Enzymes

How enzymes work?

Enzymes

1. Catalytic RNA (RNA as a catalyst, substrate is RNA)
   - Transesterification and phosphodiester bond hydrolysis (cleavage)
     - Self-splicing group I intron
     - RNase P in *E. coli*
     - Hammerhead ribozyme
2. Catalytic antibody (abzyme)
   - Ab generated with the transition-state analog as Ag
3. Proteins (in their native conformations)

Enzyme
= Protein
= Protein + cofactor (inorganic ions)
= Protein + coenzyme (organic molecules)

Tightly bound to Enz. ➔ Prosthetic group

Holoenzyme = Apoenzyme + cofactor/coenzyme

Complete, catalytically active

Naming of enzymes

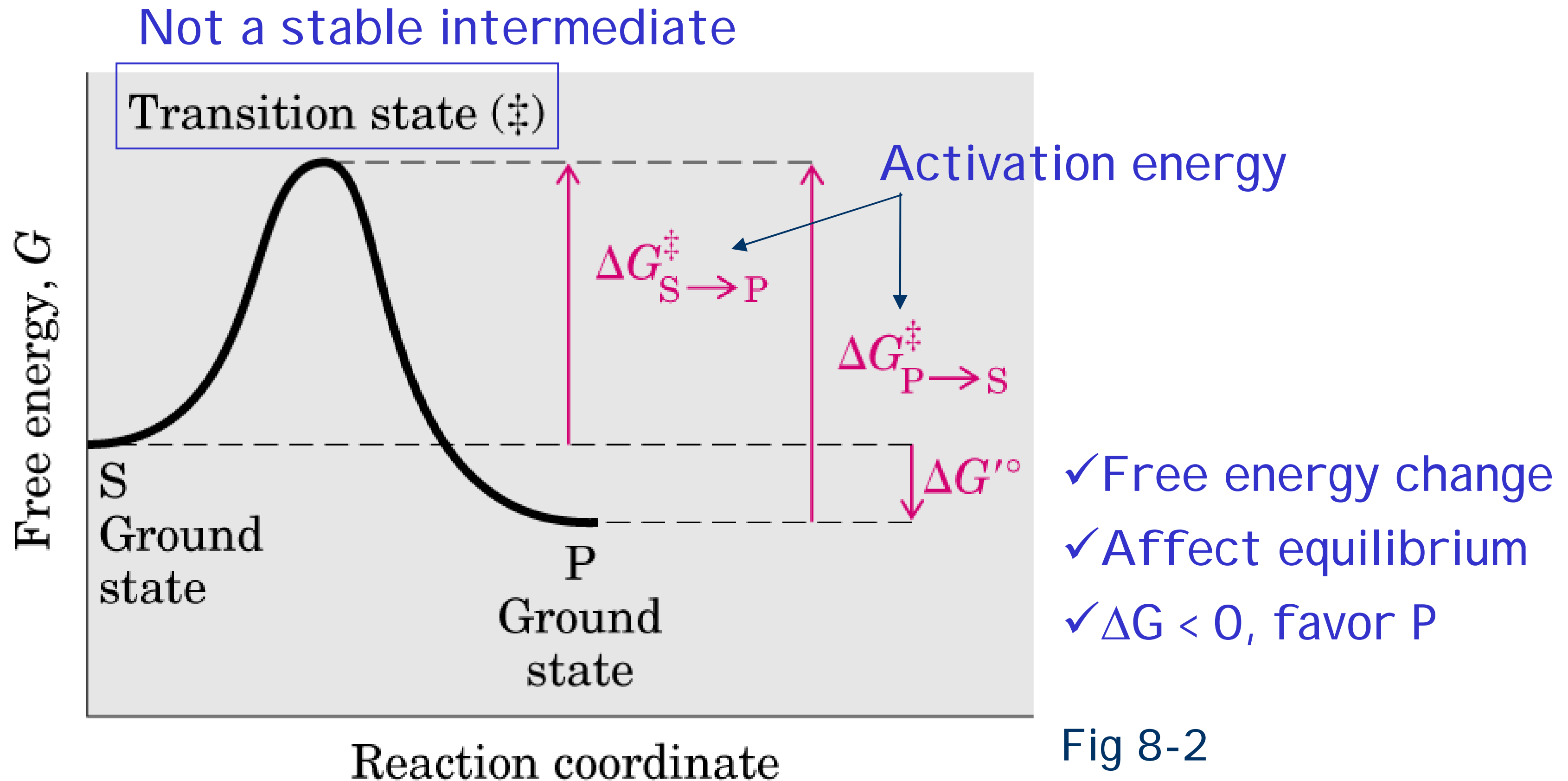
p. 192

- Reactant + *-ase*
- 6 classes (Table 6.3), based on the reaction type
  - Oxidoreductase, 氧化還原酶, ($A^- + B \rightleftharpoons A + B^+$)
  - Transferase, 轉移酶, ($A–B + C \rightleftharpoons A + B-C$)
  - Hydrolase, 水解酶, ($A–B + H_2O \rightleftharpoons A-H + B-OH$)
  - Lyase, 裂解酶, ($\underset{X\ \ Y}{A–B} \rightleftharpoons A=B + X-Y$)
  - Isomerase, 異構酶, ($\underset{X\ \ Y}{A-B} \rightleftharpoons \underset{Y\ \ X}{A-B}$)
  - Ligase, 接合酶, (synthetase) ($A + B \rightleftharpoons A-B$)

Energy Diagram of a chemical reaction

- Substrate (S) $\rightleftharpoons$ Product (P)

Not a stable intermediate



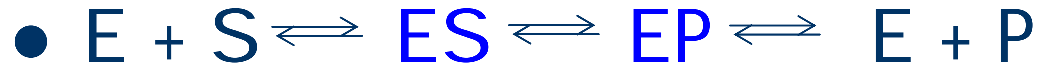
✓Free energy change

✓Affect equilibrium

✓$\Delta G < 0$, favor P

Fig 8-2

Enzymes lowers the activation energy

- 

✓Reflect reaction rate

✓do not change equilibrium

Transition state (‡)

$\Delta G^{‡}_{uncat}$

$\Delta G^{*‡}_{cat}$

Free energy, G

S

ES

EP

P

Intermediates, local min. energy

Reaction coordinate

Fig 8-3

- The binding energy (ΔG_B) released = Lowered ΔG^*
- ΔG_B: from multiple weak E-S interactions
  - **Catalysis** and **specificity**

Catalytic power vs. Specificity

- Enzyme-substrate interaction:
  - "Lock and Key" hypothesis
    - Enzymes are structurally complementary to their substrates.
  - Induced-fit hypothesis
    - A conformational change of E is induced by initial binding with S, which optimize the ES interaction.

From Stryer

Enzyme kinetics

- S $\overset{E}{\longleftrightarrow}$ P, measure the initial rate (V_o)
- Experiment:
  - [E]: fixed
  - [S]: increasing
  - Measure V_o = [P]/time

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

Michaelis-Menten equation

At low [S], $V_o \propto$ [S]

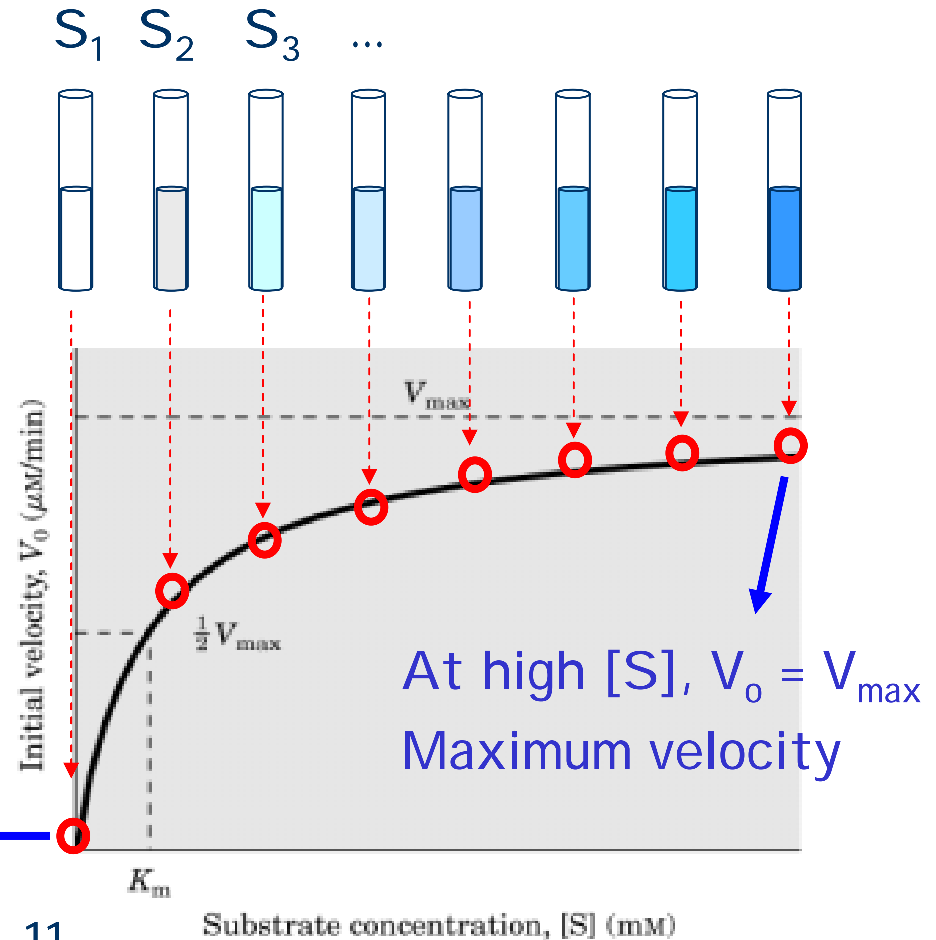
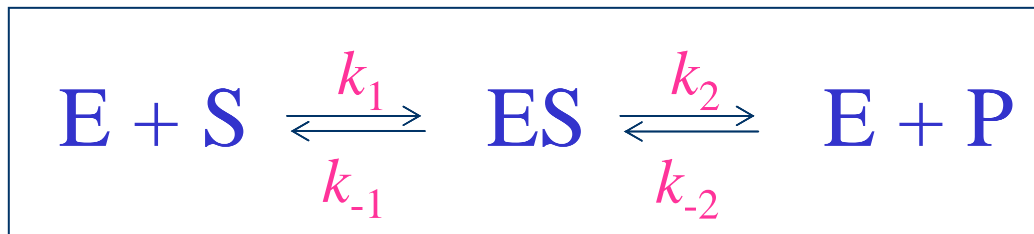


Fig 8-11

Kinetic model

- [S], V_o, V_{max}, and K_m can be determined by exp.
- Michaelis-Menten kinetics
- Steady-state kinetics
  - Before ES builds up: pre-steady state
  - After [ES] reaches const. : steady state

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



p. 259, (8-10)

fast Slow ← Rate limiting step

Michaelis-Menten kinetics

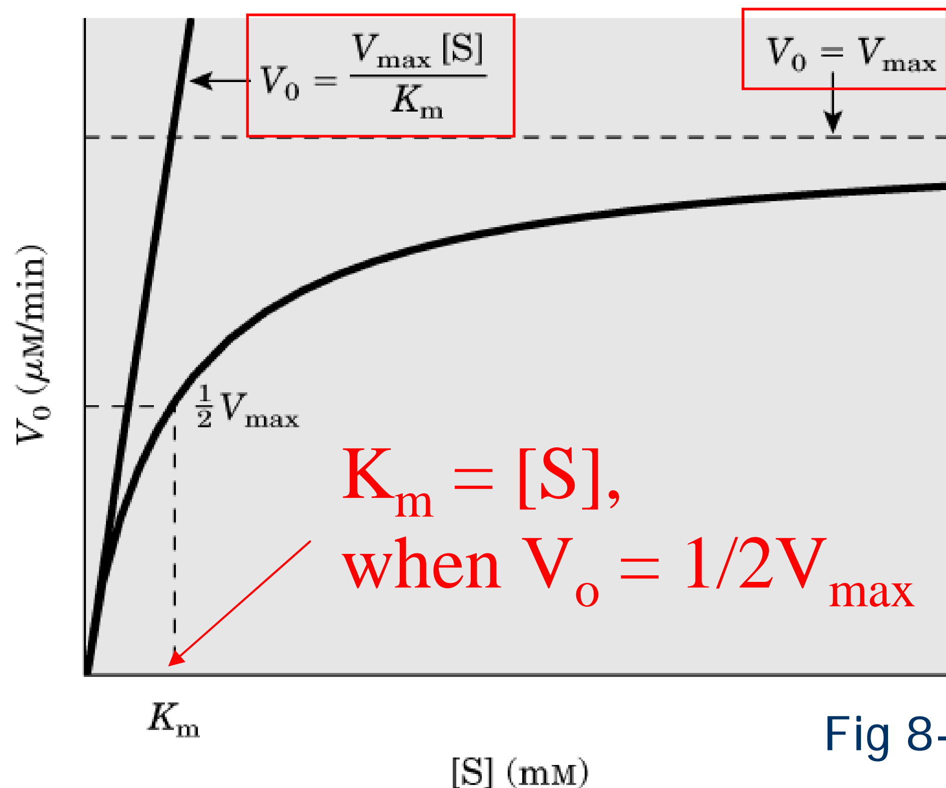


Fig 8-12

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

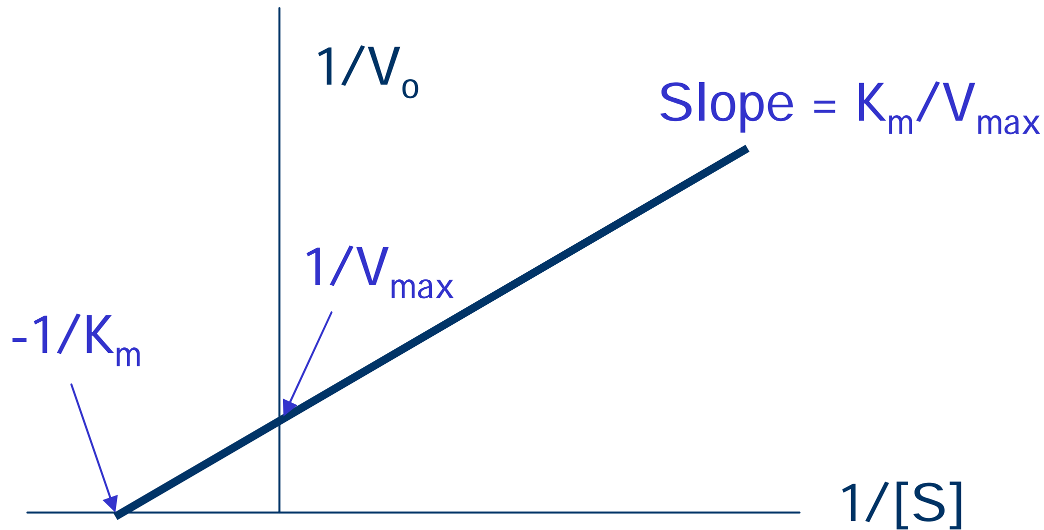
- K_m: Michaelis constant
  - The conc. of substrate that will produce ½V_{max}.

Lineweaver-Burk equation

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

Plot 1/V vs. 1/[S]

- y-intercept: $1/V_{max}$
- x-intercept: $-1/K_m$
- Slope: K_m/V_{max}



See Box 8-1

Double-reciprocal plot

Exercise

A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetic.

a) Please make a Michaelis-Menten plot.
b) Please make a Lineweaver-Burk plot (double reciprocal plot).
c) V_{max} for the enzyme is __________.
d) K_m for the enzyme is ___________.

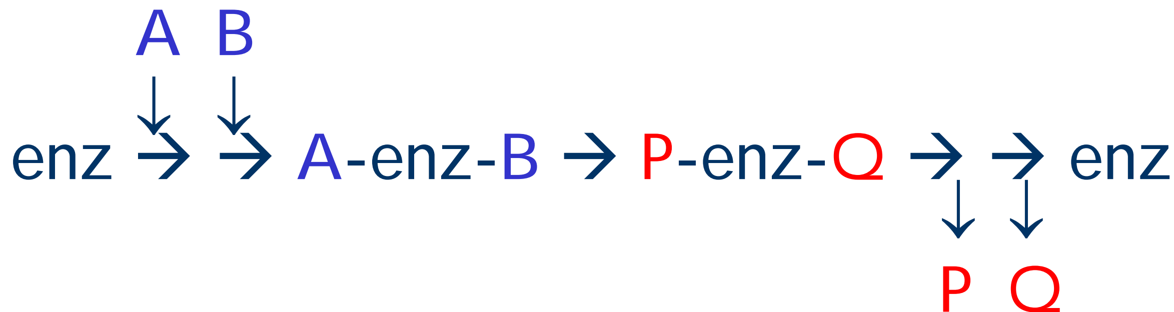
Substrate conc. [S], μM	Initial velocity V_o (μmole/min)
1	49
2	96
8	349
50	621
100	676
1,000	698
5,000	699

k_{cat} and k_{cat}/K_m

- k_{cat}, rate constant or turnover number (轉換數)
  - k_{cat} (s^{-1}) = V_{max}/[E_{total}]
    - At saturation, $k_{cat} = k_2$, $V_{max} = k_{cat}[E_{total}]$
  - The limiting rate of any enzyme-catalyzed reaction *at saturation.*
  - Enzyme efficiency: the number of S → P in a given unit of time when the E is saturated with S.
- Specificity constant: k_{cat}/K_m
  - Used to compare different enzymes
  - Upper limit: 10^8-10^9 $M^{-1}s^{-1}$, diffusion-controlled

Second-order reaction (I)

- A + B $\underset{}{\overset{E}{\rightleftharpoons}}$ P + Q (bi-substrate)
- **Single-displacement (sequential)** reaction
  - Ternary complex formation
  - Both substrates must bind to the enzyme before any products are released
  - The addition of A and B may be **ordered or random**, so is the release of products P and Q (Fig 8-13a, 8-14a)

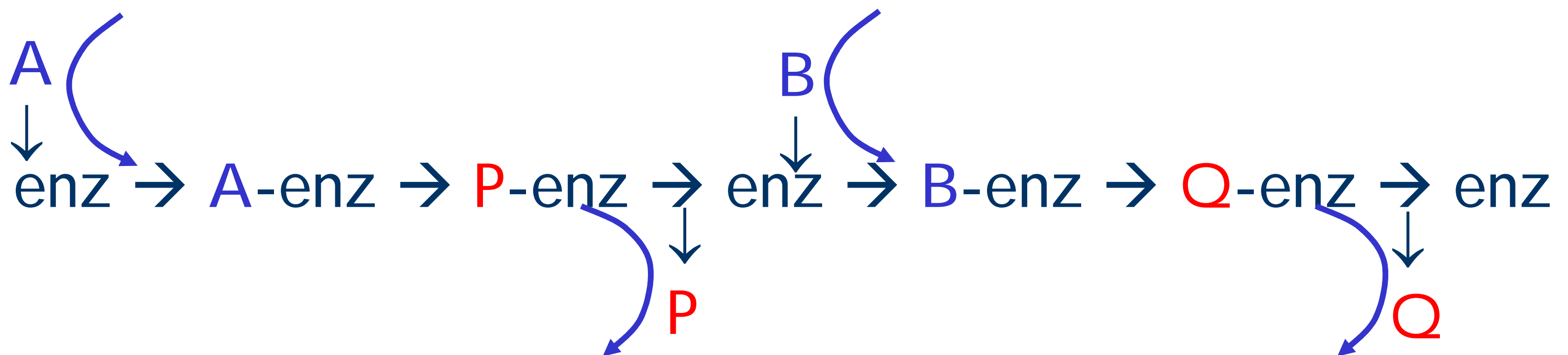


Compulsory order (Ordered Bi Bi)

Random order (Random Bi Bi)

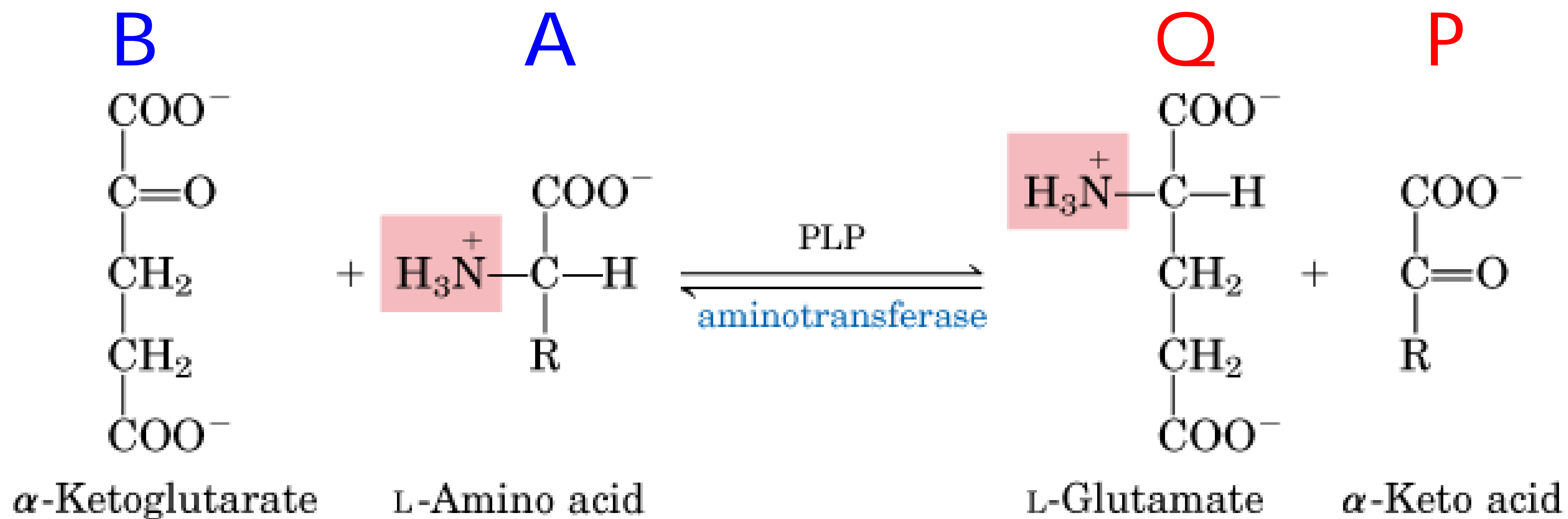
Second-order reaction (II)

- A + B $\underset{}{\overset{E}{\rightleftharpoons}}$ P + Q (bi-substrate)
- **Double-displacement** (*ping-pong*) reaction
  - One substrate binds to the enzyme and one product is released before the second substrate binds (no ternary complex formed) (Fig 8-13b, 8-14b)

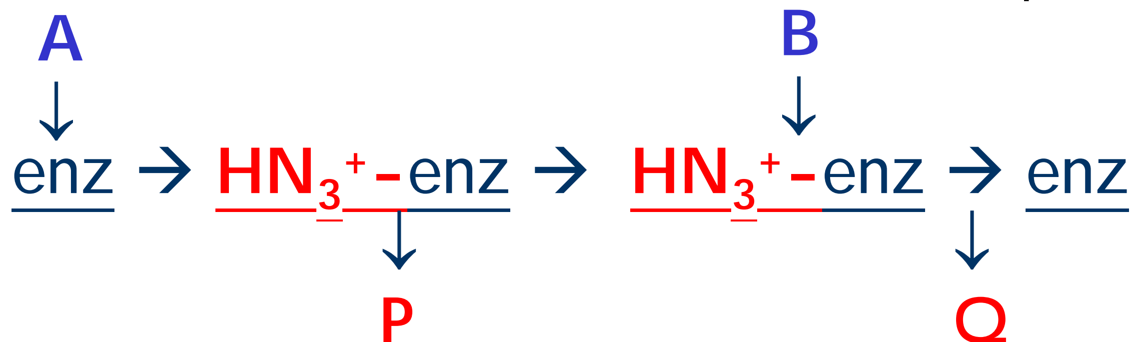


Example of a Ping-Pong reaction

- 1st step of amino acid catabolism in liver: transamination by aminotransferase (transaminase)

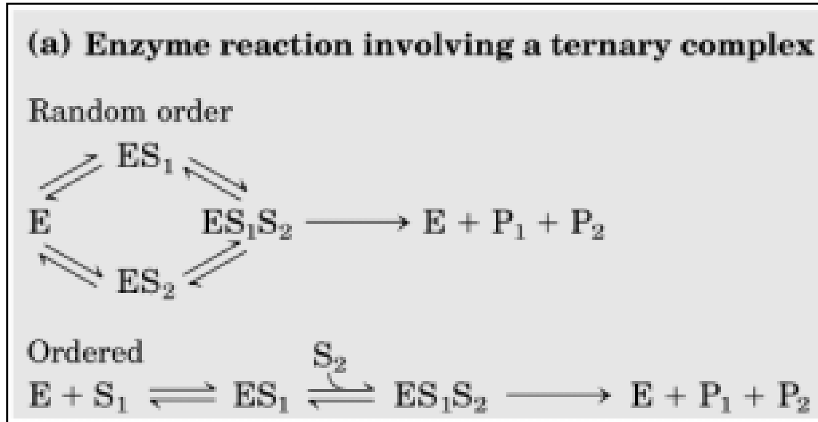


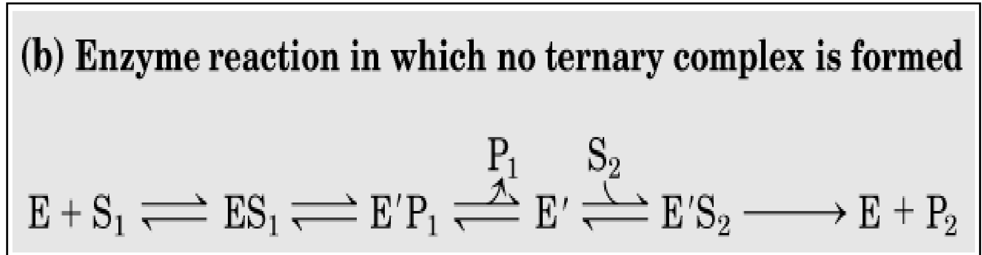
p. 628, Fig 18-4

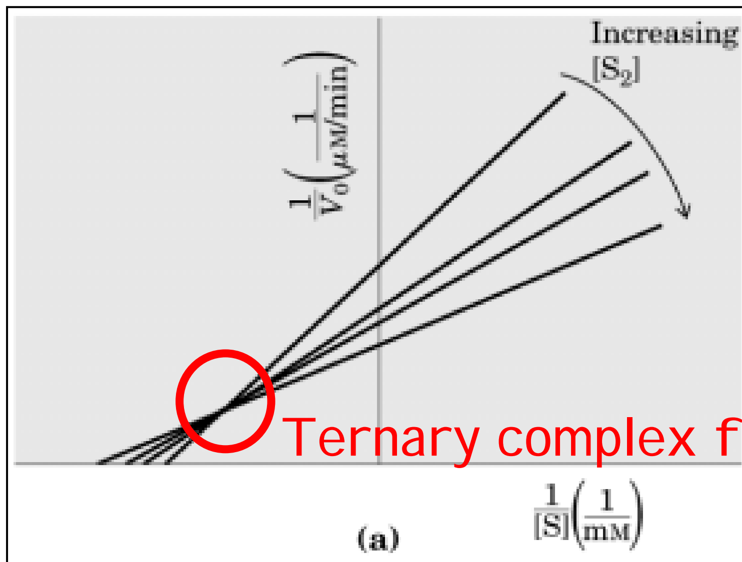


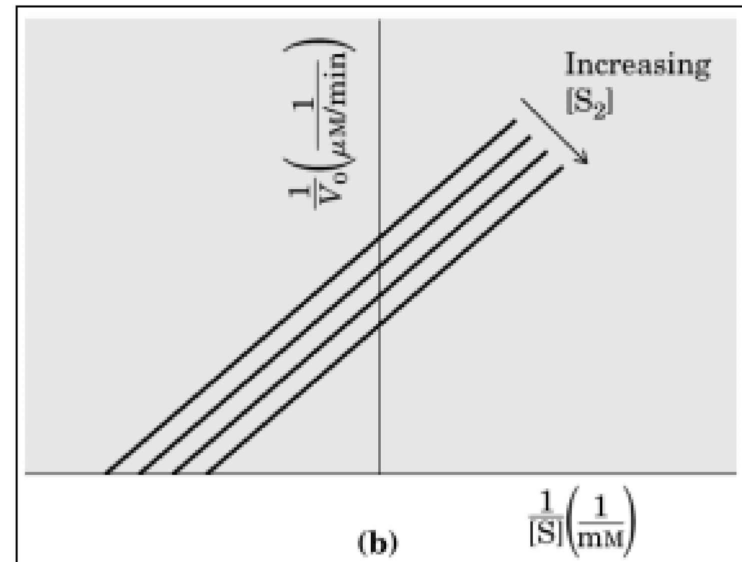
Bisubstrate reactions

Fig 8-13, 8-14









Enzyme and inhibitors

- Irreversible inhibition (p. 268)
  - Inhibitors bind and destroy the active sites
  - e.g. Nerve gas (DIFP) and ACE
    - ACE: acetylcholinesterase, catalyze the hydrolysis of acetylcholine (a neurotransmitter)
    - Chymotrypsin (Fig 8-16)
  - e.g. Asprin and prostaglandin synthet*ase*
    - Prostaglandin => pain ...
  - Suicide or mechanism-based inactivators
    - Drug design
- Reversible inhibition (p. 266)
  - Competitive
  - Uncompetitive
  - Mixed (non-competitive)

Competitive inhibition

- Inhibitor (I) competes with S for the same active site on E to form EI
- I has similar structure as S

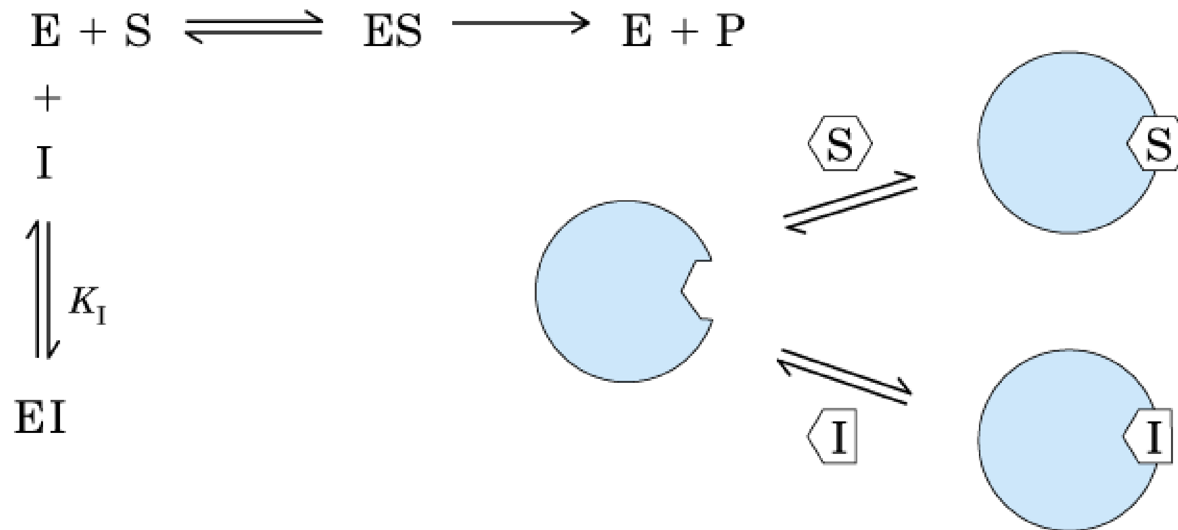
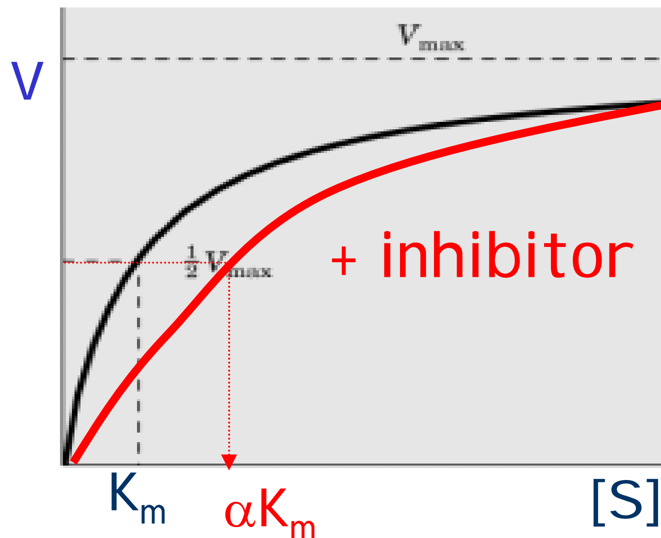


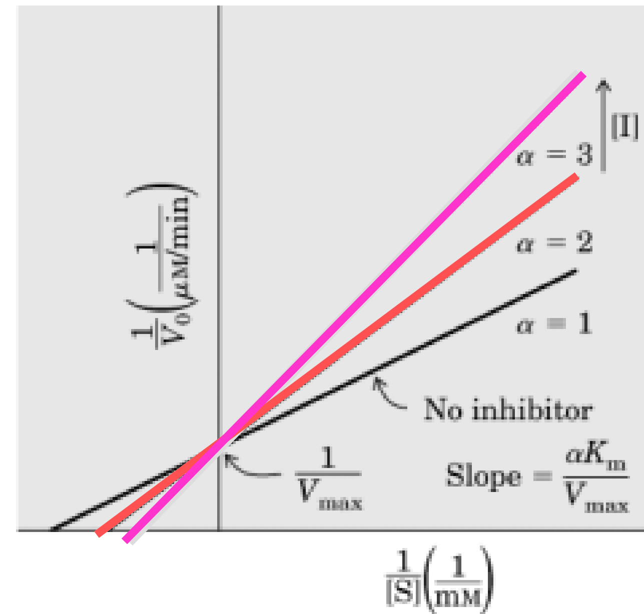
Fig 8-15a

Competitive inhibition

In presence of a competitive inhibitor, [E] constant

- V_{max} unchanged, K_m increased





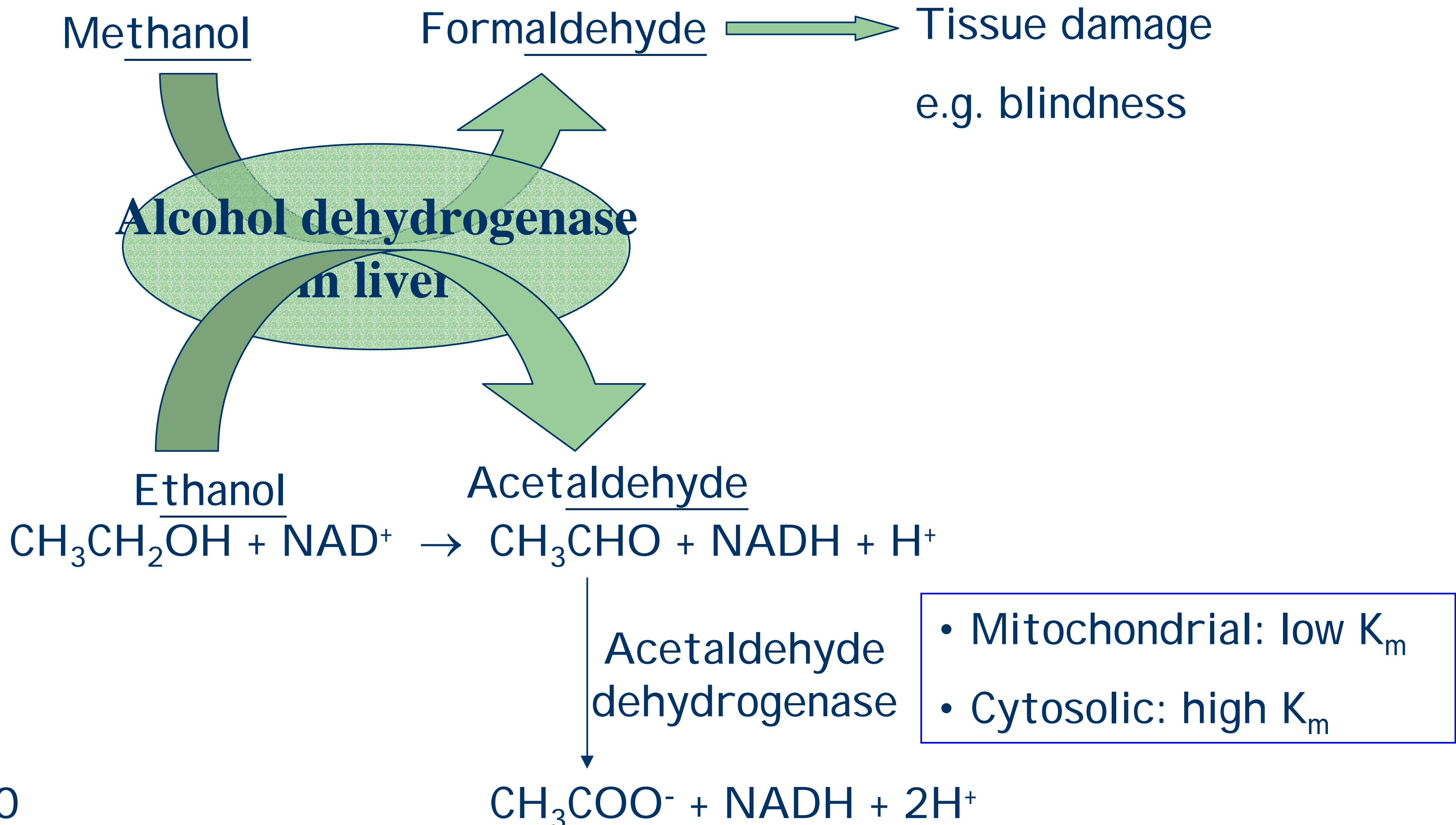
Box 8-2, fig 1

$$V_o = \frac{V_{max}\,[S]}{\alpha K_m + [S]} \qquad (8\text{-}28)$$

Apparent K_m (exp. determined)

Competitive inhibition

- Medical application



Uncompetitive inhibition

- Inhibitor (I) binds to a different site from S
- I binds ES complex to form ESI

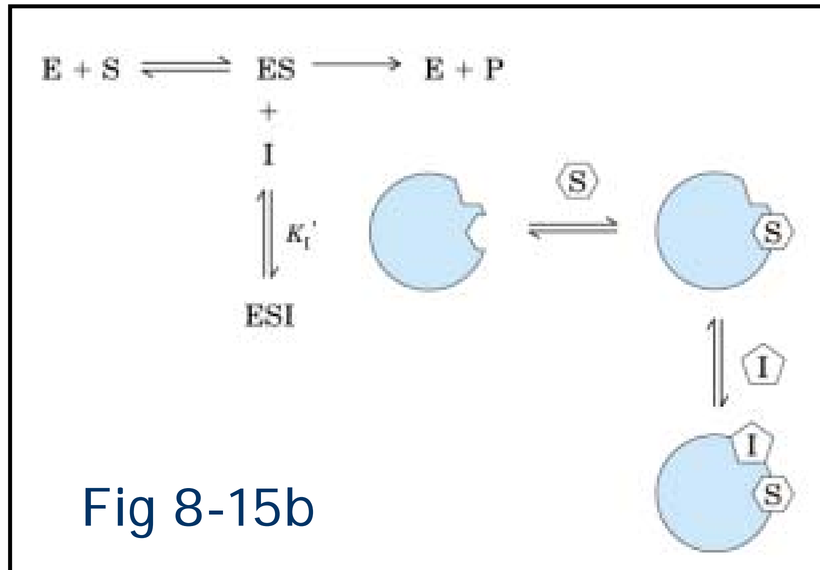
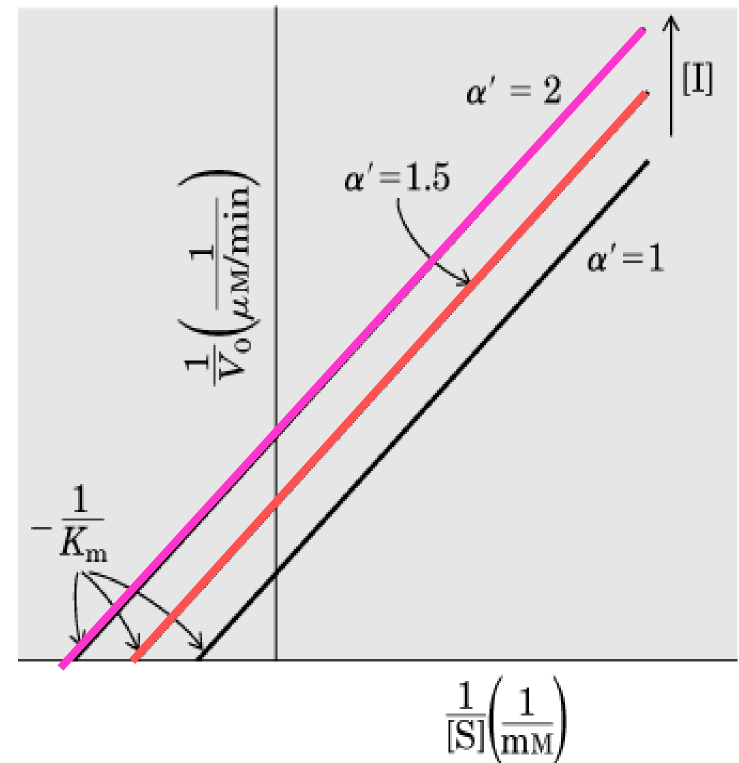


Fig 8-15b



Box 8-2, fig 2

- Both K_m and V_{max} decreased.
- Parallel lines

Mixed inhibition

- Inhibitor (I) binds a different site from S
- I binds both E and ES
  - Noncompetitive inhibition (a special case)

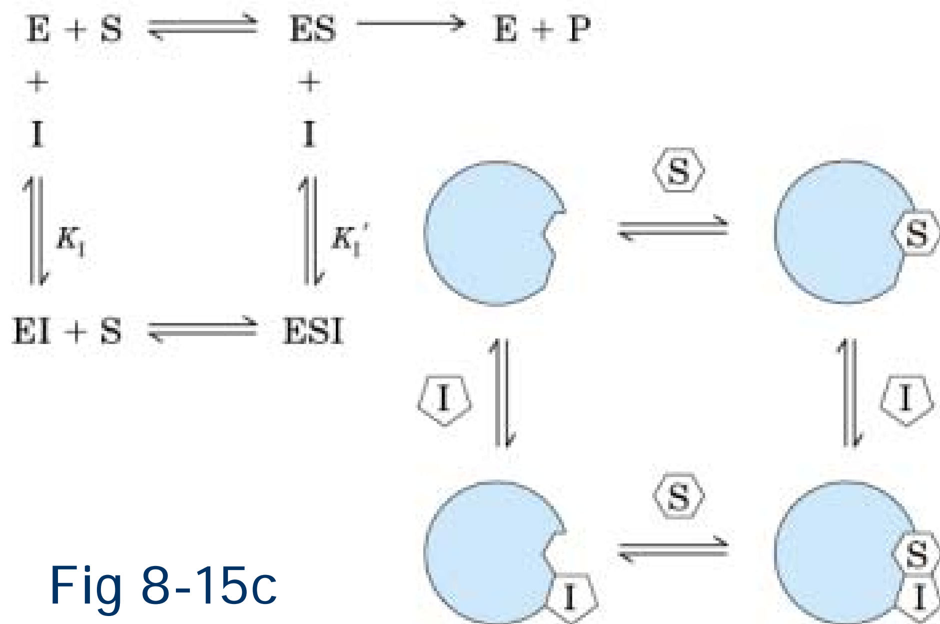
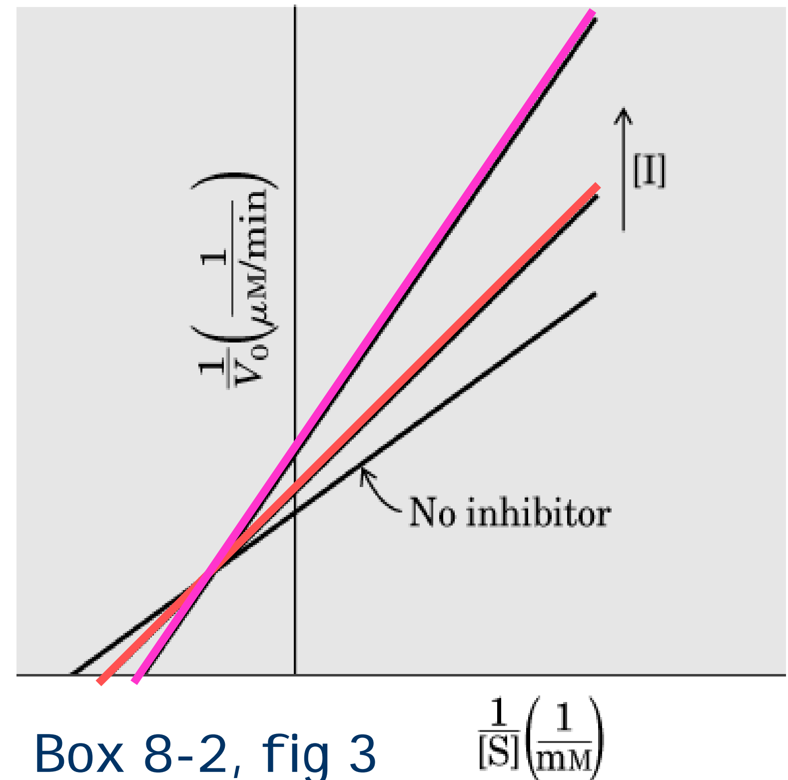


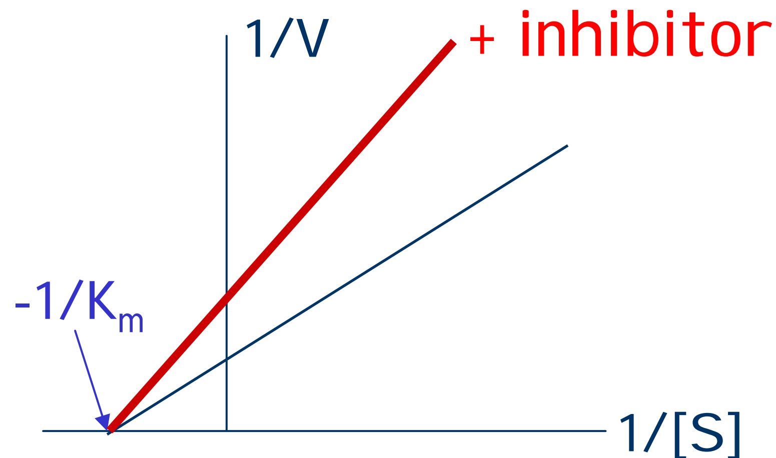
Fig 8-15c



Box 8-2, fig 3

Non-competitive inhibition

In presence of a non-competitive inhibitor

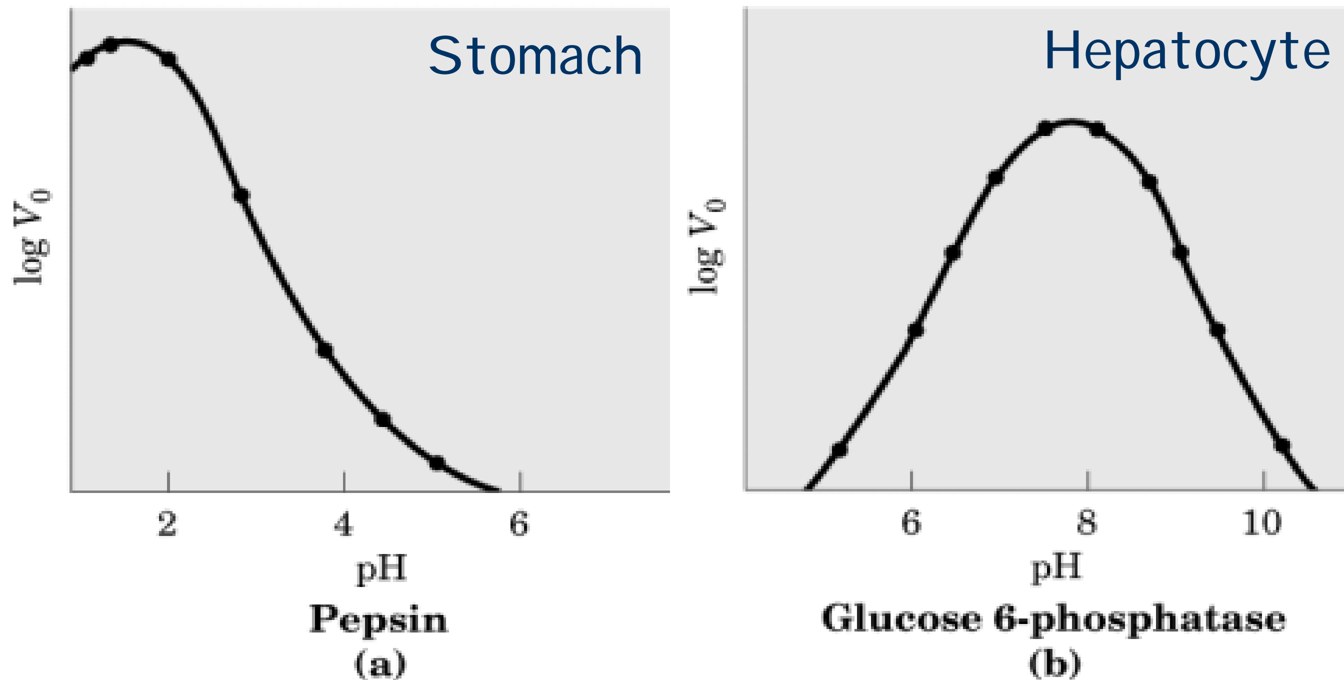


- A special case of mixed inhibition
- K_m unchanged, V_{max} decreased

Enzyme activity is affected by pH

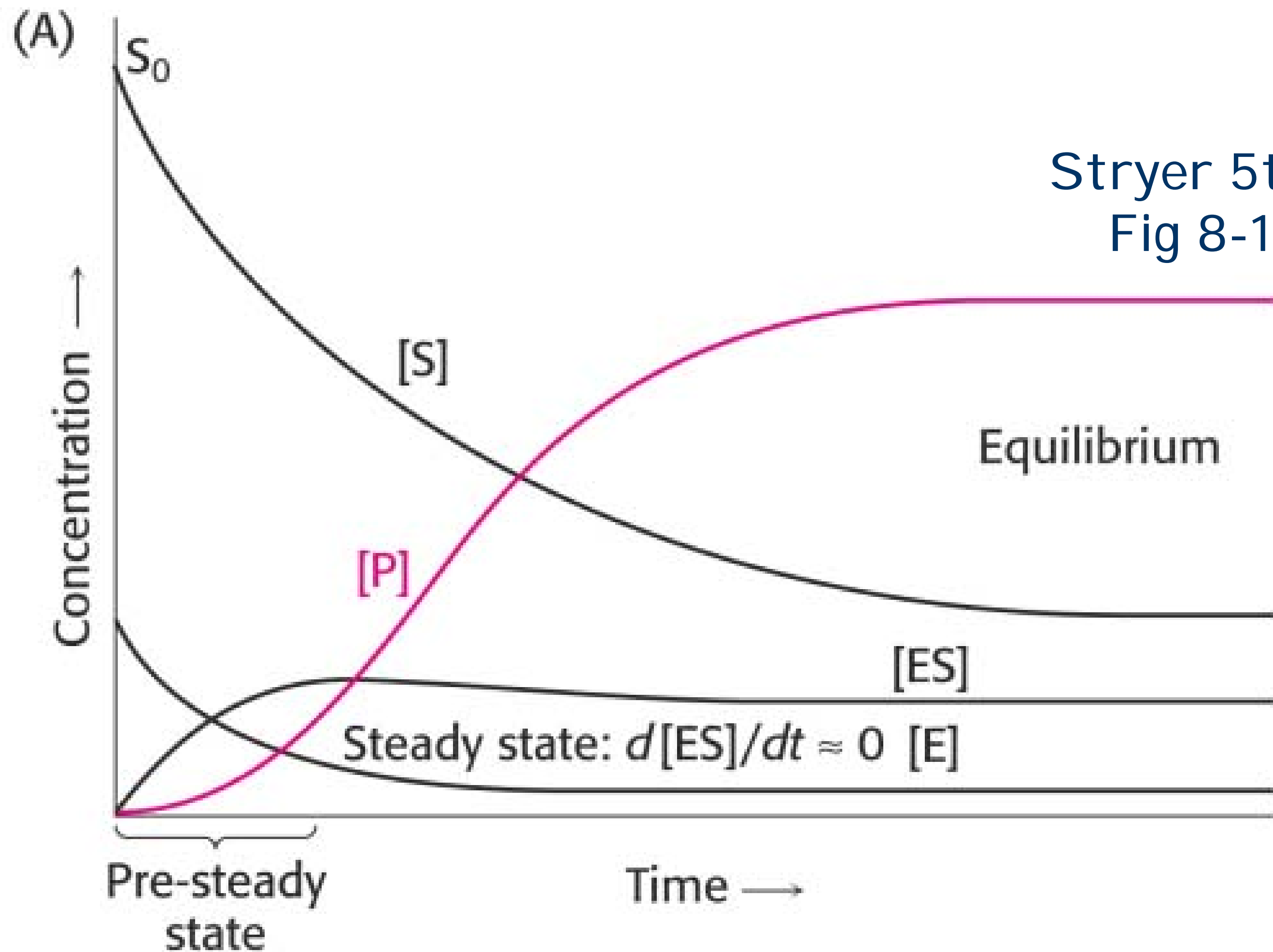
- Ionization state of key a.a.
  - In the active site
  - In structural recognition

Fig 8-17



Steady-state vs. Pre-steady state

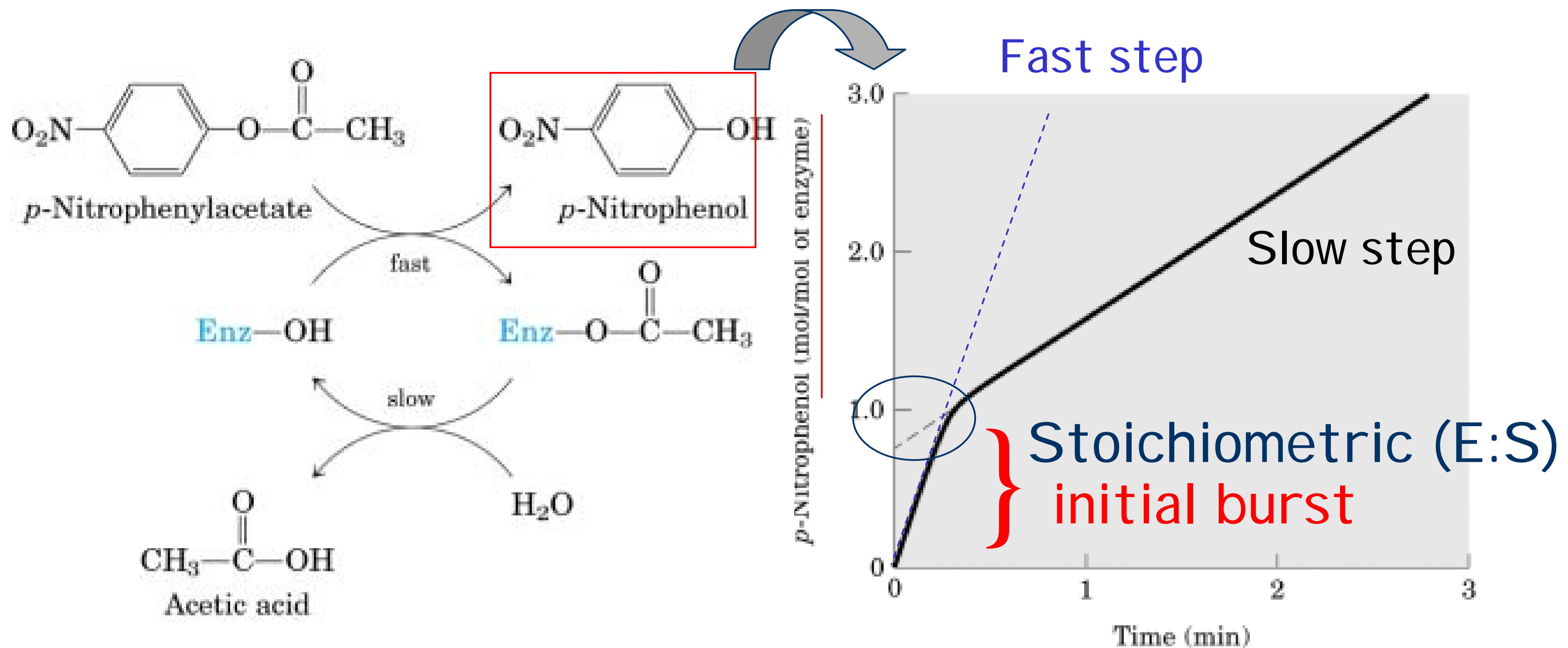
- Before [ES] reaches constant



Stryer 5th ed.
Fig 8-13A

Pre-steady state kinetics

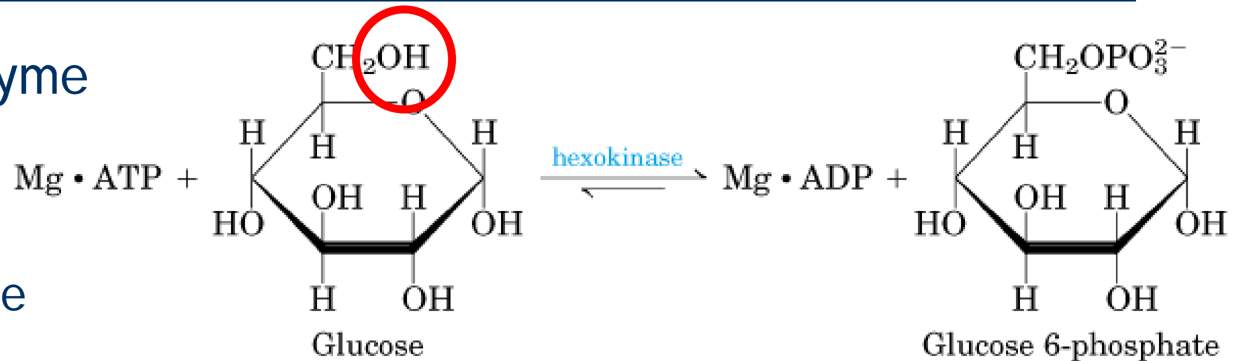
- P-nitrophenylacetate hydrolysis by chymotrypsin
  - Acylation – fast (initial burst)
  - Deacylation – slow

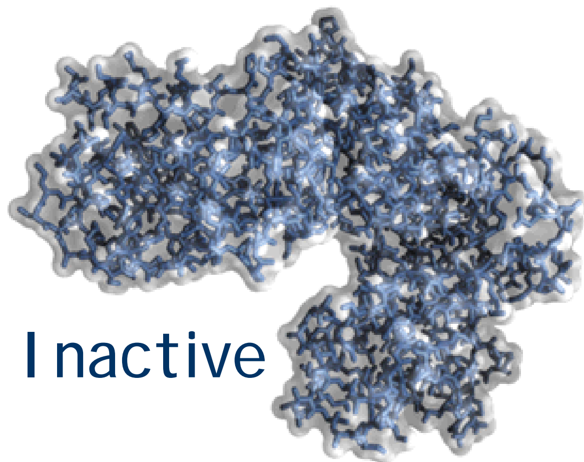


p. 275, Fig 8-20

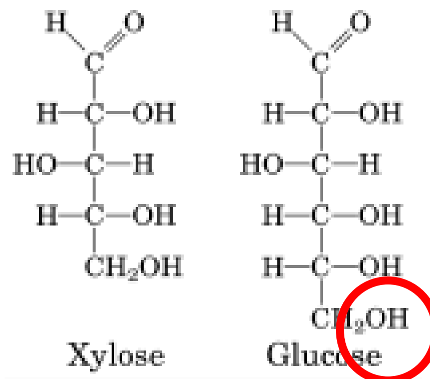
Hexokinase (p. 275-276)

- A bisubstrate enzyme
- Induced fit
  - Glucose vs. H_2O
  - Glucose vs. xylose





D-glucose



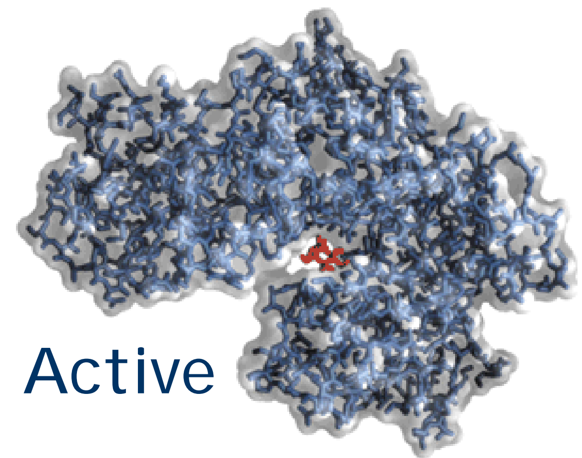
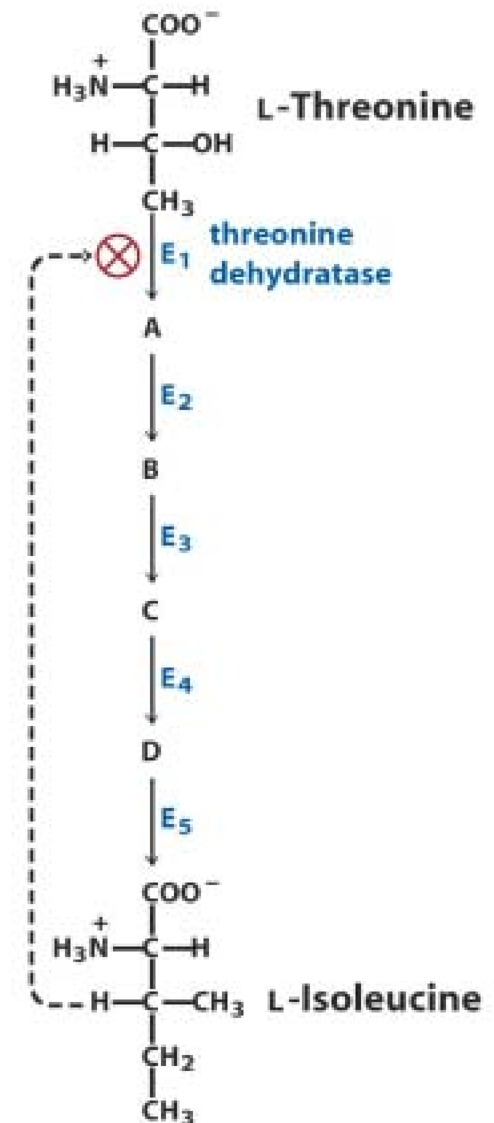


Fig 8-21

Regulatory enzymes (I)

- Allosteric enzyme
  - Conformational change
  - Does not follow M-M kinetics
  - Non-covalent modification
    - Homotropic: substrate = modulator,
      - e.g. O_2 binding of Hb
    - Heterotropic: substrate ≠ modulator
      - e.g. feedback inhibition (Fig 6-??)

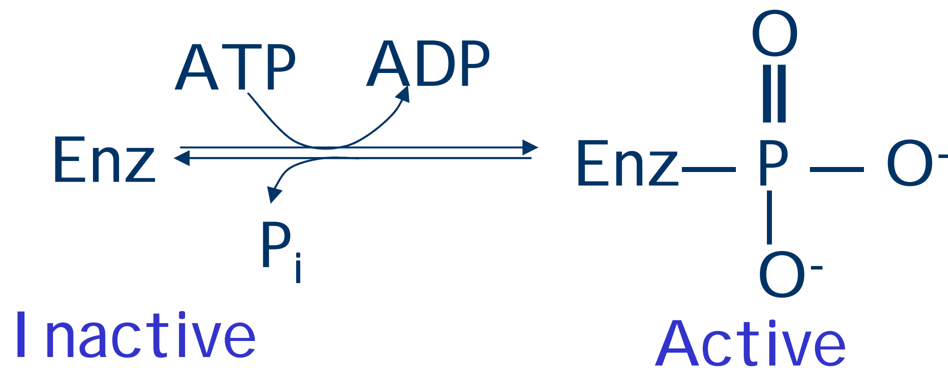


E_1 is an allosteric enzyme:
S = Thr, M = Ile

Regulatory enzymes (II)

- Covalent modification ➔ all-or-none (Fig 6-30)
  - Reversible
  - e.g. phosphorylation/dephosphorylation (Fig 6-31)

Fig 6-30 (1)



Regulatory enzymes (III)

- Polypeptide cleavage (Fig 6-33)
  - Inactive form → active form
    - e.g. chymotrypsinogen → chymotrypsin
    - e.g. trypsinogen → trypsin
  - Inactive precursor: zymogen, proenzyme, proprotein
  - Irreversible activation → inactivated by inhibitors

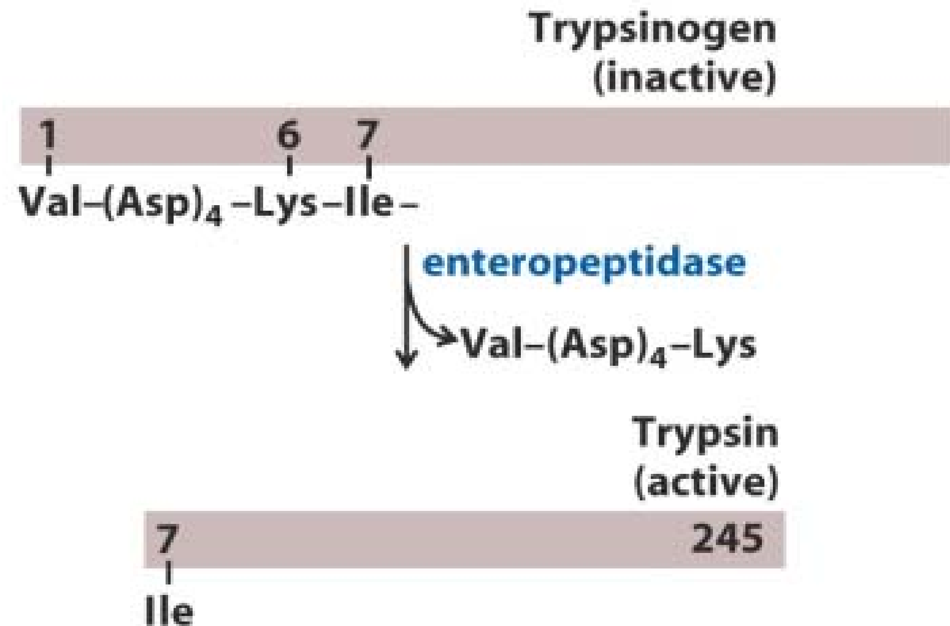


Fig 6-33, right