第五單元 蛋白質功能 - 酵素 Enzymes

學習目標：

1. 瞭解酵素的特性
2. 瞭解酵素催化反應過程的能量變化圖
3. 瞭解酵素動力學 (kinetics)
4. 瞭解 Inhibitor 的種類及對 V_{max}, K_m 的影響
5. 熟悉調控(regulation)酵素活性的方式

天堂筆記：

1. Properties of enzymes
   - Higher reaction rate
   - Milder reaction condition
   - Greater reaction specificity
   - Capacity for regulation

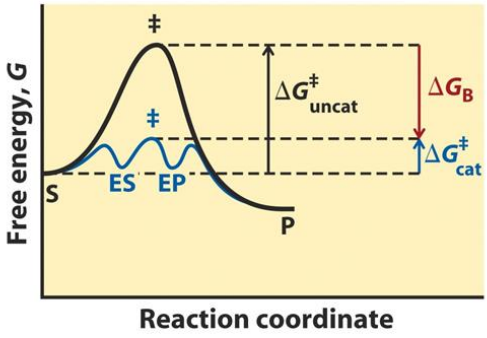


Figure 1 Reaction coordinate diagram.

2. Enzyme as catalyst (催化劑)
   - Ribozyme (catalytic RNA)
   - Protein
     - Holoenzyme (holoprotein) = Prosthetic group + Apoenzyme (apoprotein)
       - Prosthetic group: coenzyme (organic molecule) or cofactor (metal ion).

3. Enzyme classification

Classification	Type of Reaction Catalyzed
1. Oxidoreductase, 氧化還原酶	Oxidation-reduction reactions, $A^- + B \rightleftharpoons A + B^+$
2. Transferase, 轉移酶	Transfer of functional groups, $A\text{-}B + C \rightleftharpoons A + B\text{-}C$
3. Hydrolase, 水解酶	Hydrolysis reactions, $A\text{-}B + H_2O \rightleftharpoons A\text{-}H + B\text{-}OH$
4. Lyase, 裂解酶	Group elimination to form double bonds, $\overset{X\ Y}{\underset{A\text{-}B}{|\ |}} \rightleftharpoons A{=}B + X\text{-}Y$
5. Isomerase, 異構酶	Isomerization, $\overset{X\ Y}{\underset{A\text{-}B}{|\ |}} \rightleftharpoons \overset{Y\ X}{\underset{A\text{-}B}{|\ |}}$
6. Ligase, 接合酶, (synthetase)	Bond formation coupled with ATP hydrolysis, $A + B \rightleftharpoons A\text{-}B$

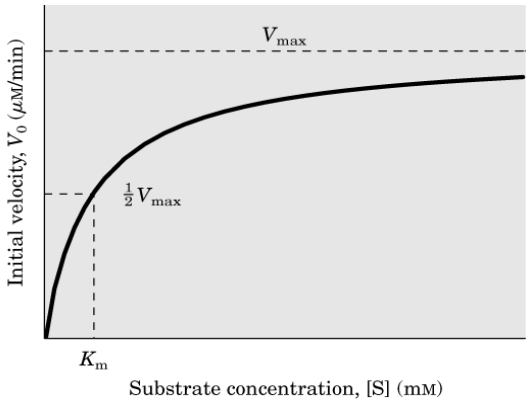
4. Enzymes stabilize transition state (Figure 1) → $\Delta G^{\ddagger}\downarrow$ → Reaction rate ↑
   - E: enzyme; S: substrate; ES: E-S complex
   - Standard free energy change (ΔG, 自由能變化) → Equilibrium 平衡
   - Activation energy ($\Delta G^{\ddagger}$, 活化能) → Reaction rate 反應速率
   - Binding energy (ΔG_B): result from multiple weak E-S interactions.

5. Enzyme kinetics 酵素動力學
   - 又稱 steady-state kinetics, Michaelis-Menten kinetics
   - Assumptions:
     - When [S] >>[E], V_o is determined at the beginning of the reaction when the [P] is infinitely small and the back reaction (S ← P) can be ignored.
     - [ES] is constant
   - Hyperbolic plot 雙曲線 (V_o vs [S])
     - K_m = [S], when V_o = ½ V_{max}
     - V_o = V_{max}, when [S] >> K_m
     - $V_o \propto$ [S], when [S] << K_m

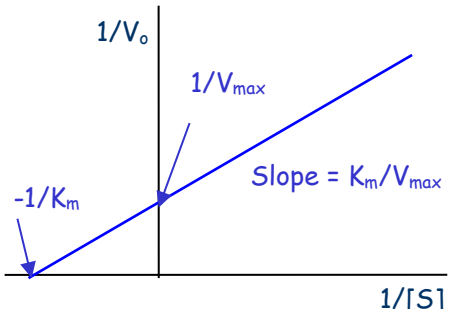
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

6. Lineweaver-Burk plot, double reciprocal plot, $1/V_o$ vs $1/[S]$
   - X-intercept: $1/V_{max}$
   - Y-intercept: $-1/K_m$

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

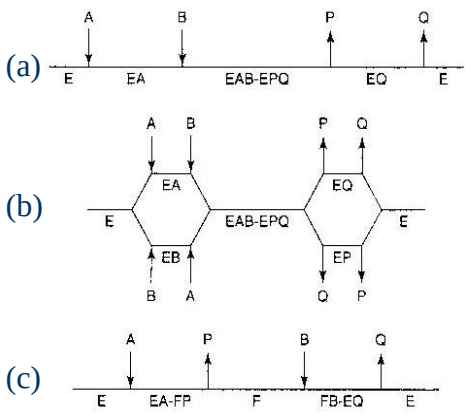


$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



7. Characteristics of an enzyme:
   - K_m: affinity of enzyme for substrate
   - k_{cat}: turnover number 轉換數,
     - k_{cat} = V_{max} / [E_T], 最大反應速率/總酵素濃度
     - The number of S → P in a given unit of time when the E is saturated with S.
     - *Used to compare catalytic efficiency for different substrates of the same enzyme*
   - k_{cat}/K_m: the specificity constant,
     - *Used to compare catalytic efficiency of different enzymes*

8. Bi-substrate and Bi-product reaction (Bi-Bi reaction)
   - A + B ↔ P + Q, catalyzed by enzyme E
   - Sequential displacement (form ternary complex)
     - Compulsory order (ordered Bi-Bi)
       (a) LDH (pyruvate + NADH → lactate + NAD^+)
     - Random order (random Bi-Bi)
       (b) Creatine kinase (ATP + Cr → PCr + ADP)
   - Ping-Pong reaction (double displacement reaction, no ternary complex)
       (c) Aminotransferases

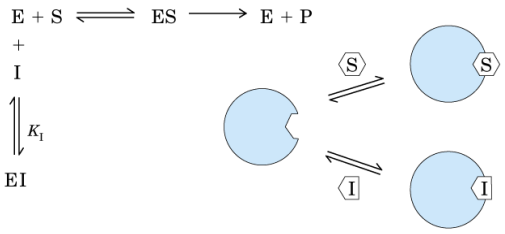


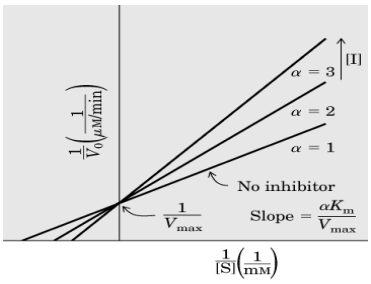
9. Irreversible inhibitor (destroy the active site):
   - Group specific reagent: react with specific R-group of a.a.
     - DIFP (diisopropylphosphofluoridate) inhibits chymotrypsin (serine protease), and acetylcholinesterase.
   - Suicide inhibitor (mechanism-based)
     - Penicillin →acts by covalently modifying transpeptidase (suicide inhibitor)

10. Reversible inhibitor (I, 抑制物)

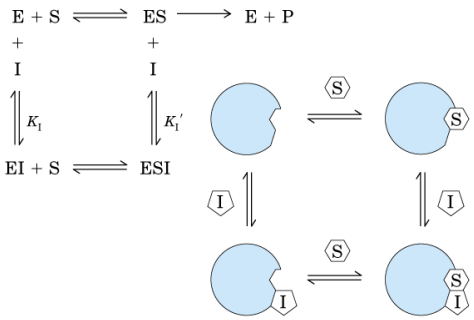
Inhibitor type	Binding site on enzyme	Kinetic effect
Competitive	Specifically at the catalytic (active site). Inhibitor has a similar structure as the substrate. Inhibition is reversed by substrate	V_{max} unchanged, K_m increased
Noncompetitive (mixed type)	I binds E or ES complex other than the active site. Inhibition can not be reversed by substrate.	V_{max} decreased, K_m unchanged
Uncompetitive	I binds only to ES complex other than the active site. Inhibition can not be reversed by substrate.	V_{max} decreased, K_m decreased

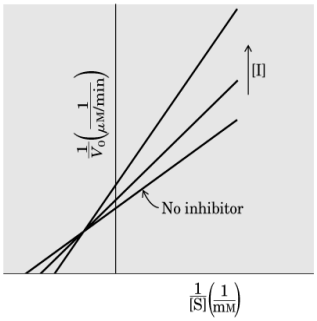
- <u>Competitive:</u>
  - Methanol vs ethanol and alcohol dehydrogenase
  - CO vs O_2

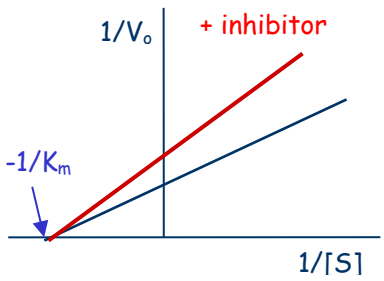




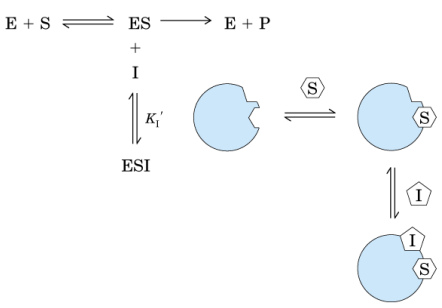
- <u>Mixed (non-competitive, a special case):</u>

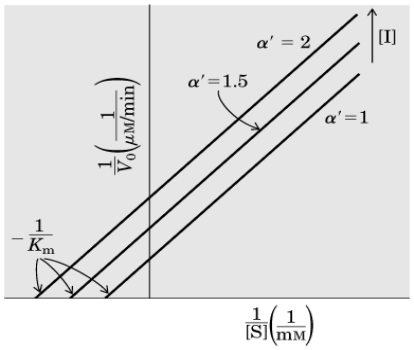




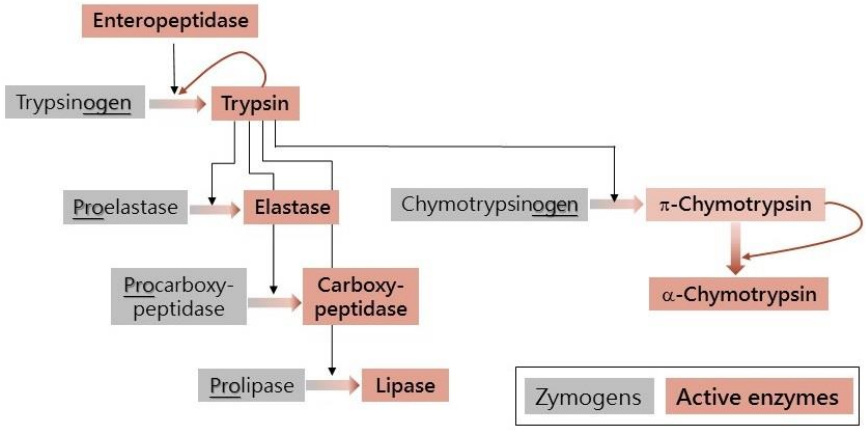


- <u>Uncompetitive:</u>





11. Regulation of enzyme activity
- pH, temperature
- Control of enzyme availability
  - Rate of synthesis
  - Rate of degradation
  - Differential subcellular expression
- Regulation type:
  - Isoenzyme, or isozyme (caused by gene duplication)
    - ◊ Properties:
      - ♦ Enzyme catalyze the same reaction
      - ♦ Different kinetic property: K_m, V_{max}
      - ♦ Different physical property: molecular weight
      - ♦ Different chemical property: amino acid sequence
      - ♦ Different tissue specificity: location
    - ◊ L-lactate dehydrogenase: lactate + NADH → pyruvate + NAD^+
      - ♦ H, heart and M, muscle
      - ♦ LDH (a tetramer): HHHH, HHHM, HHMM, HMMM, MMMM
  - Allosteric (non-covalent)
    - ◊ Hemoglobin; Aspartate transcamoylase (ATCase)
  - Covalent modification
    - ◊ Phosphorylation-dephosphorylation (kinase-phosphatase)
  - Peptide bond cleavage (proteolytic cleavage)
    - ◊ Zymogen, proenzyme, proprotein (inactive precursor) → active enzyme
    - ◊ Digestive enzymes (proteases)
      - ♦ Trypsin formed by enteropeptidase (master activation step)
        - Coordinated control of digestive enzymes (figure below):



    - ◊ Proteins in the blood-clotting cascade
      - ♦ *Serine proteases*: enzymes has *serine* in the active site
      - ♦ *Zymogen*: factors circulate in blood as *inactive form*
      - ♦ *Cascade* reaction:
        - ◊ 1st factor (small amount) → end reaction (large amount)
        - ◊ Rapid response → limiting blood loss
    - ◊ Other examples:
      - ♦ Procaspase → caspase, (programmed cell death, apoptosis)
      - ♦ Proinsulin → insulin (hormone)
      - ♦ Procollagen → collagen (Procollagenase → collagenase)
      - ♦ Timed tissue remodeling in development
        - ◊ Metamorphosis of a tadpole into a frog
        - ◊ Mammalian uterus after delivery

魔咒關鍵詞：

Holoenzyme (holoprotein) = Prosthetic group + Apoenzyme (apoprotein)
Prosthetic group, coenzyme or cofactor
Enzyme (E), Substrate (S), Enzyme-Substrate complex (E-S complex), Transition state
    Standard free energy change (ΔG), Equilibrium
    Activation energy ($\Delta G^{\ddagger}$)
    Binding energy (ΔG_B)
Enzyme kinetics (steady-state kinetics, Michaelis-Menten kinetics)
    Hyperbolic plot, V_o, K_m, V_{max}
    Lineweaver-Burk plot (double reciprocal plot)
    k_{cat}, k_{cat}/K_m
Reversible inhibitor (I):
    Competitive, Uncompetitive, Non-competitive
Regulation
    Isoenzyme (isozyme)
    Allosteric enzyme (non-covalent)
    Covalent modification
    Peptide bond cleavage (proteolytic cleavage)
        Inactive precursor: zymogen, proenzyme, proprotein
        Cascade reaction

魔法參考書目:

1. 台大莊榮輝教授教學網頁: http://juang.bst.ntu.edu.tw/BCbasics/index.htm
2. Lehninger Principles of Biochemistry (2013), 6th ed, David L. Nelson, and Michael M. Cox, Freeman and Company, New York.
3. Principles of Biochemistry (2013) 4th ed. Voet, Voet, and Pratt. Wiley.
4. Biochemistry, a short course. (2015) John L. Tymoczko, Jeremy M. Berg, Lubert Stryer (3rd ed) W.H. Freeman & Company.

魔法練習題:

1. 酵素對化學反應的反應平衡、反應速率、自由能變化(ΔG,) 、活化能($\Delta G^{\ddagger}$)等參數有何影響？（增加、減少、或不變）。
2. 生化學家研究一個遵循Michaelis-Menten kinetic的酵素，得到下列數據：

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

   (a) 請作Michaelis-Menten 的圖 (反應速率V_o 對受質濃度[S]作圖)。
   (b) 請作Lineweaver-Burk 的圖 ($1/V_o$ 對1/[S]作圖) 。
   (c) 請問這個酵素的最大反應速率V_{max} 是多少？K_m 是多少？
   (d) 加入抑制物X後再測量，得到V_{max} 為700 μmole/min, K_m 為10 μM, 請問X是屬於哪一類型的抑制物？這種抑制物的特徵為何？
3. 調控酵素活性的方式有哪些？