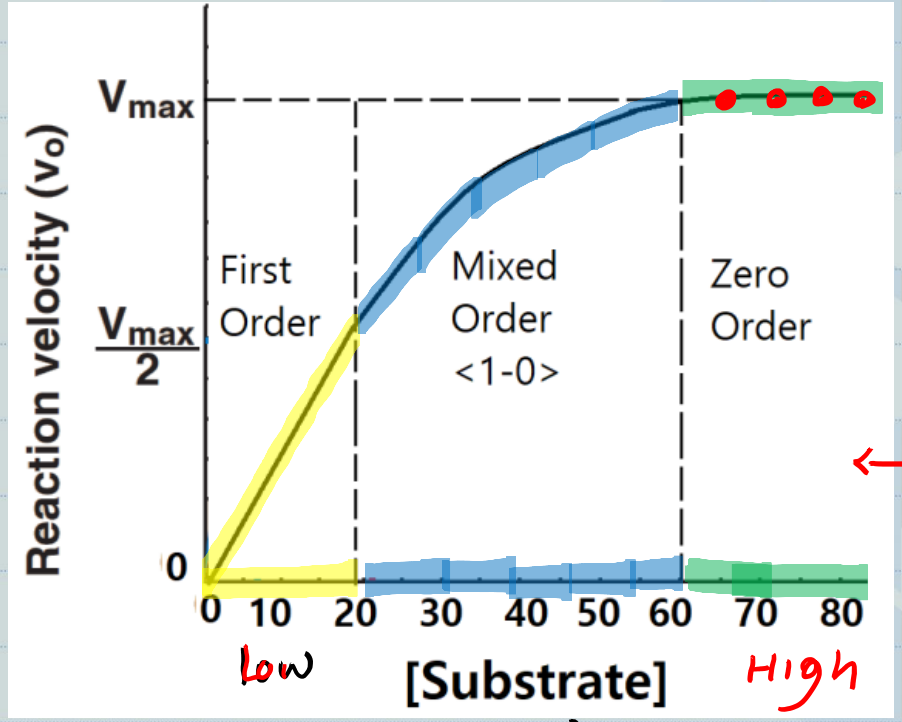
P_2

+

Binding energy due to multiple weak interactions with substrate, decreases activation energy required to achieve transition state

FACTORS AFFECTING REACTION VELOCITY

A. Substrate concentration



Low [S] = First order

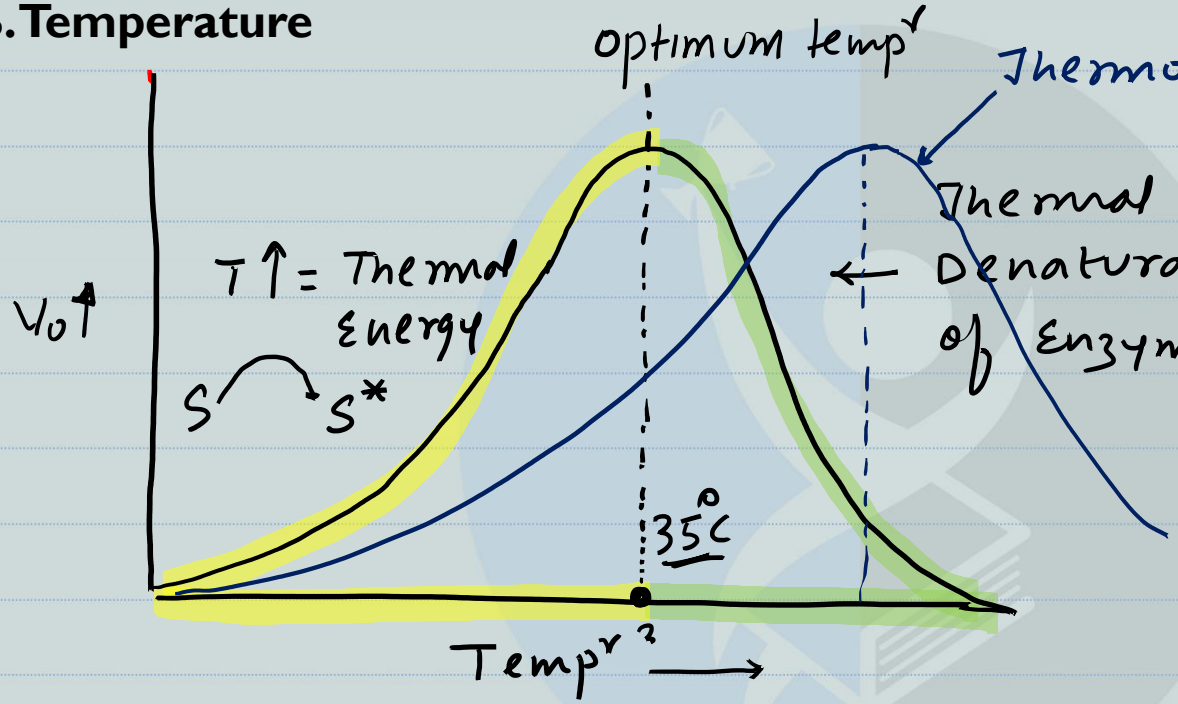
High [S] = Zero Order

Intermediate <1 — 0>

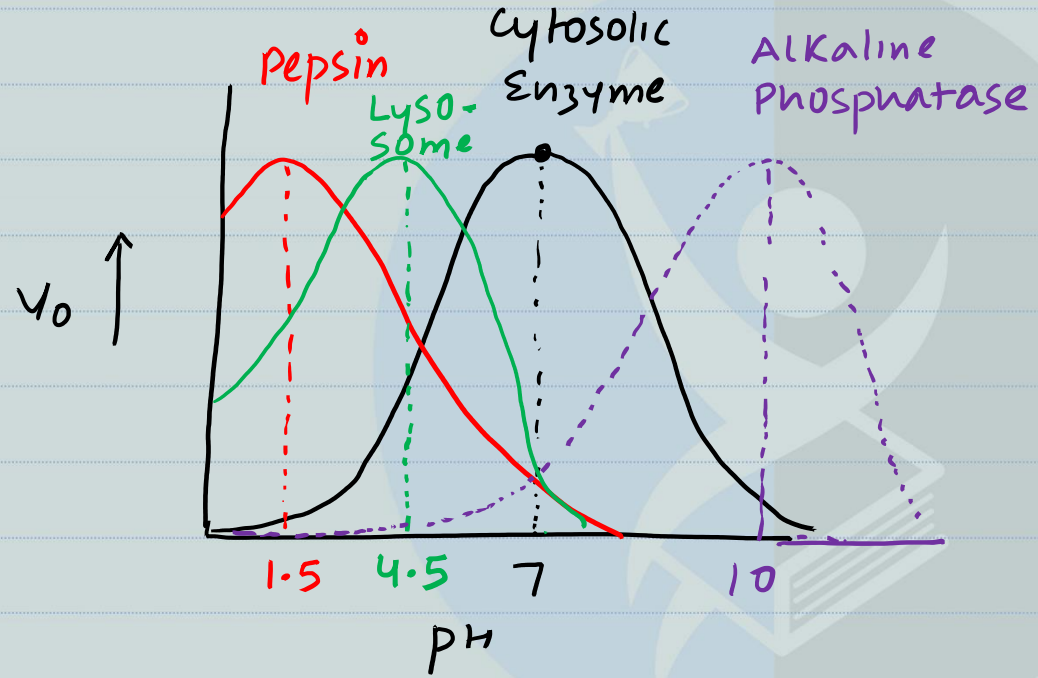
- Mixed order

← Rectangular Hyperbolic Curve

B. Temperature



C. pH : Enzymes work at optimum pH.



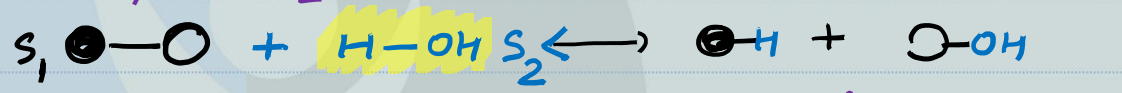
Enzyme Classes: International Union of Biochemistry and Molecular Biology (IUMB)

1 Class : Oxido-reductase $S_1 \xrightarrow{e^-} S_2$ eg Dehydrogenase, Oxidase

Transfer of electron bet^n substrates

2 Class : Transferase  eg Kinase (Phosphate)

Transfer some group from S_1 to S_2.

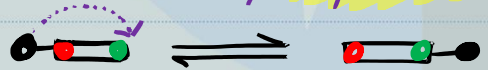
3 Class : Hydrolase 

Use water to break bond, water is release during formation of bond eg. Digestive Enzyme, Polymerase, Glycogen Synthase

4 Class : Lyase  eg A

NO use of water for bond break, no release of water during bond formation

eg - Aldolase, fumarase, Synthase, RUBISCO

5. Isomerase : 

convert one isomeric form to another (eg) Isomerase, mutase Epimerase, Racemase

6. Ligase : $A + B \xrightarrow[ATP \quad ADP]{} A-B$

Form bond with investment of Energy (ATP, GTP ....)

eg Synthetase, ligase

1	2	3	4	5	6
O	T	H	L	I	L

EC Number

Alcohol dehydrogenase 1.1.1.1

order of discovery

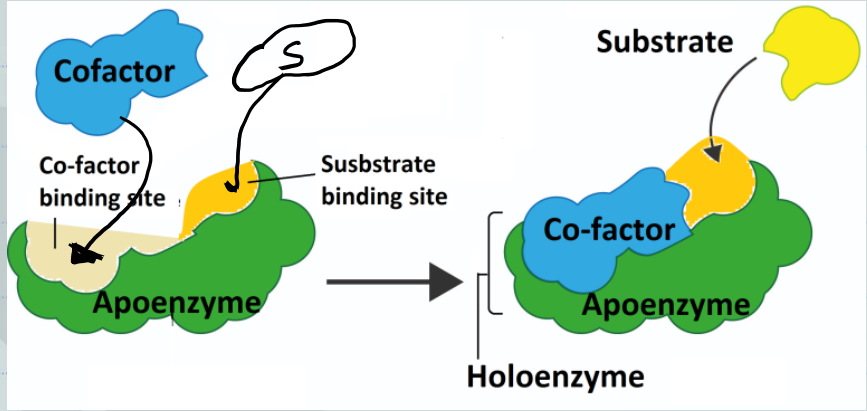
1 → Class

1 → Sub-class

1 → Sub-sub class

Cofactor

- **Inorganic**
  - Metals
  - mg^{2+}, Ni^{2+}
- **Organic**
  - Co-enzymes
    - Weak binding (Co-Enzyme)
    - Strong binding Prosthetic group



Holo-Enzyme (Active) = Apo-Enzyme (Inactive) + Co-Enzyme (co-factor)

Inorganic Co-factors ⟶ metal ions

⟶ most of Divalent cations

Mg^{2+} : Kinase, Nuclease, Polymerase

Fe^{2+} : Cytochrome oxidase, Aconitase, Prolyl hydroxylase

Zn^{2+} : Carbonic anhydrase, Alcohol dehydrogenese

Cu^{2+} : Lysyl oxidase, Tyrosinase, cyt c oxidase

Ni^{2+} : Urease

Se^{2+} : Glutathione Peroxidase

Mn^{2+} : Super oxide dismutase, water splitting enzyme (PS II)

Mo^{2+} : Nitrogenase, Nitrate reductase

K^{+} : Pyruvate Kinase

Co-enzyme: carriers of specific chemical groups

↳ organic, derived from vitamins (B, C)
water soluble

Co-Enzyme Q
vitamin K
(fat soluble)

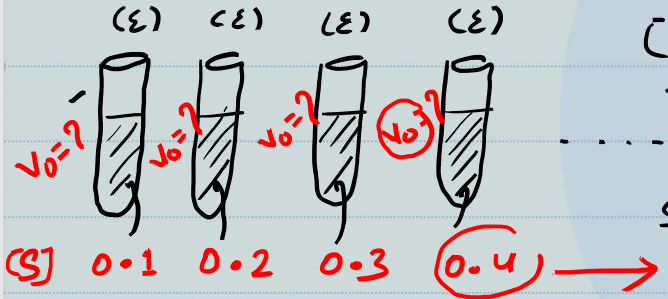
- Thiamine Pyrophosphate (TPP): Aldehyde group transfer or C-C cleavage or formation reaction
- FAD, FMN: electron transfer reaction in form of H-atom.
- NAD, NADP: electron transfer (oxidation-Reduction) → Hydride ion $H^{\ddot{}}$
- Pyridoxal Phosphate (PLP): Amino group transfer
- Tetra hydrofolate (THF): 1 carbon transfer ox^n (formyl, methyl)
- Cobalt amine: methyl group transfer
- S-adenosyl methionine: methyl group transfer
- Biotin: carboxylation reaction
- Co-Enzyme A: Acyl group transfer reaction.

ENZYME KINETICS — Michaelis-Menten

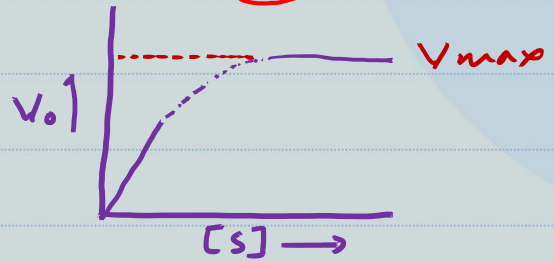
$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

- How increase in [S] effect velocity of enzyme catalyzed reaction

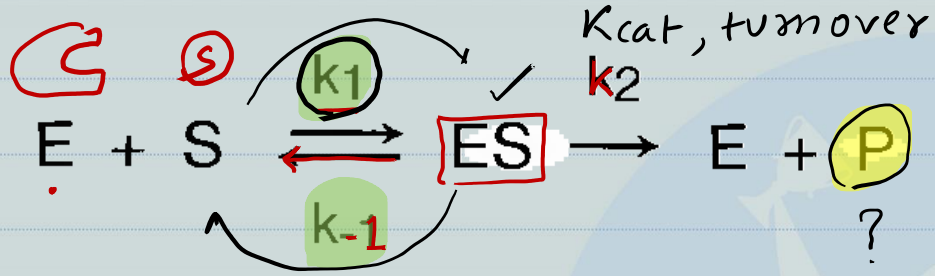
Where, Vo = initial reaction velocity (variable)
Vmax = maximal velocity (constant)
Km = Michaelis constant = $(k_{-1} + k_2)/k_1$
[S] = substrate concentration (variable)



[Enzyme] = same
Temp^r = same
pH = same
Solvent = same



Assumptions of Michaelis and Menten Equation



Kcat, turnover number

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

1. **Initial velocity** (V_o) : No product
2. **[S]>>[E]**
3. **ES dissociation is insignificant**
4. **Steady-state assumption:**
5. **Rate limiting step is K2** $K_{-1} >>> K_2$ (slow)

K_1 = Rate constant for formation of ES complex

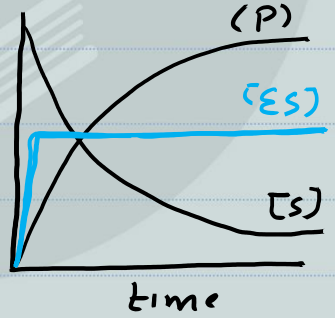
K_{-1} = Dissociation of ES to E+S

K_2 = Dissociation of ES to E+P

$K_{-1} + K_2 = K_1$

$$K_S = \frac{K_{-1}}{K_1} \quad \frac{\text{Dissociation}}{\text{Formation}}$$

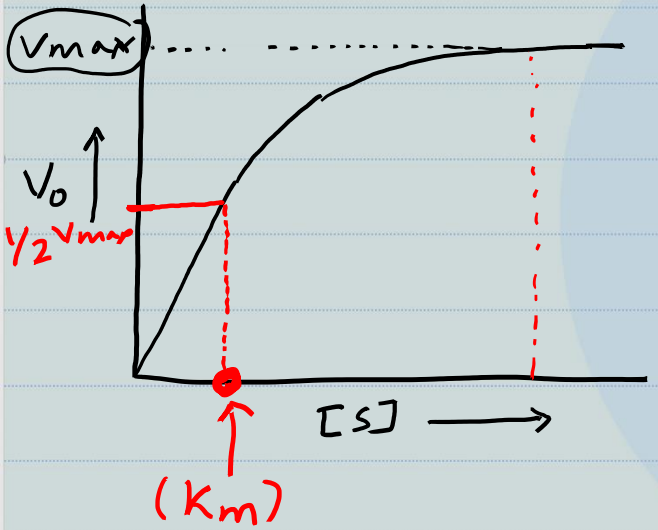
$$K_m = \frac{K_{-1} + K_2}{K_1} \quad \frac{\text{Dissociation}}{\text{formation}}$$



What is Km?

$$K_m = \frac{K_{-1} + K_2}{K_1}$$

- ✓ Dissociation constant for ES complex
- ✓ Substrate concentration where Vo=0.5 Vmax



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

$$0.5\,V_{max} = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{0.5\,\cancel{V_{max}}}{\cancel{V_{max}}} = \frac{[S]}{K_m + [S]}$$

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

$$K_m + [S] = 2[S]$$

$$K_m = 2[S] - [S]$$

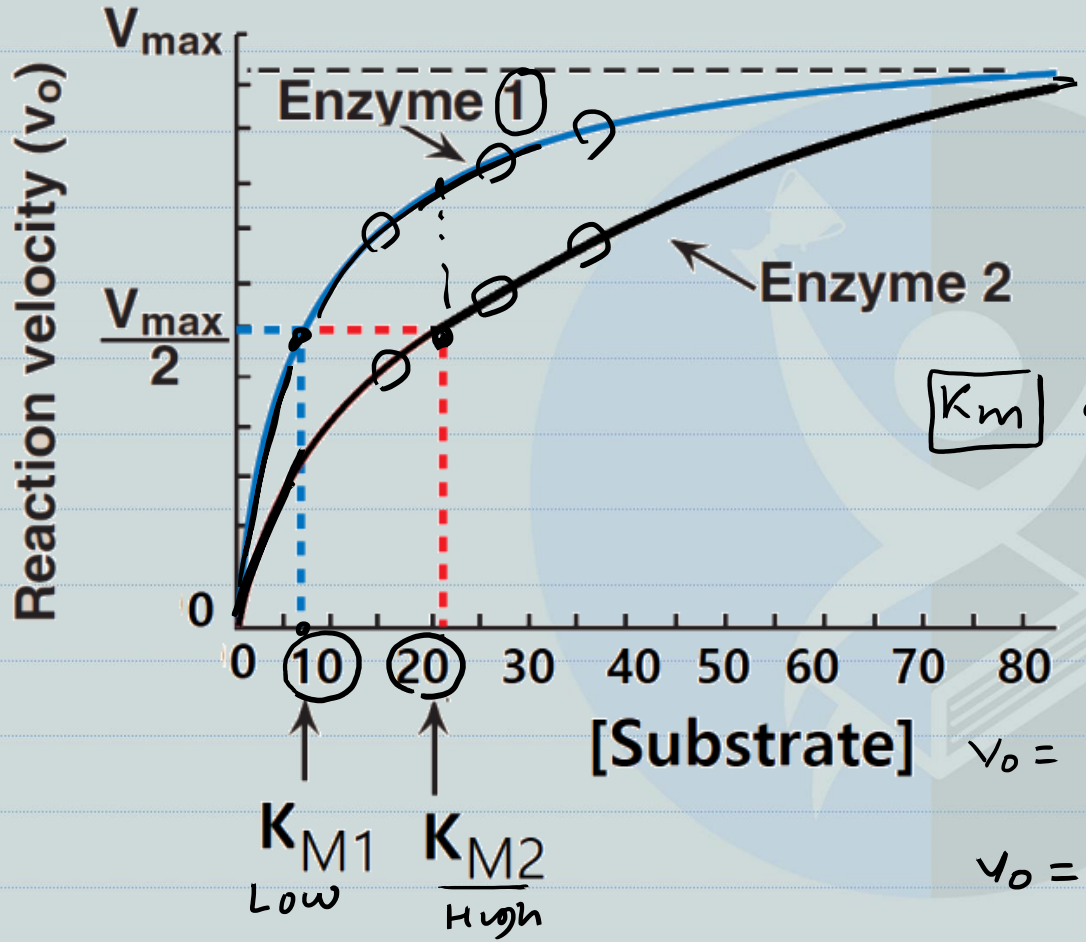
$$K_m = [S]$$

$[S] = K_m \quad V_0 = \frac{1}{2} V_{max}$

$[S] < K_m \quad V_0 < \frac{1}{2} V_{max}$

$[S] > K_m \quad V_0 > \frac{1}{2} V_{max}$

K_M does not vary with the concentration of enzyme.



- Km is constant
- donot vary with Substrate concr or Enzyme concr

$K_m \propto \frac{1}{\text{Affinity for substrate}}$

$[S] = K_m \quad V_0 = \frac{1}{2} V_{max}$

$[S] = 2K_m$

$V_0 = \frac{V_{max}(S)}{K_m + (S)}$

$V_0 = \frac{V_{max} \cdot 2K_m}{K_m + 2K_m}$

$V_0 = V_{max} \cdot \frac{2K_m}{3K_m}$

$V_0 = \frac{2}{3} V_{max}$

$= 66\% V_{max}$

$$V_0 = \frac{V_{max}\,(S)}{K_m + (S)}$$

Apply your Mind

The enzyme alkaline phosphatase was tested for its catalytic activity using the substrate para-nitrophenylphosphate. The Km obtained was 10 mM and Vmax was 100 umol/min. Which one of the following options represents the initial velocity of the reaction at a substrate concentration of 10 mM?

(1) 50 µmol/min
(2) 100 µmol/min
(3) 500 µmol/min
(4) 20 µmol/min

$K_m = 10\ mM$

$S = 10\ mM$

$K_m = [S]$

$V_0 = 1/2\ V_{max}$

$V_0 = 1/2 \times 100\ \mu mol/min$

$= 50\ \mu mol/min$

Case 1 [S] = 0.5 Km V_0 = ?

Apply your Mind

Case 2 [S] = 2 Km ✓ V_0 = ?

What is the fold difference between v at [S] = ½ Km and v at [S] = 2Km where v is the initial velocity of an enzyme catalyzed reaction, [S] is substrate concentration and Km is the Michaelis constant?

(1) 2
(2) 4
(3) 10
(4) 100

Case 1: $V_0 = V_{max}\frac{(S)}{Km+(S)} = V_{max}\frac{0.5\,Km}{1\,Km+0.5\,Km} = V_{max}\frac{0.5}{1.5} = \frac{1}{3}V_{max}$

Case 2: $V_0 = V_{max}\frac{2\,Km}{1\,Km+2\,Km} = V_{max}\frac{2}{3} = \frac{2}{3}V_{max}$

$\frac{1}{3}V_{max}$ → $\frac{2}{3}V_{max}$

0.33 V_{max} → 0.66 V_{max}

What is Kcat or turnover number?

speed of single Enzyme

$K_{cat} = V_{max}/E_T$

$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow[\text{(Slow)}]{K_2} E + P$

$\frac{V_{max}}{E_T} = K_{cat}$

$V_{max} = K_2 \cdot E_T$

Fast and slow enzymes

i) Enzyme	ii) Turnover number (Kcat)
Catalase	4.0×10^7
Carbonic anhydrase	1.0×10^6
Acetylcholine esterase	2.5×10^4
Lactate dehydrogenase	1.0×10^3
Urease	1.0×10^4
Chymotrypsin	100.0
Rubisco	3.0 — slow
Lysozyme	0.5 — slow

*For Ribozymes and restriction endonuclease, it is expressed per minute

Kcat = constant
fast enzyme = Kcat ↑
Slow enzyme = Kcat ↓
→ donot vary with substrate conc^r or enzyme conc^r.

Specificity constant or catalytic efficiency

Km = ↓ Kcat = ↑
↑
Desire

Kcat/Km is rate constant that refers to properties and reactions of free enzyme and free substrate

Enzyme	Substrate	$K_M\,(M)$	$k_{cat}\ (s^{-1})$	Kcat/km
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	~ 2×10^8
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	~ 2×10^8
Superoxide dismutase	Superoxide ion ($O_2^{-\cdot}$)	3.6×10^{-4}	1.0×10^{6}	~ 2×10^8
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	

Kcat/Km of 2 X 10^8 for enzyme indicates that the value is close to diffusion controlled rate of encounter



HAPPY LEARNING

THANKS

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