Slide : Enzymes 4

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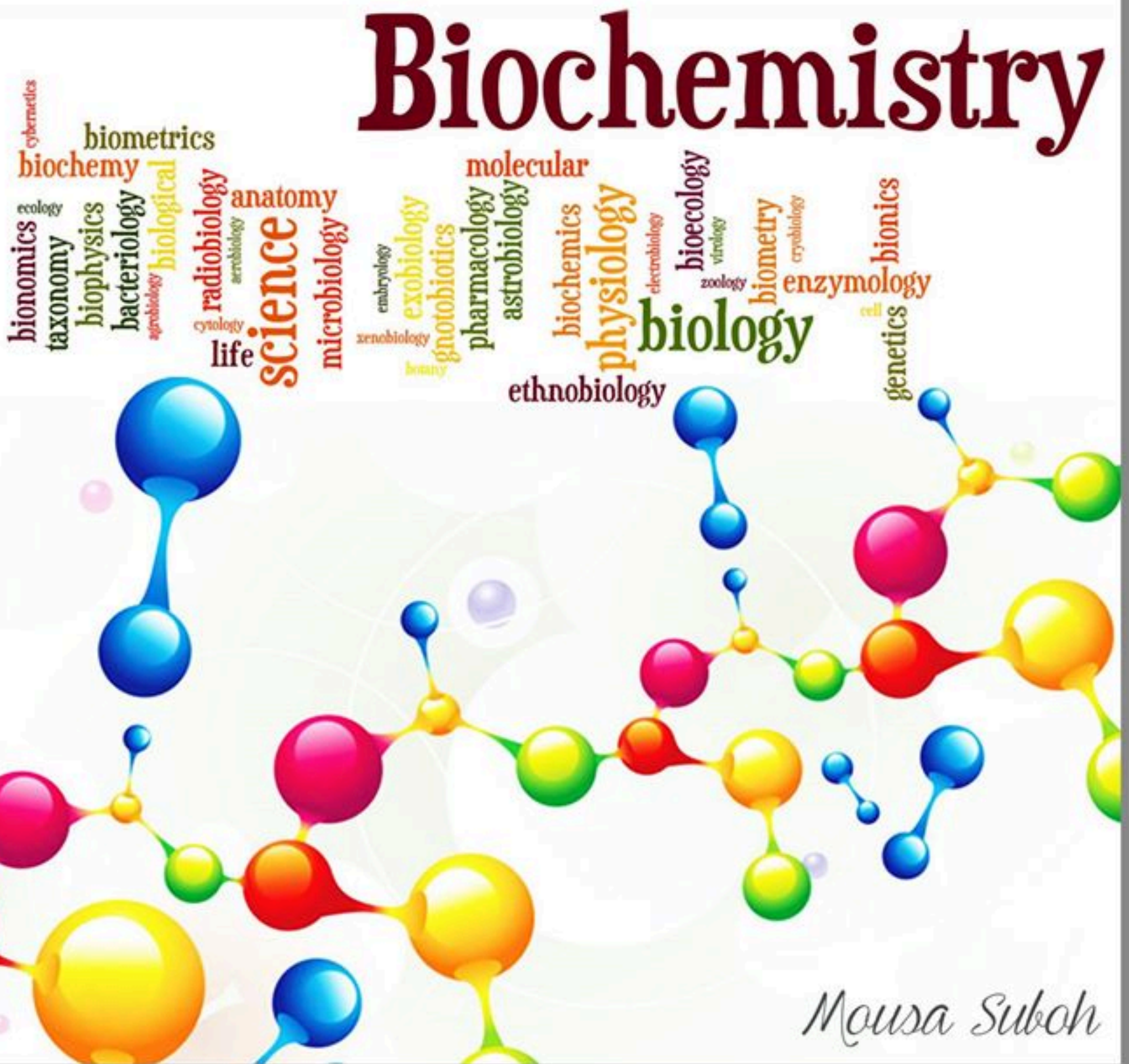
Sections : 4,5,6

■ Slide □ Sheet

Medical Committee
The University of Jordan



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
- $[S] >> [E]$
- Initial rate i.e $[P]$ is negligible
- Course of reaction for a short time.
- Steady state of $[ES]$

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

$$v = k_3 [ES]$$

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

The Michaelis-Menten Equation

- The Steady-State Assumption

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow[(K_{cat})]{k_3} E + P$$

(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. $K_m = \dfrac{k_2 + k_3}{k_1}$

$$E = E_T - [ES]$$

on substituting and rearrangement

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

by substituting

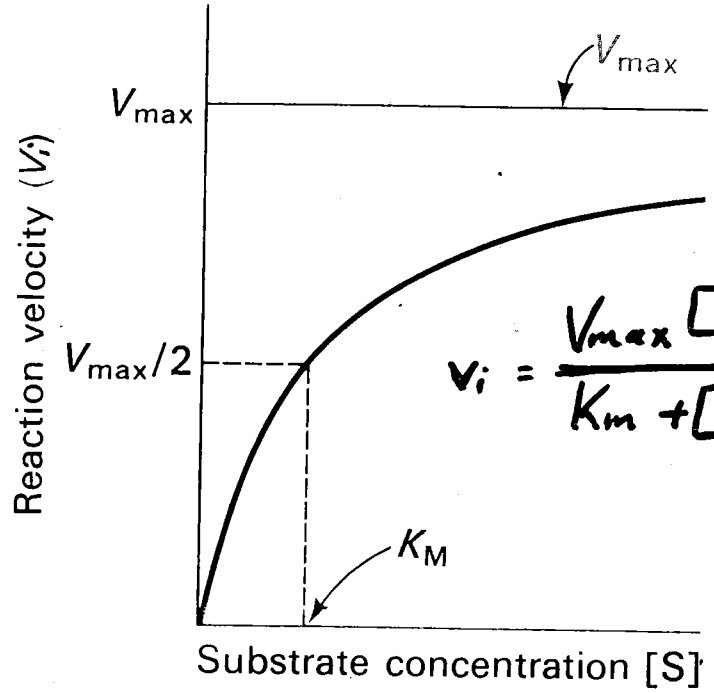
$$v = k_3[ES]$$

$$v = k_3[E_t]\frac{[S]}{[S] + K_m}$$

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

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

Velocity and Substrate Concentration 3

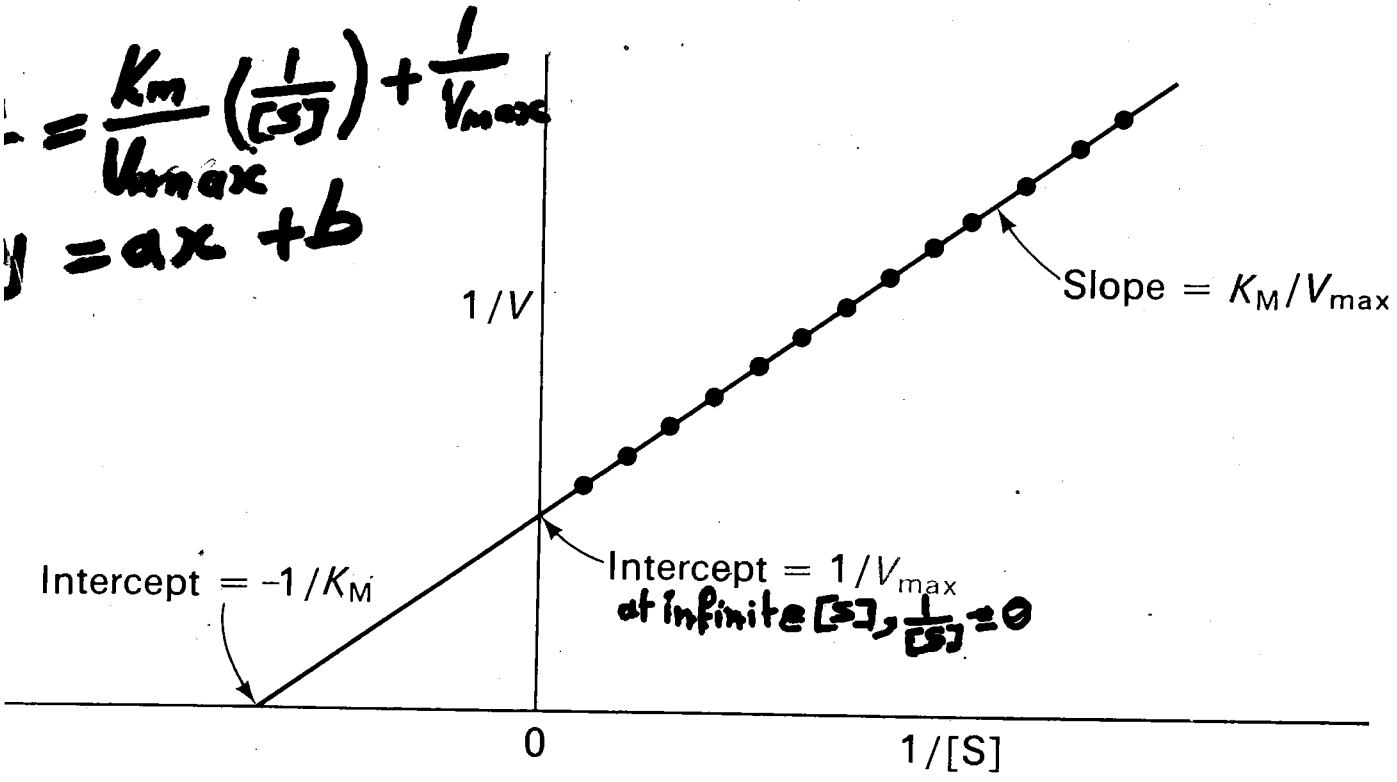


$$v_i = \frac{V_{max}[S]}{K_m + [S]}$$

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

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

$$y = ax + b$$



igures 8-15 and 8-16

tryer: *Biochemistry*, Third Edition

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

$V = \frac{Vmax}{Km + [S]} \rightarrow V = [S] \frac{Vmax}{Km}$

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

$V = Vmax$

3. When $[S] = Km$

$V = Vmax/2$

Km values depends

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

- Enzyme Units

Unit = production of 1 μmole product per min

- Specific Activity

= units/mg protein

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 $S \rightarrow ES$

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

$K_m = \frac{K_2 + K_3}{K_1} \approx \frac{K_2}{K_1} = K_d$ 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 0.6M 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

1) 10 μg of pure carbonic anhydrase (H.W. 30,000) catalyzes the hydration of 0.3 gr of CO_2 (H.W. 44) in 1 min at 37°C under optimal conditions. Calculate (K_{cat}) turnover number of C.A. (in units of min^{-1}).

$$H_2O + CO_2 \underset{}{\overset{C.A.}{\rightleftharpoons}} H_2CO_3$$

$$K_{cat} = \frac{0.3}{44} \Big/ \frac{10 \times 10^{-6}}{3 \times 10^4}$$

$$= \frac{0.9 \times 10^4}{44 \times 10^{-5}} = \frac{90}{44} \times 10^7$$

$$= \sim 2 \times 10^7 \ min^{-1}$$

2) At what substrate conc. will an enzyme having a k_{cat} of $30\ s^{-1}$ and a K_m of 0.005 M show one-quarter of its maximum rate

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

$$\frac{1}{4} V_{max} = \frac{V_{max} \times [S]}{0.005 + [S]}$$

$$[S] = \sim 1.7 \times 10^{-3}$$

3) Determine the fraction of V_{max} when

$$[S] = 2\ K_m,\ 10\ K_m$$

A pure enzyme solution of 100 µg catalyzes the production of 1 mmole product in 10 min.

1) What are the total units (min^{-1})

2) What is the specific activity

1. Total units

$$= \frac{1 \times 1000}{10} = 100 \text{ units}$$

(1 unit ≡ production of 1 µmole per min or (unit time)

2. Sp. Activity

$$= \frac{\text{no. of units}}{1 \text{ mg protein}}$$

$$= \frac{100}{0.1 (\text{mg})} = 1000 \text{ units/mg}$$