Slides Nayef 8

ENZYMES

REGULATION OF ENZYMES

- Introduction
- Mechanism of Regulation
  - Substrate conc.
  - Reversible Inhibition by products or other compounds
  - Regulation through Conformational change
    - Allosteric Activation or Inhibition
    - Covalent Modification
    - Modulator Protein Binding
    - Proteolytic Cleavage
  - Enzyme concentration
- Regulation of Metabolic pathways

Many enzymatic reactions can be described by Michaelis-Menten kinetics :-

M-M analyzed enzymatic reactions under simplified assumptions :-

- The reaction has only one substrate
- $[S] \gg [E]$
- Initial rate i.e $[P]$ is negligible
- Course of reaction for a short time.

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

$$v = k_3[ES]$$

$$V_{max} = k_3[E_T]$$

The Michaelis-Menten Equation

- The Steady-State Assumption

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow[(Kcat)]{K_3} E + P$$

(velocity) $v = K_3[ES]$

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

Rate of Breakdown of $ES = [K_2 + K_3][ES]$

At steady state $[ES]$ remains constt.

$$[ES] = \frac{[E][S]}{K_2 + K_3/K_1} = \frac{[E][S]}{K_m}$$

The Michaelis Constt. $K_m = \frac{K_2 + K_3}{K_1}$

$$E = E_T - [ES]$$

on substituting and rearrangement

$$[ES] = [E_t]\frac{[S]}{[S] + K_m}$$

by substituting

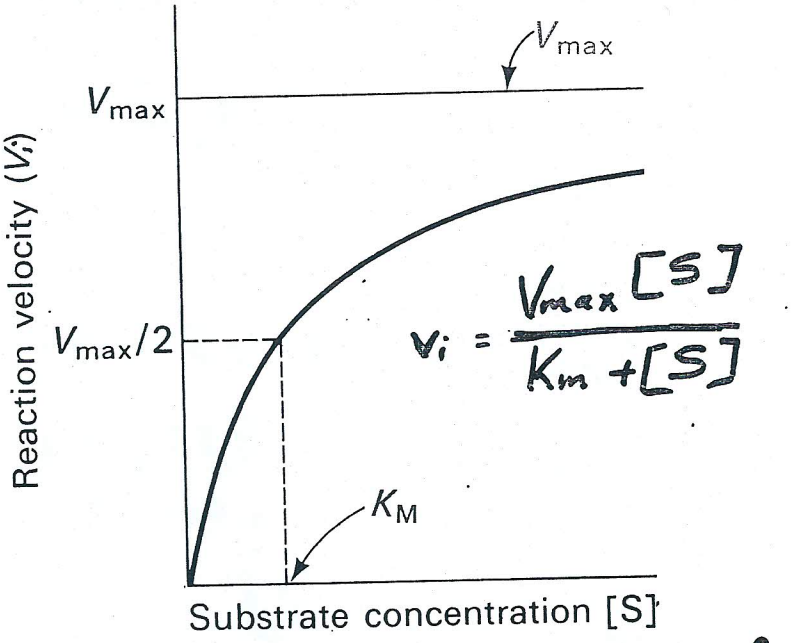
$$v = K_3[ES]$$

$$v = K_3[E_t]\frac{[S]}{[S] + K_m}$$

$$V_{max} = K_3[E_t]$$

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

Velocity and Substrate Concentration 3

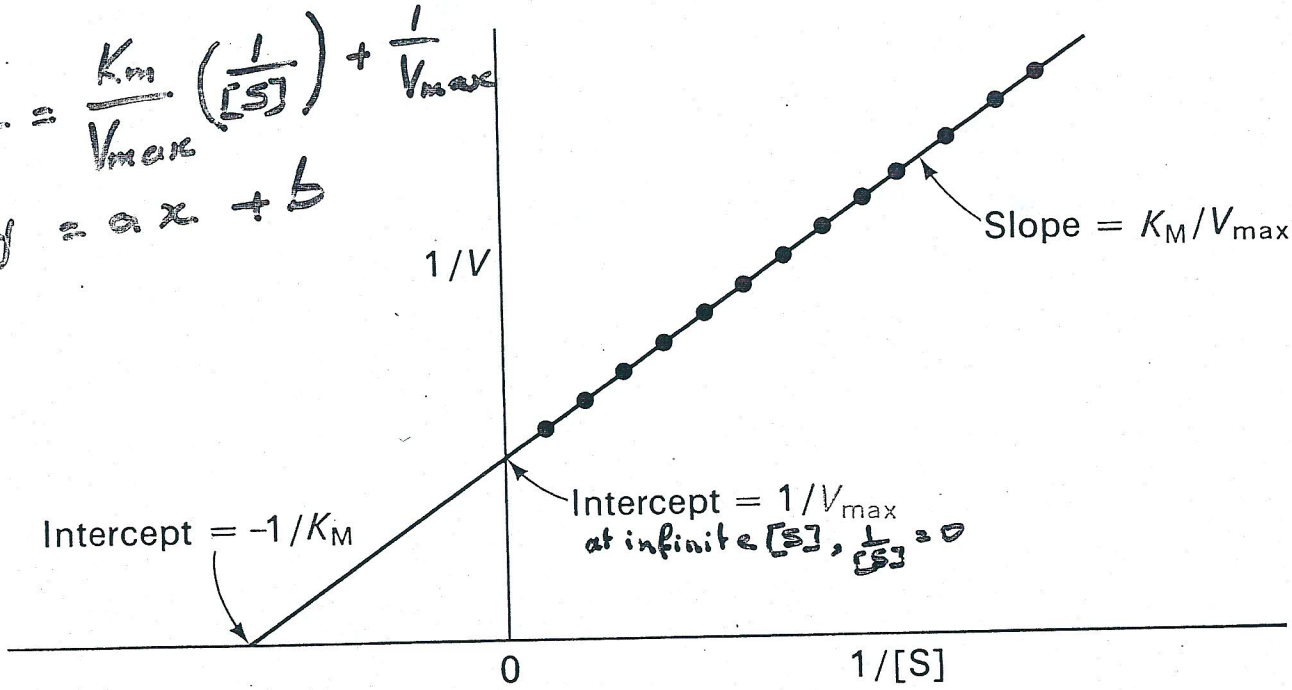


$$v_i = \frac{V_{max}[S]}{K_m + [S]}$$

Lineweaver-Burk transformation (plot)
(double reciprocal plot)

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

$$y = ax + b$$



Figures 8-15 and 8-16

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1. At very low [S] i.e. $K_m \gg [S]$

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

2. At very high [S] i.e. $[S] \gg K_m$

$V = V_{max}$

3. When $[S] = K_m$

$V = V_{max}/2$

Km values depends

1. Particular substrate
2. Presence of I or activator
3. pH
4. Temp
5. Ionic strength

- Enzyme units
  1 unit = production of 1 μmole product per min

- Specific Activity
  = units/mg protein

When $K_2 \gg K_3$ $\Rightarrow$ $E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_3} E + P$

$K_m = \frac{K_2 + K_3}{K_1} \approx \frac{K_2}{K_1} = K_d$ if K_3 is much smaller than K_2

Under this condition

K_m is a measure of the strength of ES complex

High K_m — weak binding

Low K_m — strong binding

- K_m indicates the affinity of ES complex when $K_2 \gg K_3$

Turnover number of an enzyme :- K_3

number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate

$V_{max} = K_3[E_T] \rightarrow K_3 = V_{max}/[E_T]$

e.g. 10^{-6} M of C.A catalyzes the formation of 0.6 M H_2CO_3 per sec. when saturated with S

$$\text{Turnover number} = K_3 = \frac{6 \times 10^{-1}}{10^{-6}} = 6 \times 10^5 \ s^{-1}$$

∴ Each round of catalysis occurs in a time equal to $\frac{1}{K_3} = \frac{1}{6 \times 10^5} = 1.7\ \mu s$

Examples:

Enz.	Turn over number (per sec)
C.A	600,000
Acetylcholinesterase	25,000
LDH	1,000
DNA polymerase I	15
Lysozyme	0.5

Significance of K_m and V_{max}

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

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

$$V = k_3[Et]\frac{[S]}{[S] + K_m} \qquad V_{max} = k_3[Et]$$

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

(1) f_{ES} = fraction of sites filled at any $[S]$ can be calculated if K_m is known

$$f_{ES} = \frac{V}{V_{max}} = \frac{[S]}{[S] + K_m}$$

(2) When $k_2 \gg k_3$ (a limiting case)

$$K_m = \frac{k_2}{k_1} = K_d$$

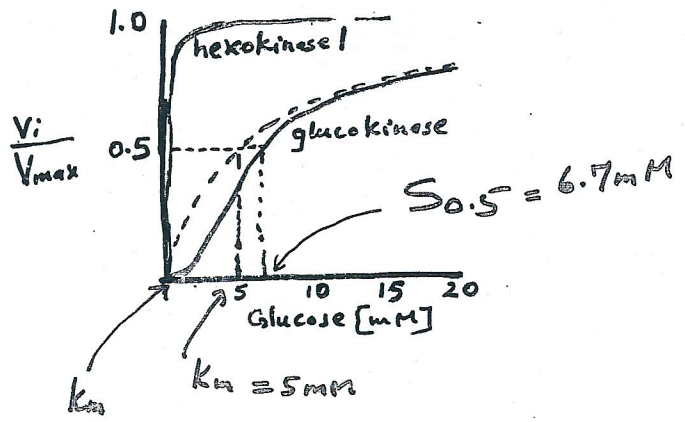
K_m indicates affinity of E to $S \rightarrow ES$

Hexokinase Isozymes have different Km values for glucose

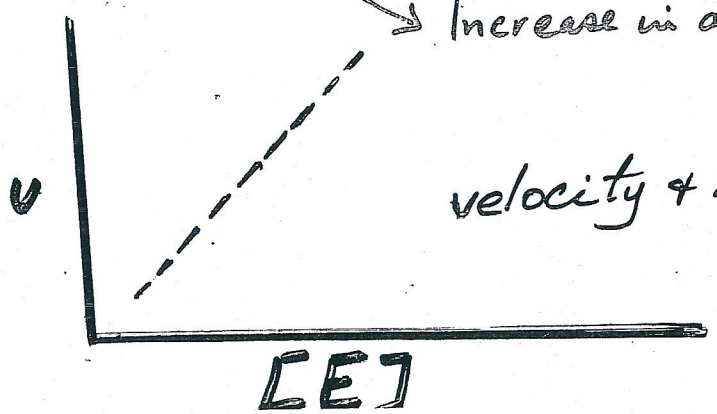
HK-I (110 kD) in erythrocytes, muscle, brain & most other tissues
Km 0.02 — 0.13 mM

GK isozyme (55 kD) in liver: 5 to 6 mM (Km)

Km for GK is calculated from Michaelis-Menten Eq.



- Which enzyme activity increases after a high carbohydrate meal

- $V_{max} = k_3 [E_T]$

Increase in V_{max} → Increase in Catalytic Power, K_{cat} and/or Increase in amount of enzyme