Chapter 13

Enzyme – Kinetics (動力學) and Specificity (特異性、專一性)

Biochemistry

by

Reginald Garrett and Charles Grisham

Essential Question

- What are enzymes?

  Features, Classification, Terms, Definition

- What do they do?

  Mechanism, Theory

13.1 – What Characteristic Features Define Enzymes?

I. Enzymes endow cells with the remarkable capacity to exert kinetic control over thermodynamic potentiality

II. Enzymes are the agents of metabolic function

*** Biological catalysts that function in

- dilute,
- aqueous solutions
- under mild conditions (e.g., pH)
- low temperatures to increase reaction rate

***細胞代謝酵素 是“生物性催化劑”作用條件:
在 稀釋(低濃度)、水相 、溫和pH、 低溫 下增加反應速率

II. Thermodynamic potentiality of enzyme

生物系統 利用酵素
“加速” 與 “控制” 生化反應

II. Enzymes are the agent of metabolic functions

酵素 是代謝功能中的”反應劑”

*** 酵素 有三獨特性***

(1) Catalytic power (催化力)

*Ratio of catalyzed rate to uncatalyzed rate

*Rate acceleration up to 10^{26} power

(2) Specificity (專一性)

*The reactant in an enzyme-catalyzed reaction is called the substrate (基質).

*Substrate specificity (selectivity, 分子相互辨認, 結構互補)

*Active site (酶分子上基質結合部位 環境有關)

* Induced-fit by “dynamic recognition” through conformation flexibility (構形彈性 作動能性辨認)

(3) Regulation (調節性)

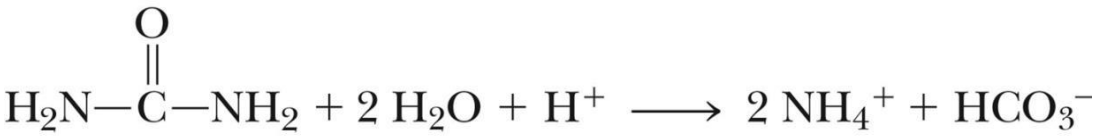
*Regulate enzyme activity (沒有副產物之浪費)

*Inhibition, Activation

Catalytic power (催化力)

- Enzymes can accelerate reactions as much as 10^{26} over uncatalyzed rates!
  However, the uppor limit on Kcat/Km (vatalytic efficiency) is about 10^{9}

- Urease is a good example:



  - Catalyzed rate: $3x10^{4}$/sec
  - Uncatalyzed rate: $3x10^{-10}$ /sec
  - Ratio is $1x10^{14}$

Enzyme lowers free energy of activation ($\Delta G^{\ddagger}$) Accelerate rate

降低活化自由能 ($\Delta G^{\ddagger}$)

不改變標準自由能(ΔG)

(指的是$\Delta G^{o'}$)

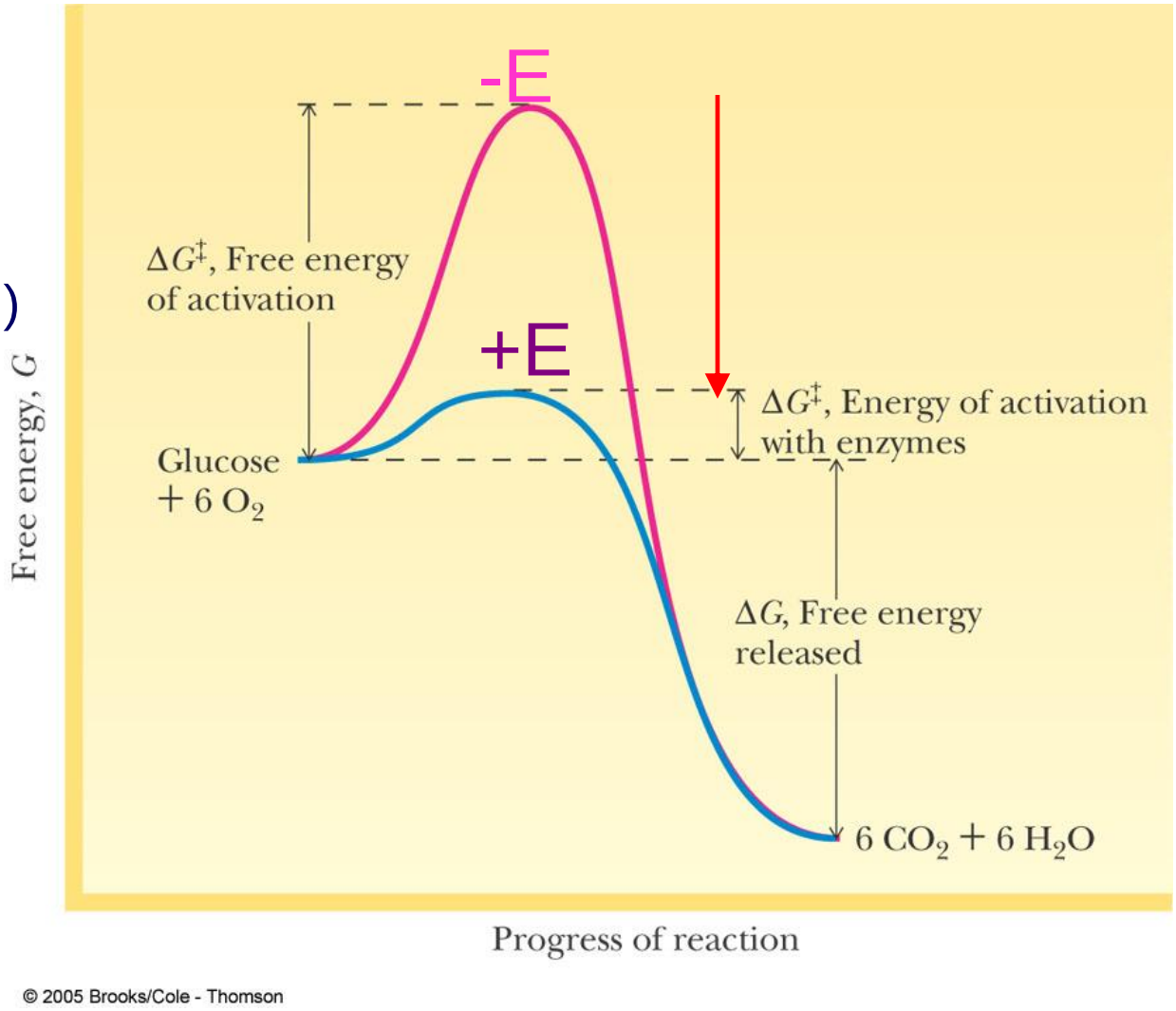


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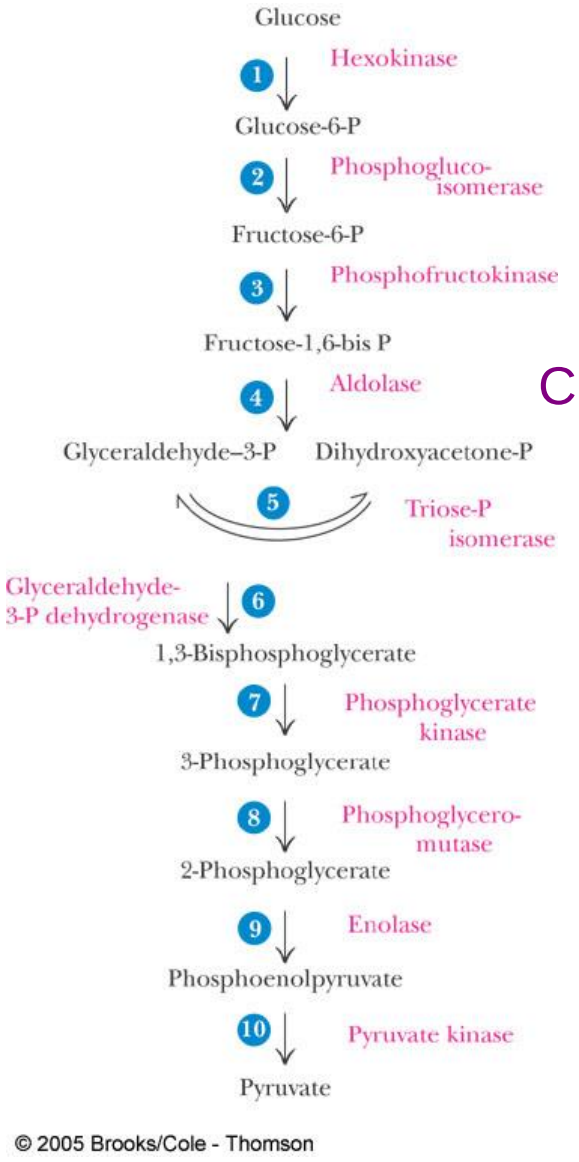
Figure 13.1
Reaction profile showing large $\Delta G^{\ddagger}$ for glucose oxidation, free energy change of -2,870 kJ/mol; catalysts lower $\Delta G^{\ddagger}$, thereby accelerating rate.

Figure 13.2

The breakdown of glucose by *glycolysis* provides a prime example of a metabolic pathway.

Ten enzymes mediate the reactions of glycolysis. Enzyme 4, *fructose* 1,6, *biphosphate aldolase*, catalyzes the C-C bond- breaking reaction in this pathway.

糖解作用中有10個酵素參與, 第四個F-1,6-BP aldolase將1分子C6 葡萄糖斷裂成2分子C3, 再轉換成 丙酮酸



Nomenclature (命名)

- Formally, enzymes are classified by a system of nomenclature based on the particular reaction they catalyze, although certain trivial names for enzymes often enjoy common usage.

Nomenclature (命名)

- International Commission on Enzymes

E.C. (enzyme commission number國際酵素委員彙編 碼)

- Since 1956
- According to the reaction they catalyzed
- Six classes

- Systematic name (系統名)
- Trivial name or Common names—”ase” urase, kinase (俗名)

E.C. Six classes (Table 13.1)

1. Oxidoreductase (氧化還原酶)
   - Oxidation-reduction rxn
2. Transferase (轉移酶)
   - Transfer of functional groups
3. Hydrolase (水解酶)
   - Hydrolysis rxn
4. Lyase (裂合酶)
   - Addition of double bonds
5. Isomerase (異構酶)
   - isomerization
6. Ligase (接合酶)
   - Formation of bonds with ATP cleavage

E.C.2.7.1. 代表什麼?

- E.C. Enzyme Commission
- Class: 2. Transferase
- Subclass: 7. P-containing group transfer, phosphotransferase
- Sub-subclass: 1. with an alcohol group as an acceptor
- Sub-sub-sub class: 2. ATP:D-g-6-p

Xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

- Systematic name: ATP:D-glucose-6-phosphotransferase
- Trivial name: glucokinase (E.C.2.7.1.2.)

完整表示:
glucokinase (E.C.2.7.1.2. ; ATP:D-glucose-6-phosphotransferase)

葡萄糖激酶 轉移ATP上的˙P到D-glucose第6C上的OH

xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
Sub-sub-sub class 上碼1, 不限定6C糖種類 反應物 與轉移位置 較廣
Trivial name: hexokinaes (E.C.2.7.1.1.)
Systematic name: kinase: ATP-dependent phosphotransferase)

TABLE 13.1 Systematic Classification of Enzymes According to the Enzyme Commission

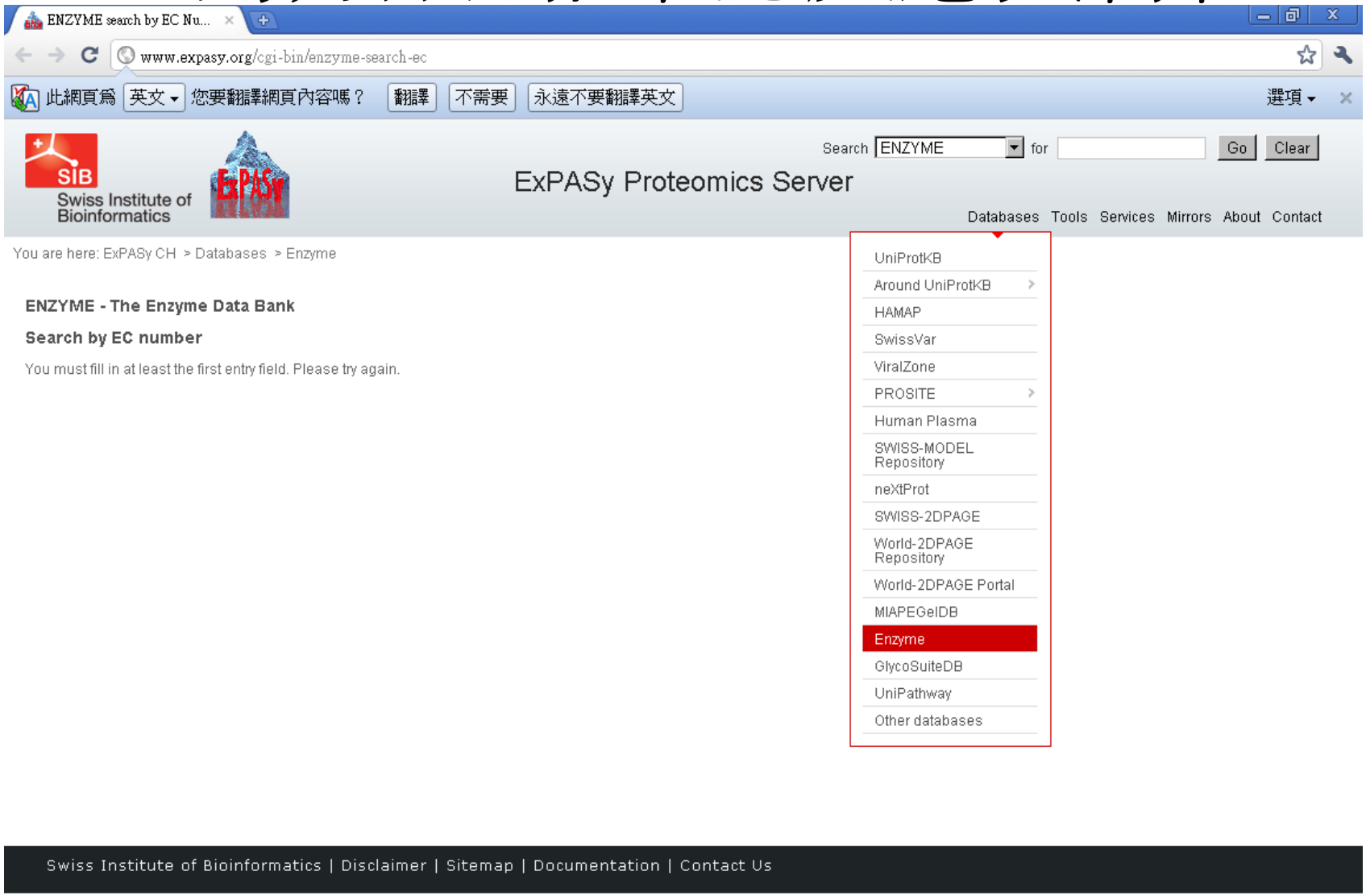
E.C. Number	Systematic Name and Subclasses
1	*Oxidoreductases* (oxidation–reduction reactions)
1.1	Acting on CH—OH group of donors
1.1.1	With NAD or NADP as acceptor
1.1.3	With O_2 as acceptor
1.2	Acting on the $\rangle$C=O group of donors
1.2.3	With O_2 as acceptor
1.3	Acting on the CH—CH group of donors
1.3.1	With NAD or NADP as acceptor
2	*Transferases* (transfer of functional groups)
2.1	Transferring C-1 groups
2.1.1	Methyltransferases
2.1.2	Hydroxymethyltransferases and formyltransferases
2.1.3	Carboxyltransferases and carbamoyltransferases
2.2	Transferring aldehydic or ketonic residues
2.3	Acyltransferases
2.4	Glycosyltransferases
2.6	Transferring N-containing groups
2.6.1	Aminotransferases
2.7	Transferring P-containing groups
2.7.1	With an alcohol group as acceptor
3	*Hydrolases* (hydrolysis reactions)
3.1	Cleaving ester linkage
3.1.1	Carboxylic ester hydrolases
3.1.3	Phosphoric monoester hydrolases
3.1.4	Phosphoric diester hydrolases

E.C. Number	Systematic Name and Subclasses
4	*Lyases* (bond cleavage by means other than hydrolysis or oxidation)
4.1	C—C lyases
4.1.1	Carboxy lyases
4.1.2	Aldehyde lyases
4.2	C—O lyases
4.2.1	Hydrolases
4.3	C—N lyases
4.3.1	Ammonia lyases
5	*Isomerases* (isomerization reactions)
5.1	Racemases and epimerases
5.1.3	Acting on carbohydrates
5.2	*Cis-trans* isomerases
6	*Ligases* (formation of bonds with ATP cleavage)
6.1	Forming C—O bonds
6.1.1	Amino acid–RNA ligases
6.2	Forming C—S bonds
6.3	Forming C—N bonds
6.4	Forming C—C bonds
6.4.1	Carboxylases

查詢分類碼, 確認反應與特性

http://www.expasy.org/cgi-bin/enzyme-search

查詢分類碼, 確認反應與特性



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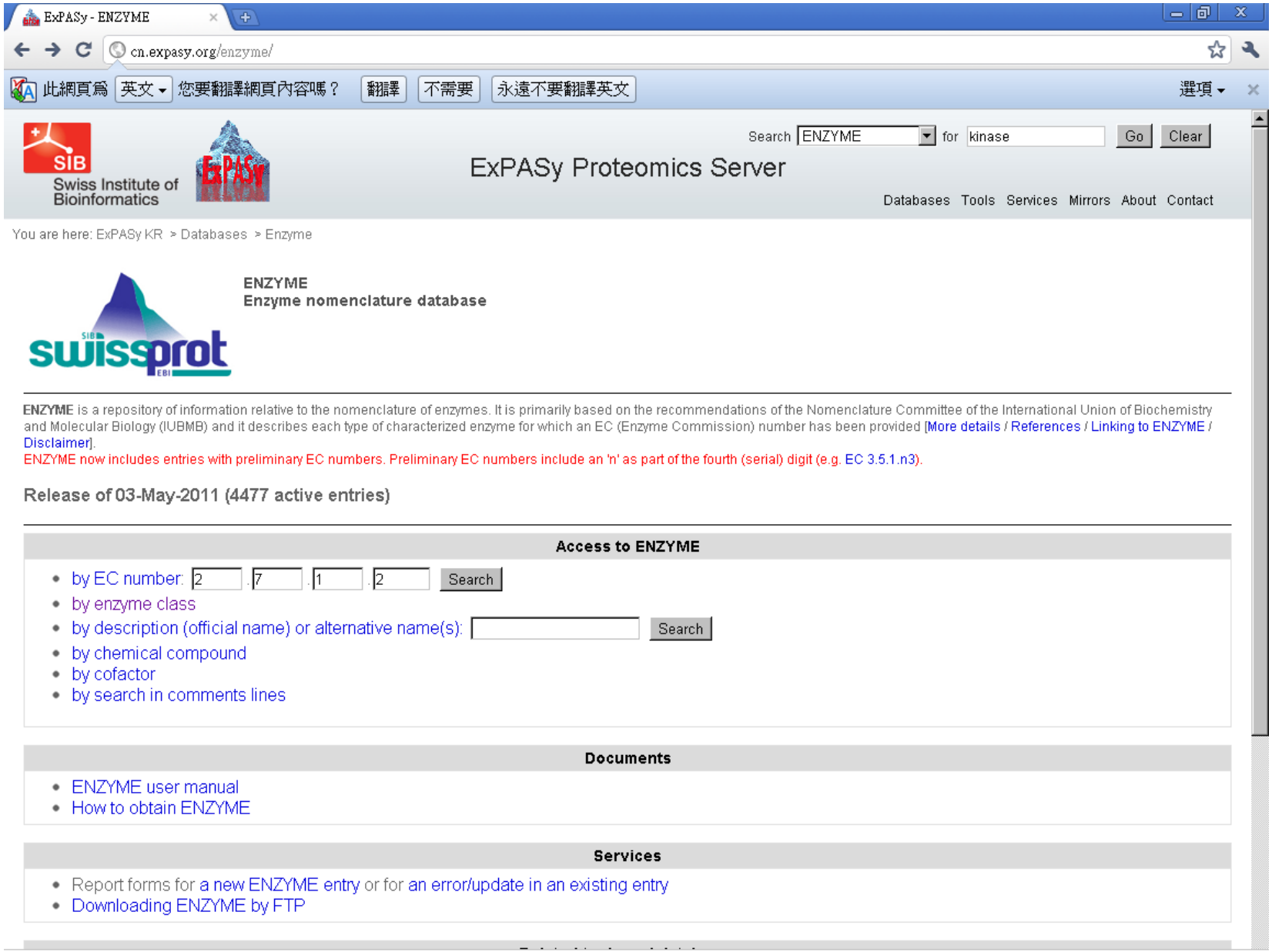
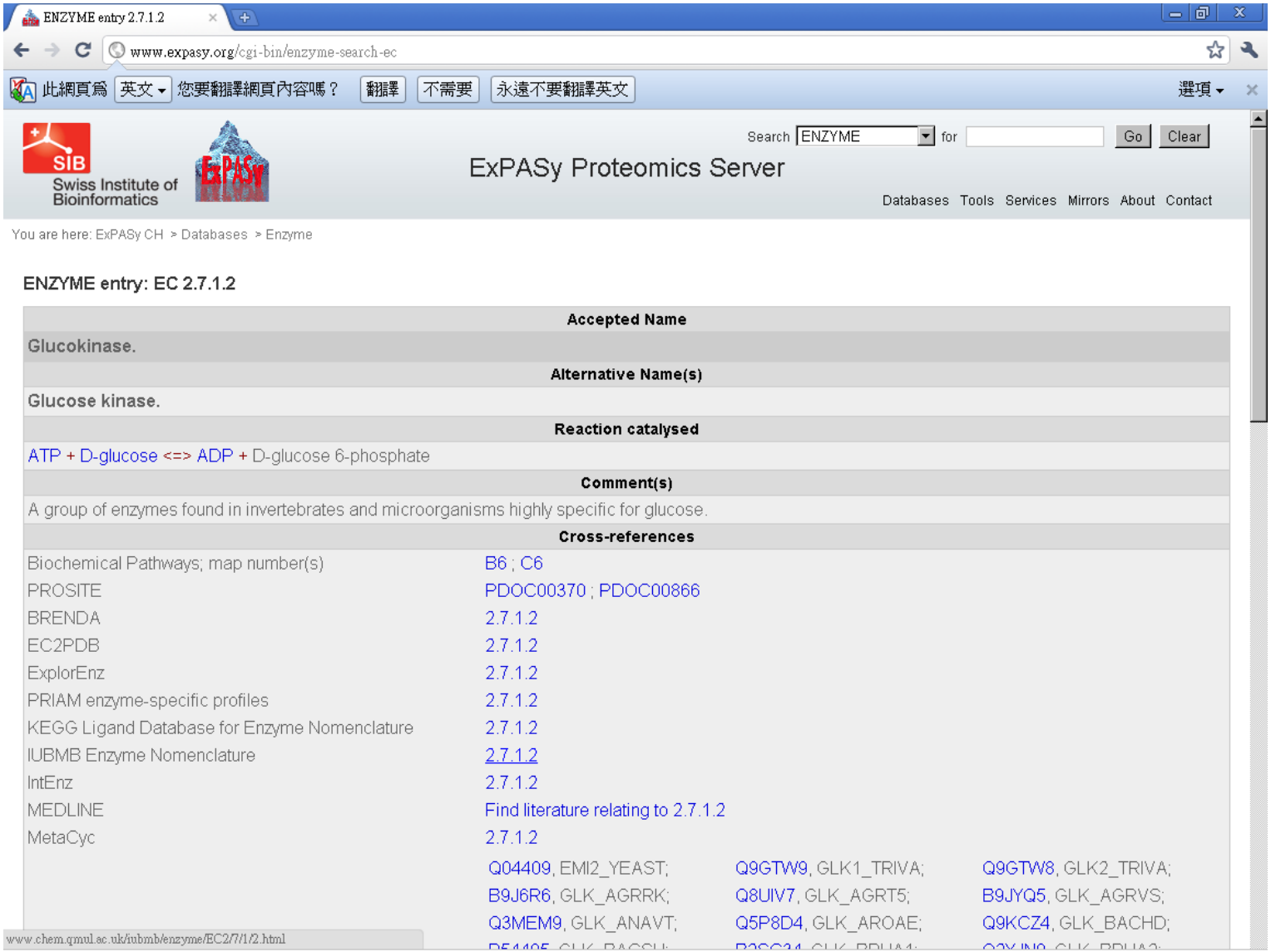


查詢分類碼, 確認反應與特性



IUBMB Enzyme Nomenclature

EC 2.7.1.2

* IUBMB 也可查
* CAS no. (Chemistry Abstracts Registry Number)
酵素為´biological catalyst, 有各自CAS 號碼

Accepted name: glucokinase

Reaction: ATP + D-glucose = ADP + D-glucose 6-phosphate

Other name(s): glucokinase (phosphorylating)

Systematic name: ATP:D-glucose 6-phosphotransferase

Comments: A group of enzymes found in invertebrates and microorganisms that are highly specific for glucose.

Links to other databases: BRENDA, EXPASY, KEGG, PDB, CAS registry number: 9001-36-9

References:

1. Baumann, P. Glucokinase of *Dictyostelium discoideum*. *Biochemistry* 8 (1969) 5011-5015. [PMID: 4312464]

2. Bueding, E. and MacKinnon, J.A. Hexokinases of *Schistosoma mansoni*. *J. Biol. Chem.* 215 (1955) 495-506.

3. Porter, E.V., Chassy, B.M. and Holmlund, C.E. Purification and kinetic characterization of a specific glucokinase from *Streptococcus mutans* OMZ70 cells. *Biochim. Biophys. Acta* 709 (1982) 178-186. [PMID: 7150605]

[EC 2.7.1.2 created 1961]

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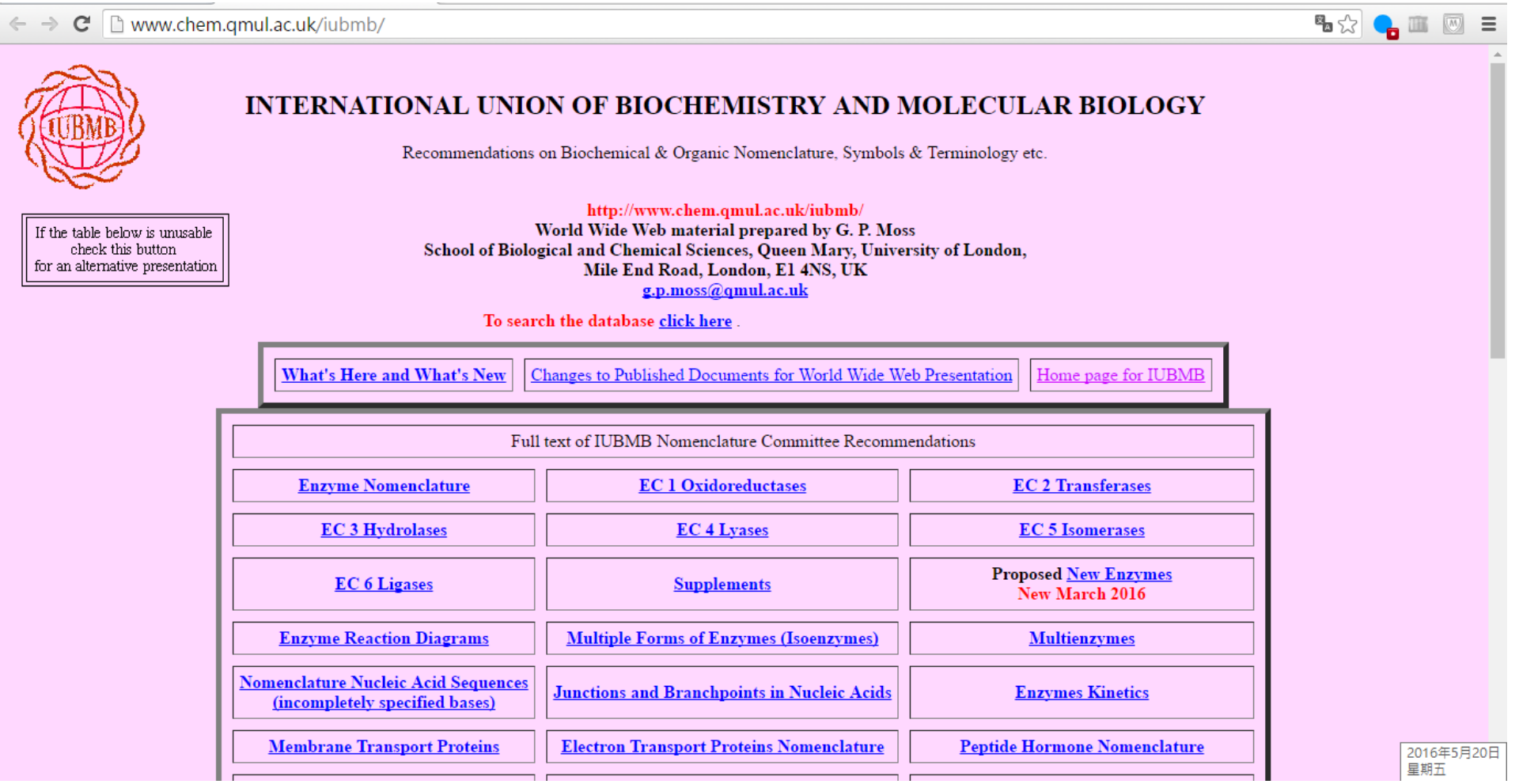
IUBMB查詢　E.C.x.x.x.x.



Terms

- Cofactor (輔因子): nonprotein component (metal ions and organic coenzyme)
- Coenzyme (輔酶): Organic compound cofactor
- Prosthetic group (輔基) tightly bound coenzyme

輔因子是酶之非蛋白質部份,包括輔酶與金屬離子

- Holoenzyme(全酶): Protein and prosthetic group
- Apoenzyme (脫輔基 酶蛋白質): Protein lacking prosthetic group

輔酶 **(Coenzyme)** 為有機分子, 通常由維生素衍生而來,以輔基 **(prothetic group)** 結合於 酵素分子中, 參與官能基轉移, 氧化還原等)

Vitamin	Coenzyme	Process
❖ Thiamine(B_1)	TTP	decarboxylation
❖ Niacin	$NAD(P)^+$	redox
❖ Riboflavin(B_6)	Pyridoxal P	amino group transfer
❖ Folic acid	THF	one-carbon transfer
❖ Vit A	retinal	vision, growth

酶以金屬離子為”輔因子”
稱為”metalloenzyme”,
為反應物

Coenzymes (輔酶)當作
特定原子 或 官能基 的短
暫攜帶者

TABLE 13.2 Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated

Metal Ions and Some Enzymes That Require Them		Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups		
Metal Ion	Enzyme	Coenzyme	Entity Transferred (被轉移的實體)	Representative Enzymes Using Coenzymes
Fe^{2+} or Fe^{3+}	Cytochrome oxidase	Thiamine pyrophosphate (TPP)	Aldehydes	Pyruvate dehydrogenase
	Catalase	Flavin adenine dinucleotide (FAD)	Hydrogen atoms	Succinate dehydrogenase
	Peroxidase	Nicotinamide adenine dinucleotide (NAD)	Hydride ion ($:H^-$)	Alcohol dehydrogenase
Cu^{2+}	Cytochrome oxidase			
Zn^{2+}	DNA polymerase	Coenzyme A (CoA)	Acyl groups	Acetyl-CoA carboxylase
	Carbonic anhydrase	Pyridoxal phosphate (PLP)	Amino groups	Aspartate aminotransferase
	Alcohol dehydrogenase			
Mg^{2+}	Hexokinase	5′-Deoxyadenosylcobalamin (vitamin B_{12})	H atoms and alkyl groups	Methylmalonyl-CoA mutase
	Glucose-6-phosphatase			
Mn^{2+}	Arginase	Biotin (biocytin)	CO_2	Propionyl-CoA carboxylase
K^+	Pyruvate kinase (also requires Mg^{2+})	Tetrahydrofolate (THF)	Other one-carbon groups, such as formyl and methyl groups	Thymidylate synthase
Ni^{2+}	Urease			
Mo	Nitrate reductase			
Se	Glutathione peroxidase			

Specificity (專一性)

- Enzymes selectively recognize proper substrates over other molecules
- Enzymes produce products in very high yields - often much greater than 95%
- Specificity is controlled by structure in the active site- the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield

全酶 (Holoenzyme) 四級結構例子:
E. coli DNA聚合酶III 全酶有17 protein subunits

DNA聚合酶I, II和V的功能在DNA修復, 聚合酶III是主要DNA複製酶,
每一大腸桿菌細胞只有40聚合酶III分子, 全酶 $(\alpha\varepsilon\theta)_2\beta_2\tau_2\gamma\delta\delta'\chi\psi$, 反應需要Zn+2 參與

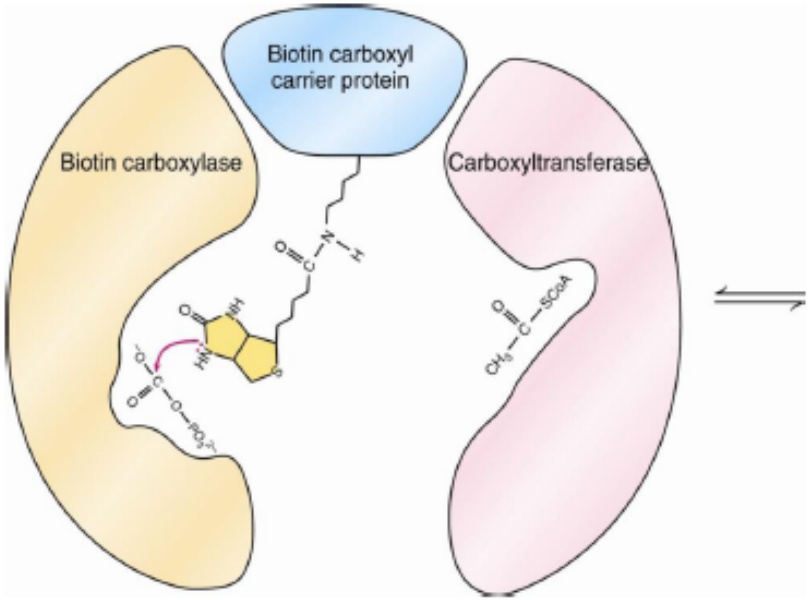
TABLE 28.2 Subunits of *E. coli* DNA Polymerase III Holoenzyme

Subunit	Mass (kD)	Function
α	130	Polymerase
ϵ	27.5	3′-Exonuclease
θ	8.6	ϵ-subunit stabilization
τ	71	DNA template binding; core enzyme dimerization
β	41	Sliding clamp, processivity
γ	47.5	Part of the γ-complex*
δ	39	Part of the γ-complex*
δ'	37	Part of the γ-complex*
χ	17	Interaction with SSB and the γ-complex
ψ	15	Interaction with χ and the γ-complex

*Subunits τ, γ, δ, δ', χ, and ψ form the so-called γ-complex responsible for adding β-subunits (the sliding clamp) to DNA and anchoring the sliding clamp to the two core DNA polymerase III structures. The γ-complex is referred to as the clamp loader.

全酶 (Holoenzyme) 有tightly bound coenzyme (prothetic group)

例子: 大腸桿菌Acetyl-CoA Carboxylase (ACC) holoenzyme 有三個subunits



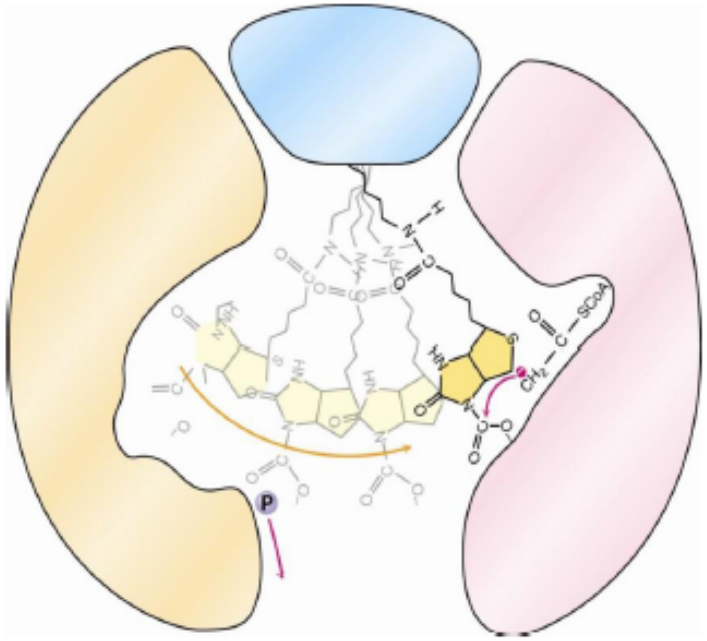


Figure 24.3 In the ACC reaction, the biotin ring acquires carboxyl groups from carbonyl-phosphate on the biotin carboxylase subunit.

Biotin on a flexible tether delivers carboxyl groups from the carboxylase to the carboxyltransferase.

糖解作用中10個酵素藉由 可逆性, 活化, 抑制, 量的調節, 使反應持續進行, 不至於終反應只有35%

Actually step 1 to step 10 has regulation to proceed continuously

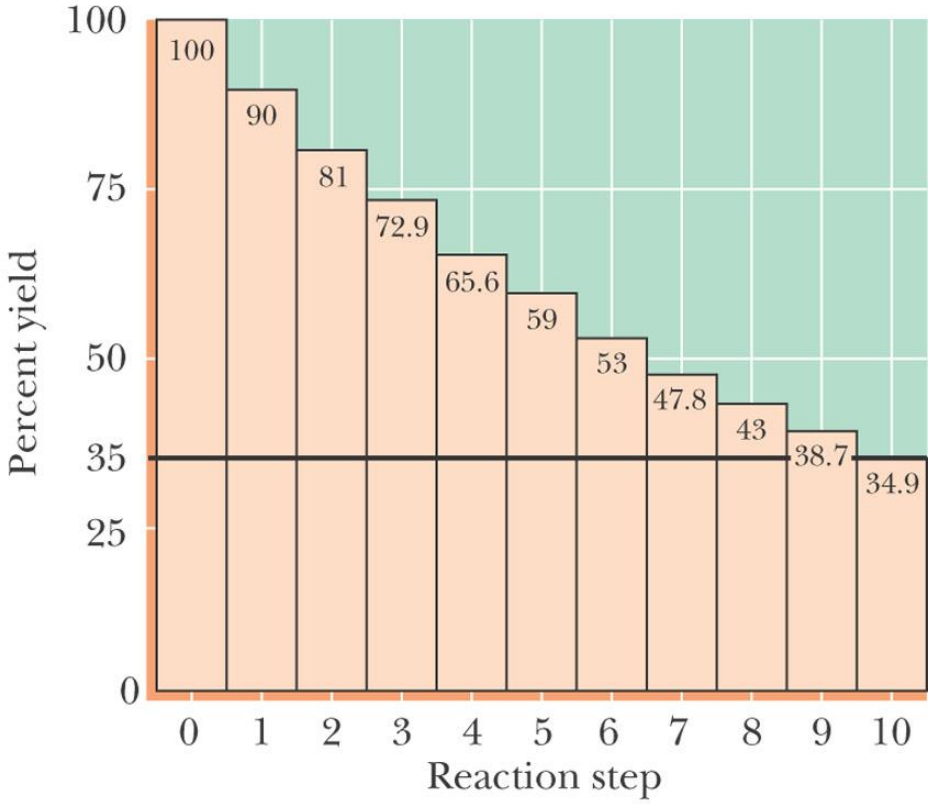


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Figure 13.3 A 90% yield over 10 steps, for example, in a metabolic pathway, gives an overall yield of 35%. Therefore, yields in biological reactions *must be substantially greater*; otherwise, unwanted by-products would accumulate to unacceptable levels.

13.2 – Can the Rate of an Enzyme-Catalyzed Reaction Be Defined in a Mathematical Way?

以數學方式定義酶的催化反應速率

Enzyme kinetics (酵素動力學)

- Kinetics is the branch of science concerned with the rate of chemical reaction.
- Enzyme kinetics addresses
  - the biological roles of enzyme catalysis
  - How they accomplish
  - Determine maximum velocity (V_{max})
  - Binding affinity for substrate and inhibitors (K_m,K_i)
  - Mechanism of catalytic action
  - Essential to understand metabolism

Terms to know

- rate or velocity (v)
- rate constant (k)
- rate law ($v = -dA/dt = k[A]^n$)
- molecularity of an enzyme reaction

  Overall stoichiometry（化學比例計量）in nature

  ≤ 3 order of a reaction

  A → P

  A → I → J → P

  A + B → P + Q

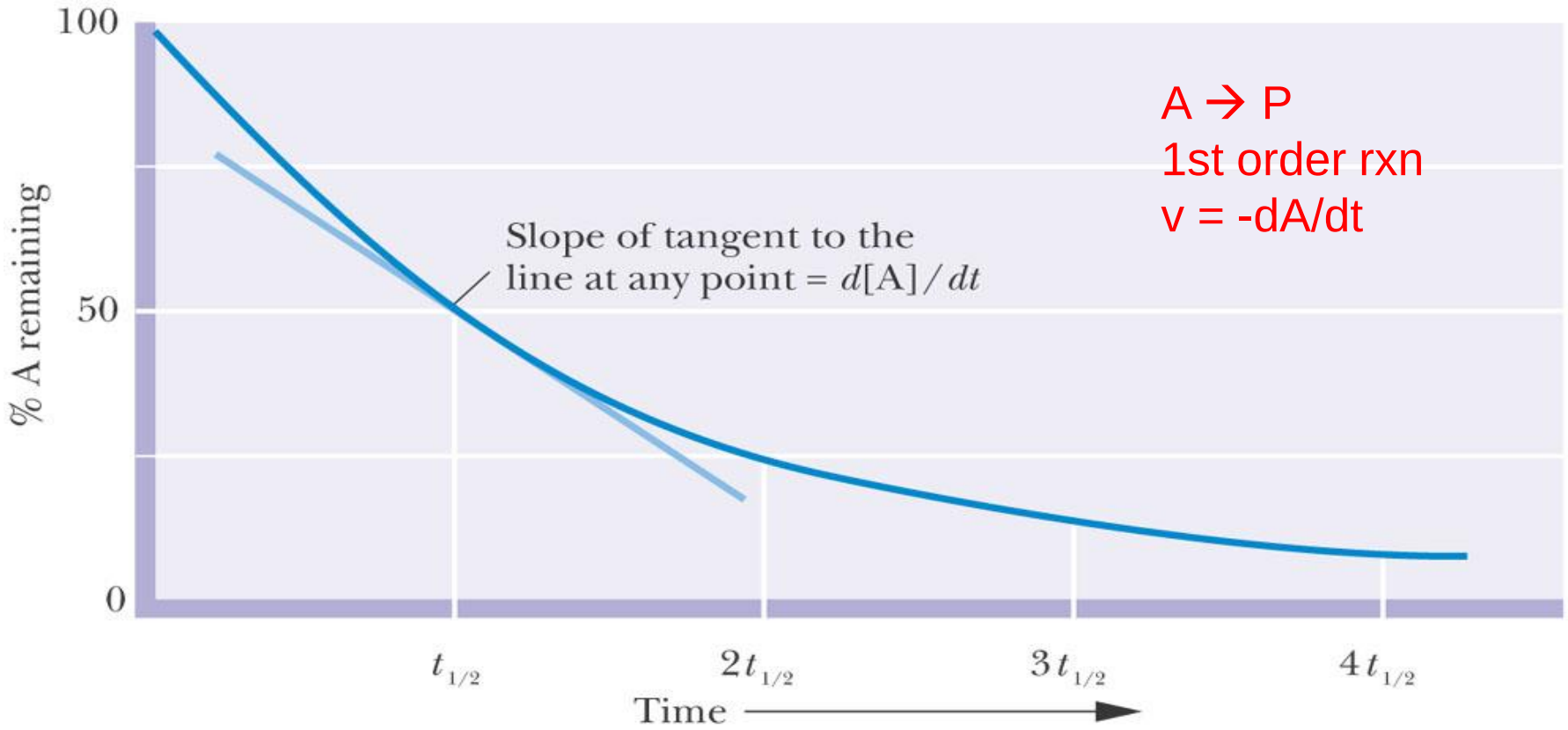


Figure 13.4
Plot of the course of a first-order reaction.

The half-time, $t_{1/2}$, is the time for one-half of the starting amount of A to disappear.

消耗一半反應物分子所需要的時間

Chemical kinetics

- Rate law: $v = -dA/dt = k[A]^n$
  - k = rate constant
  - n = order of reaction,
    - n=1, first order [A]
    - n=2, second order $[A]^2$ or [A][B] (n1for A and n1 for B)

- Molecularity: Number of molecules that must simultaneously react

(必需同時反應的分子數目）

- **ALL RATE EQUATIONS ARE DETERMINED EXPERIMENTALLY!! (由實驗決定)**

Reaction rates

- Limited by activation barrier: Free energy needed to reach transition state

- Relationship between activation energy and the rate constant k
  - Arrhenius equation $k = Ae^{-\Delta G/RT}$
  - Influenced by
    - Temperature
    - Catalysts
  - If the ΔG decrease, reaction rate increase

- 反應速律受限於活化能：自由能需要達到過渡態,
- 活化能改變 (ΔG)減少,反應速律增加
- 活化能受溫度與催化劑影響

The Transition State (過渡態)

區分ΔG (自由能差)與 $\Delta G^{\ddagger}$ (活化自由能差)

- 一級反應A → P 發生期, A分子的部分, 能量提升到反應狀態, 即過渡態, 使轉換成產物, 有助反應物基質A提升能量, 克服反應自由能的因素, 增加反應速率, 即單位時間產物生成

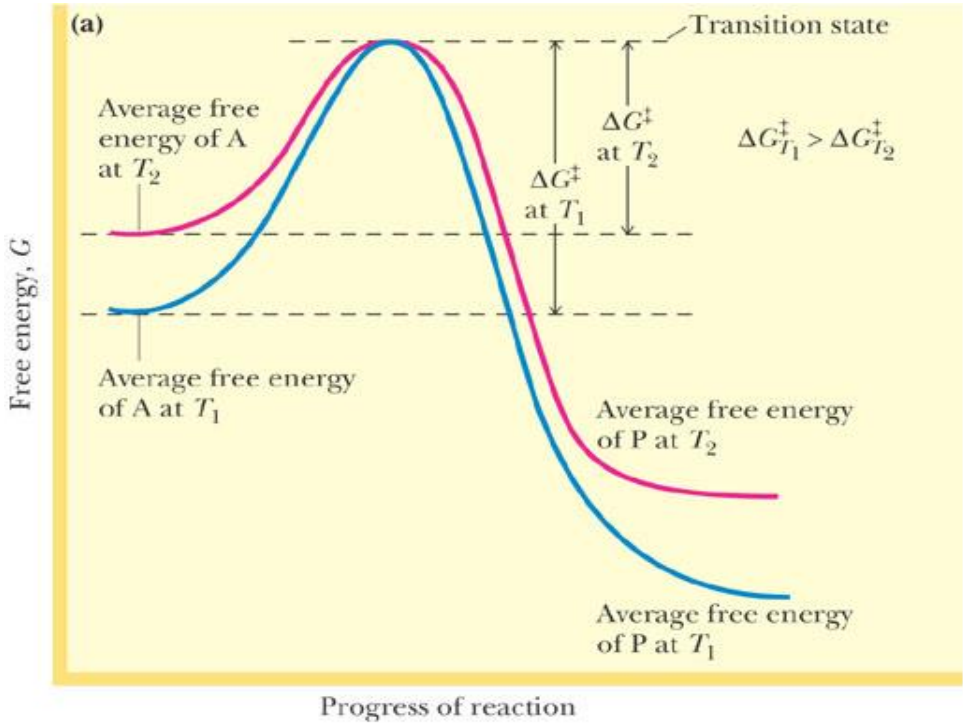
* The overall free energy change (整體自由能改變)for a reaction is related to the equilibrium constant (平衡常數)--特定反應是不變的

* The free energy of activation (活化自由能) for a reaction is related to the rate constant (速率常數)-- 受溫度與催化劑影響

由Rate law與Arrhenius equation 關係得知:

反應速律 受限於活化能：自由能需要達到過渡態,
活化能改變 (ΔG)減少, 反應速律增加
活化能受溫度與催化劑影響

Figure 13.5
Energy diagram for a chemical reaction (A→P) and the effects of **(a)** raising the temperature from T1 to T2 or **(b)** adding a catalyst.



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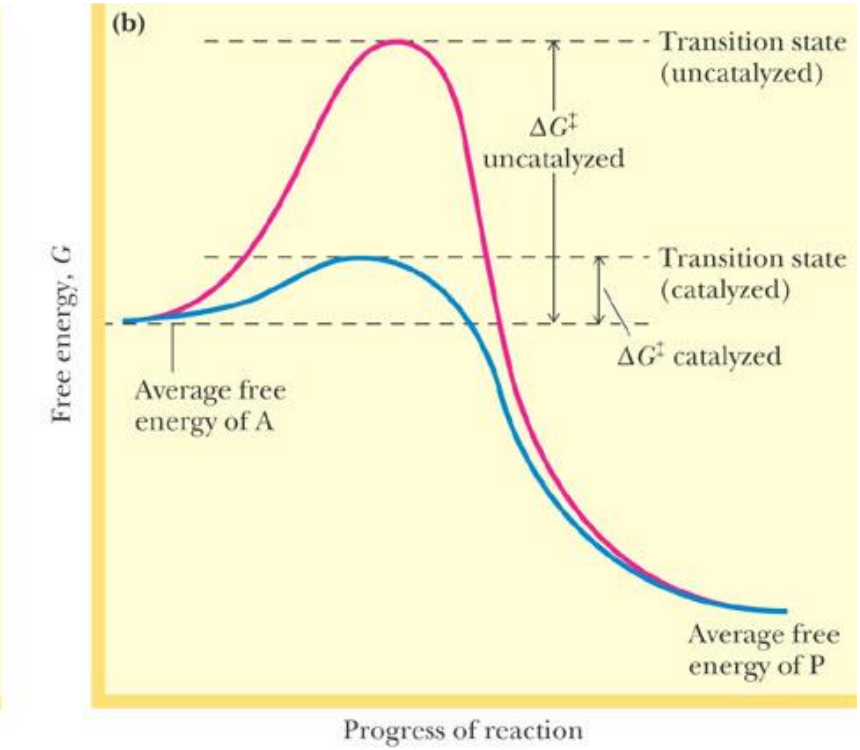
ΔG‡ decrease,
reaction rate increase

加溫

Raising the temperature raises the average energy of A molecules, which increases the population of A molecules having energies equal to the activation energy for the reaction, thereby increasing the reaction rate.

ΔG‡ decrease,
reaction rate increase

加催化劑



In contrast, the average free energy of A molecules remains the same in uncatalyzed versus catalyzed reactions (conducted at the same temperature).

The effect of the catalyst is to lower the free energy of activation for the reaction.

13.3 – What Equations Define the Kinetics of Enzyme-Catalyzed Reactions?

- Enzymes accelerate reactions by lowering the free energy of activation
- Enzymes do this by binding the transition state of the reaction better than the substrate

酶藉由降低活化的自由能, 結合反應過渡態比基質更好, 加速反應

Important terms (重要名辭)

- Active site
- K_m = Michaelis constant
- V_{max}
- Turnover number, k_{cat}
- Catalytic efficiency (k_{cat}/K_m)
- katal, Unit
- Specific activity
- Q_{10}

最適當反應條件下測得一組 酵素動力學參數

*** 改變條件會獲得一組不同參數 (Vmax, Km etc.)

(1) 固定反應條件:
酵素純度, 濃度固定 (由適量化之線性關係獲得)
特定溫度, 相同反應時間
特定反應條件 (buffer, pH, cofactors, salt/ionic etc)
反應偵測 (基質減少或產物生成) 在線性範圍內
反應操作中加成份步驟, 最後加S或E之啟動反應順序
(2) 單一變因**:** 例如 不同基質濃度, 製作 Substrate saturation curve
(3) 相同酵素在相同反應條件下, 比較變因的影響

Active Site (活化部位)

- The place on/in the enzyme where S bind
- Comprise only a small portion of the overall enzyme molecule
- The conformation of the active site is structured to form a special pocket or cleft
- Conformation change upon binding (Induced fit)
- S recognize E by structural complementary
- S bind to E through weak force:
  - H bond, ionic bond (salt bridge), van der Waals interactions

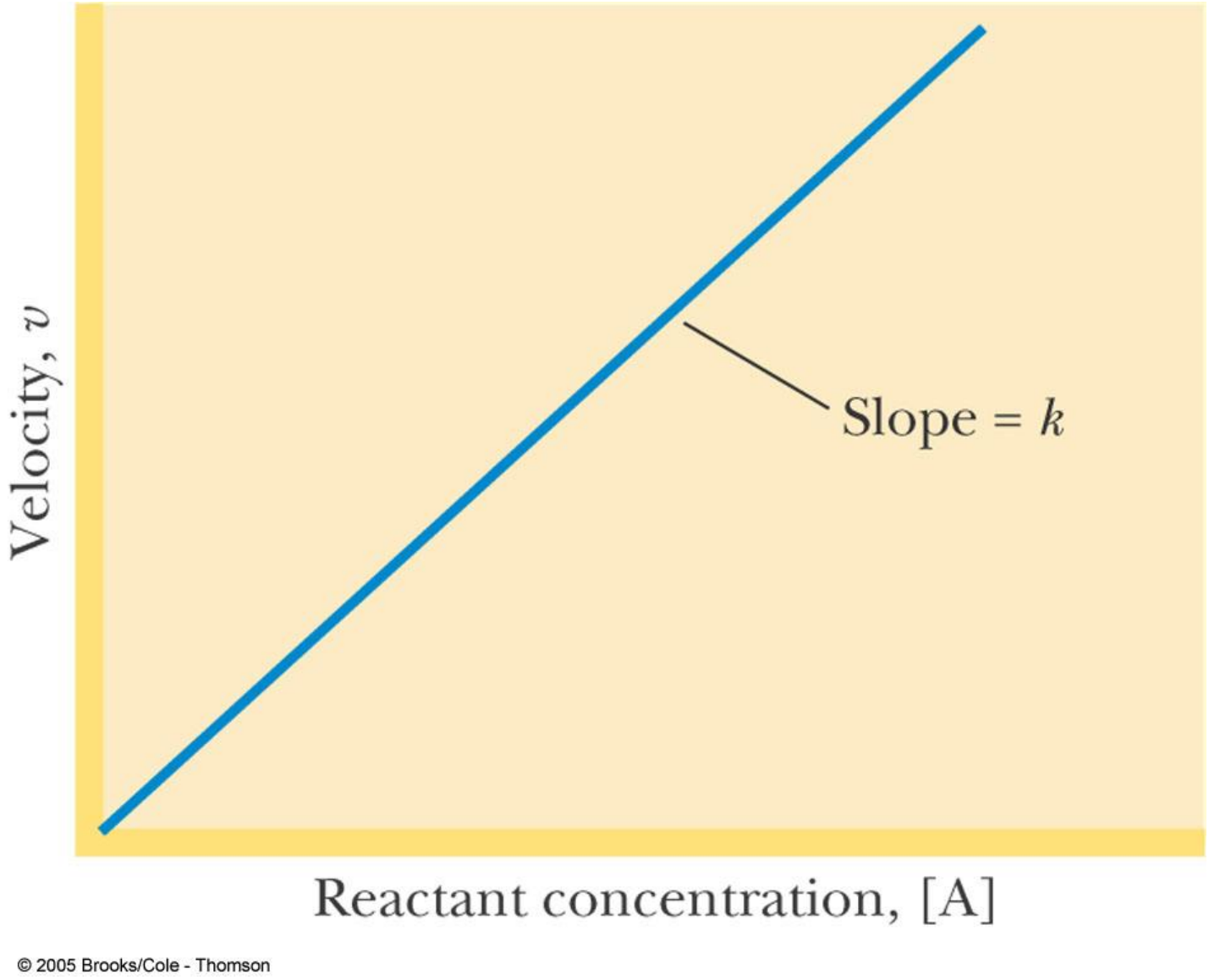


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Figure 13.6
A plot of *v* versus [A] for the unimolecular chemical reaction, A→P, yields a straight line having a slope equal to *k*.

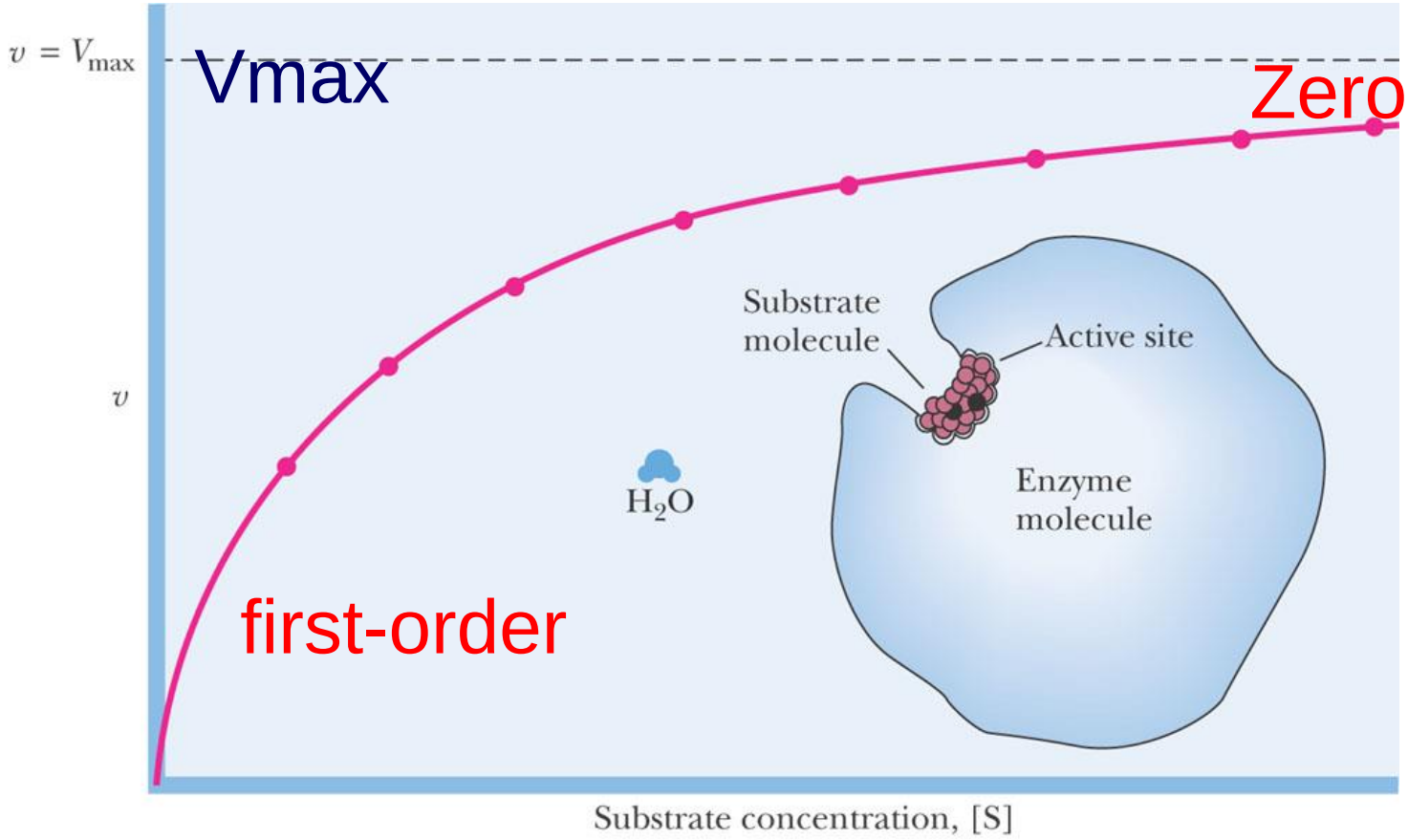
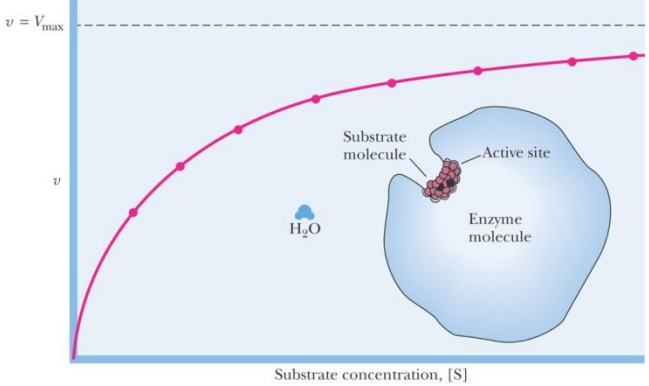


Figure 13.7 Substrate saturation curve for an enzyme-catalyzed reaction.
(基質飽和曲線)

The amount of enzyme is constant, and the velocity of the reaction is determined at various substrate concentrations.

The H2O molecule provides a rough guide to scale. The substrate is bound at the active site of the enzyme.



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The reaction rate, *v*, as a function of [S] is described by a rectangular hyperbola (矩形上拋曲線) .

At very high [S] (飽和) , *v* = Vmax. That is, the velocity is limited only by conditions (temperature, pH, ionic strength) and by the amount of enzyme present; v becomes independent of [S].

Such a condition is termed *zero-order kinetics*. Under zero-order conditions, velocity is directly dependent on [enzyme]. (酵素多,少; Vmax增,減).

Michaelis-Menten Kinetics

要能導出反應式

- Louis Michaelis and Maud Menten's theory (1913)
- Based on 3 assumptions (M-M動力學基於3個假設):

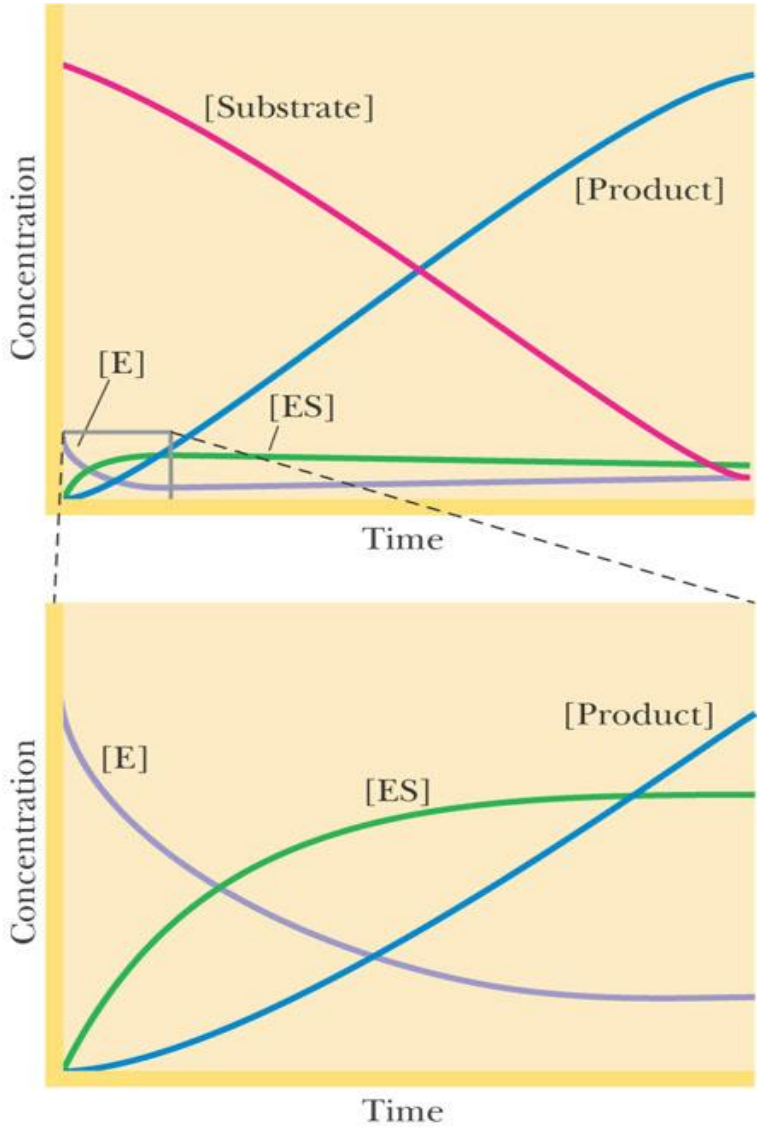
  (1) It assumes the formation of an enzyme-substrate complex (ES)

  (2) It assumes that the ES complex is in rapid equilibrium with free enzyme

  (3) Breakdown of ES to form products is assumed to be slower than
  - 1) formation of ES and
  - 2) breakdown of ES to re-form E and S

Figure 13.8
Time course for

the consumption of substrate,
the formation of product, and
the establishment of a steady-state level of the enzyme-substrate [ES] complex for a typical enzyme obeying the Michaelis-Menten, Briggs-Haldane models for enzyme kinetics.



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How Michaelis-Menten equation derive? (推導公式)

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

Total enzyme [Et] = [E] + [ES]

[E]=free enzyme = [Et]– [ES]

[ES]=bound enzyme

Rate of ES synthesis = $k_1[E][S] = k_1([Et]-[ES])[S]$

Rate of ES decomposition= $k_2\,[ES] + k_{-1}[ES]$

Rate of ES synthesis = rate of ES decomposition

$$k_1([Et]-[ES])[S] = k_2\,[ES] + k_{-1}[ES]$$

$$k_1[Et][S] - k_1[ES][S] = k_2\,[ES] + k_{-1}[ES]$$

$$k_1[Et][S] = k_1[ES][S] + k_2\,[ES] + k_{-1}[ES]$$

$$k_1[Et][S] = k_1[ES][S] + [ES](\,k_2 + k_{-1})$$

$$= [ES]\,(k_2 + k_{-1} + k_1\,[S])$$

$$k_1[Et][S] = k_1[ES][S] + [ES](k_2 + k_{-1})$$

$$= [ES](k_2 + k_{-1} + k_1[S])$$

$$[ES] = k_1[Et][S] / (k_2 + k_{-1} + k_1[S])$$

$$= [Et][S] / ((k_2 + k_{-1}) / k1 + [S])$$

Steady state [ES] = [Et], $V_0 = V_{max}$

$$\mathbf{V_0 = k_2[ES] = k_2[Et]}$$

$$= k_2([Et][S] / ((k_2 + k_{-1}) / k_1 + [S])$$

$$= V_{max}[S] / ((k_2 + k_{-1}) / k_1 + [S])$$

$$= V_{max}[S] / (K_m + [S])$$

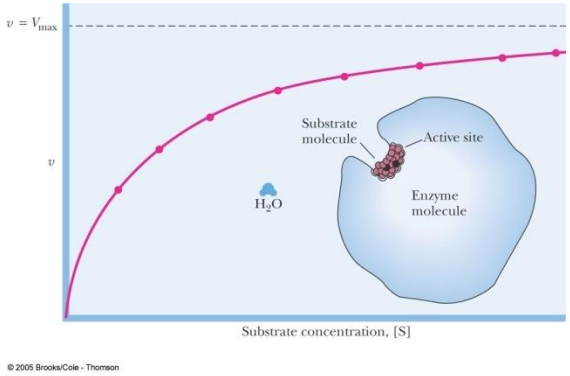
Michaelis-Menten equation

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

The dual nature of the Michaelis-Menten equation

Combination of 0-order and 1st-order kinetics

- When S is low, the equation for rate is 1st order in S
- When S is high, the equation for rate is 0-order in S
- The Michaelis-Menten equation describes a rectangular hyperbolic dependence of v on S!



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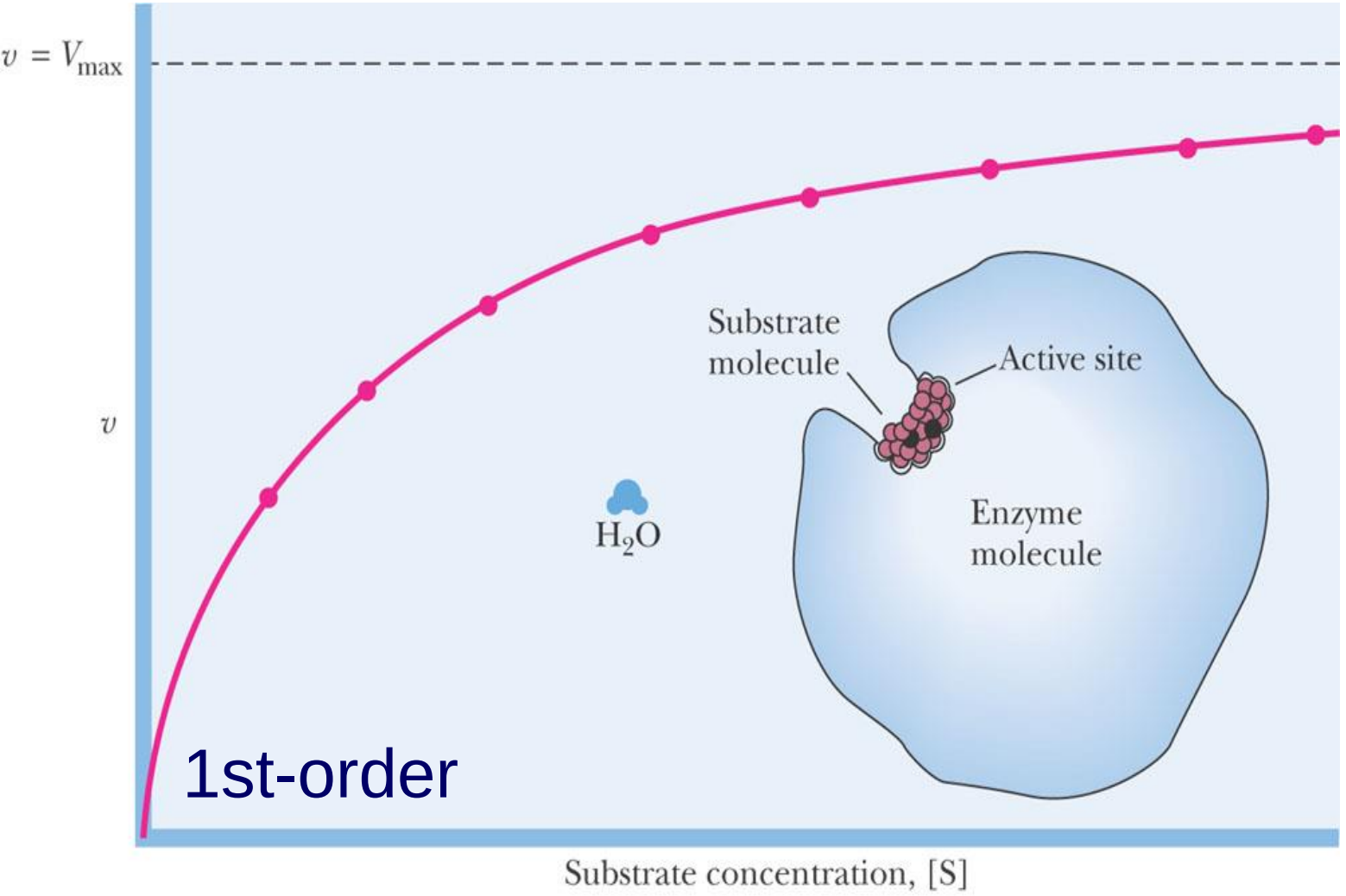
Substrate saturation curve: 基質(作用的)飽和曲線



0-order

1st-order

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K_m

也稱為 *The "kinetic activator constant"*
也稱為 *The Michaelis constant*

- K_m is a constant (基質親和性常數)
- K_m is a constant derived from rate constants
- K_m is, under true Michaelis-Menten conditions, an estimate of the dissociation constant of E from S
- Km represent the affinity between E and S
  - Small K_m means tight binding, high affinity
  - High K_m means weak binding
    - $Km = (k_{-1}+k_2)/ k_1$
    - $Km = [S]$ when $v = V_{max}/2$

TABLE 13.3 K_m Values for Some Enzymes

Enzyme	Substrate	K_m (m*M*)
Carbonic anhydrase	CO_2	12
Chymotrypsin	*N*-Benzoyltyrosinamide	2.5
	Acetyl-L-tryptophanamide	5
	N-Formyltyrosinamide	12
	N-Acetyltyrosinamide	32
	Glycyltyrosinamide	122
Hexokinase	Glucose	0.15
	Fructose	1.5
β-Galactosidase	Lactose	4
Glutamate dehydrogenase	NH_4^+	57
	Glutamate	0.12
	α-Ketoglutarate	2
	NAD^+	0.025
	NADH	0.018
Aspartate aminotransferase	Aspartate	0.9
	α-Ketoglutarate	0.1
	Oxaloacetate	0.04
	Glutamate	4
Threonine deaminase	Threonine	5
Arginyl-tRNA synthetase	Arginine	0.003
	$tRNA^{Arg}$	0.0004
	ATP	0.3
Pyruvate carboxylase	HCO_3^-	1.0
	Pyruvate	0.4
	ATP	0.06
Penicillinase	Benzylpenicillin	0.05
Lysozyme	Hexa-*N*-acetylglucosamine	0.006

V_{max} (最大反應速度, 理論值)

The theoretical maximal velocity

- V_{max} is a constant
- V_{max} is the theoretical maximal rate of the reaction - but it is NEVER achieved in reality
- To reach V_{max} would require that
  - ALL enzyme molecules are tightly bound with substrate
- V_{max} is asymptotically approached as substrate is increased

Turnover number (轉換數)

A measure of catalytic activity

- k_{cat}, the turnover number, is the number of substrate molecules converted to product per enzyme molecule per unit of time, when E is saturated with substrate.

(當E與S飽和, 單位時間每個 E 分子將S轉換成P數目)

- If the M-M model fits, $k_2 = k_{cat} = V_{max}/E_t$
- Values of k_{cat} range from less than 1/sec to many millions per sec

TABLE 13.4 Values of k_{cat} (Turnover Number) for Some Enzymes

Enzyme	k_{cat} (sec^{-1})
Catalase	40,000,000
Carbonic anhydrase	1,000,000
Acetylcholinesterase	14,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

轉換數

Catalytic efficiency (催化效率)

Name for k_{cat}/K_m

An estimate of "how perfect" the enzyme is

- k_{cat}/K_m is an apparent second-order rate constant
- It measures how the enzyme performs **when S is low** (在低基質濃度下測定)
- The upper limit for k_{cat}/K_m is the diffusion limit - the rate at which E and S diffuse together
- Enzyme this efficient have achieved called "catalytic-perfection"

k_{cat}/K_m數值代表催化效率上限，達到E和S一起結合/擴散的狀態

TABLE 13.5 Enzymes Whose k_{cat}/K_m Approaches the Diffusion-Controlled Rate of Association with Substrate

Enzyme	Substrate	k_{cat} (sec^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}\ sec^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^{4}	9×10^{-5}	1.6×10^{8}
Carbonic anhydrase	CO_2	1×10^{6}	0.012	8.3×10^{7}
	HCO_3^-	4×10^{5}	0.026	1.5×10^{7}
Catalase	H_2O_2	4×10^{7}	1.1	4×10^{7}
Crotonase	Crotonyl-CoA	5.7×10^{3}	2×10^{-5}	2.8×10^{8}
Fumarase	Fumarate	800	5×10^{-6}	1.6×10^{8}
	Malate	900	2.5×10^{-5}	3.6×10^{7}
Triosephosphate isomerase	Glyceraldehyde-3-phosphate*	4.3×10^{3}	1.8×10^{-5}	2.4×10^{8}
β-Lactamase	Benzylpenicillin	2×10^{3}	2×10^{-5}	1×10^{8}

k2=kcat = Vmax/Et

Km= [S] at 1/2 Vmax

Enzyme activity unit (活性單位)

- Unit (U) = μmole/min
  - The amount of enzyme that catalyzes the formation of 1 micromole of product per minute
- katal = mole/sec
  - The amount of enzyme that catalyzes the conversion of 1 mole of substrate to product in 1 second
- 1 katal = 6×10^7 U

Specific Activity (比活性)

- Enzyme unit per mg protein
- U/mg

- As extraneous proteins are removed in the purification process.
- S.A. represent the purity of the enzyme

Linear Plots of the Michaelis-Menten Equation

- Michael's-Menten equation

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

(1) Lineweaver-Burk Plot

$1/v$ versus $1/[S]$

(2) Hanes-Woolf Plot **(=M-M equation 兩邊乘以[S])**

$[S]/v$ versus $[S]$

Lineweaver-Burk equation

Double Reciprocal
雙倒數

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

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

$$Y = a\ X + b$$

雙倒數圖 (1/[S] , 1/v)

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

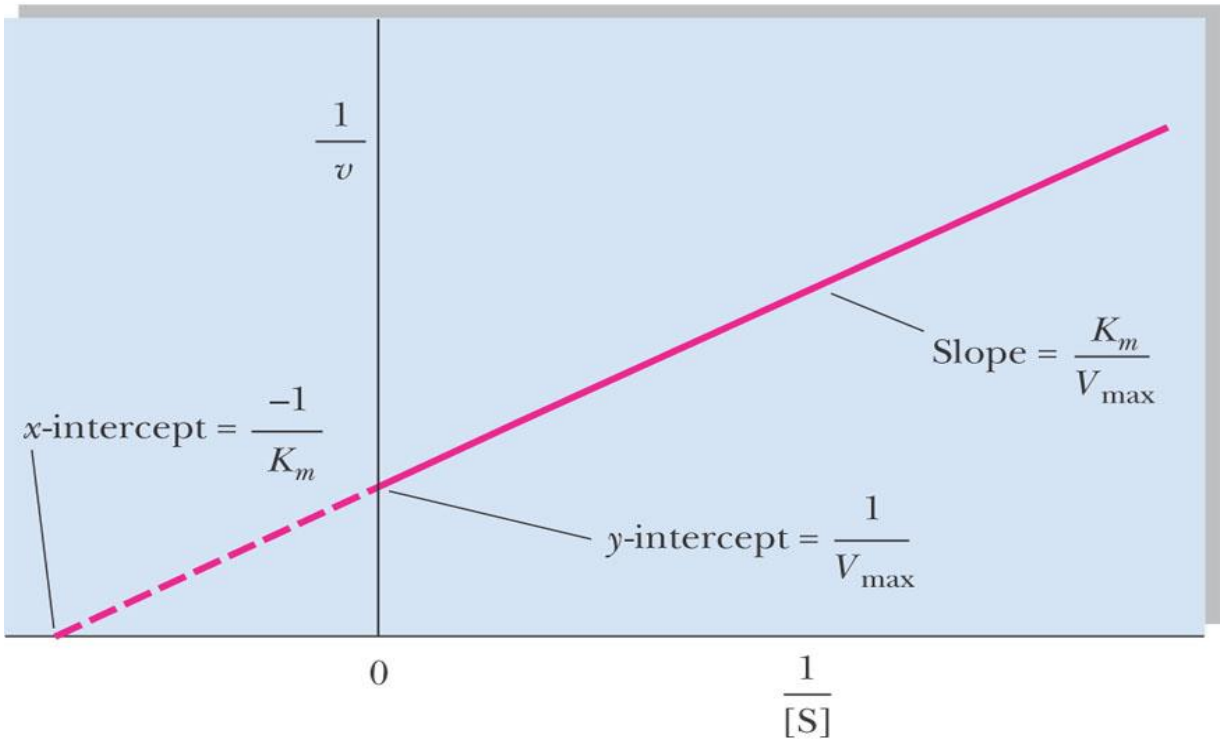

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Figure 13.9
The Lineweaver-Burk double-reciprocal plot, depicting extrapolations that allow the determination of the *x*- and *y*-intercepts and slope.

Hanes-Woolf is best - why?

- Rearrangement of Lineweaver-Burk

  M-M equation 兩邊乘以[S]

- **[S]/*v* versus [S]**

(i) Not overemphasizing the data obtained at low [S]

(ii) Allow both K_m and V_{max} to be accurately estimated

(iii) Smaller and more consistent errors across the plot

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

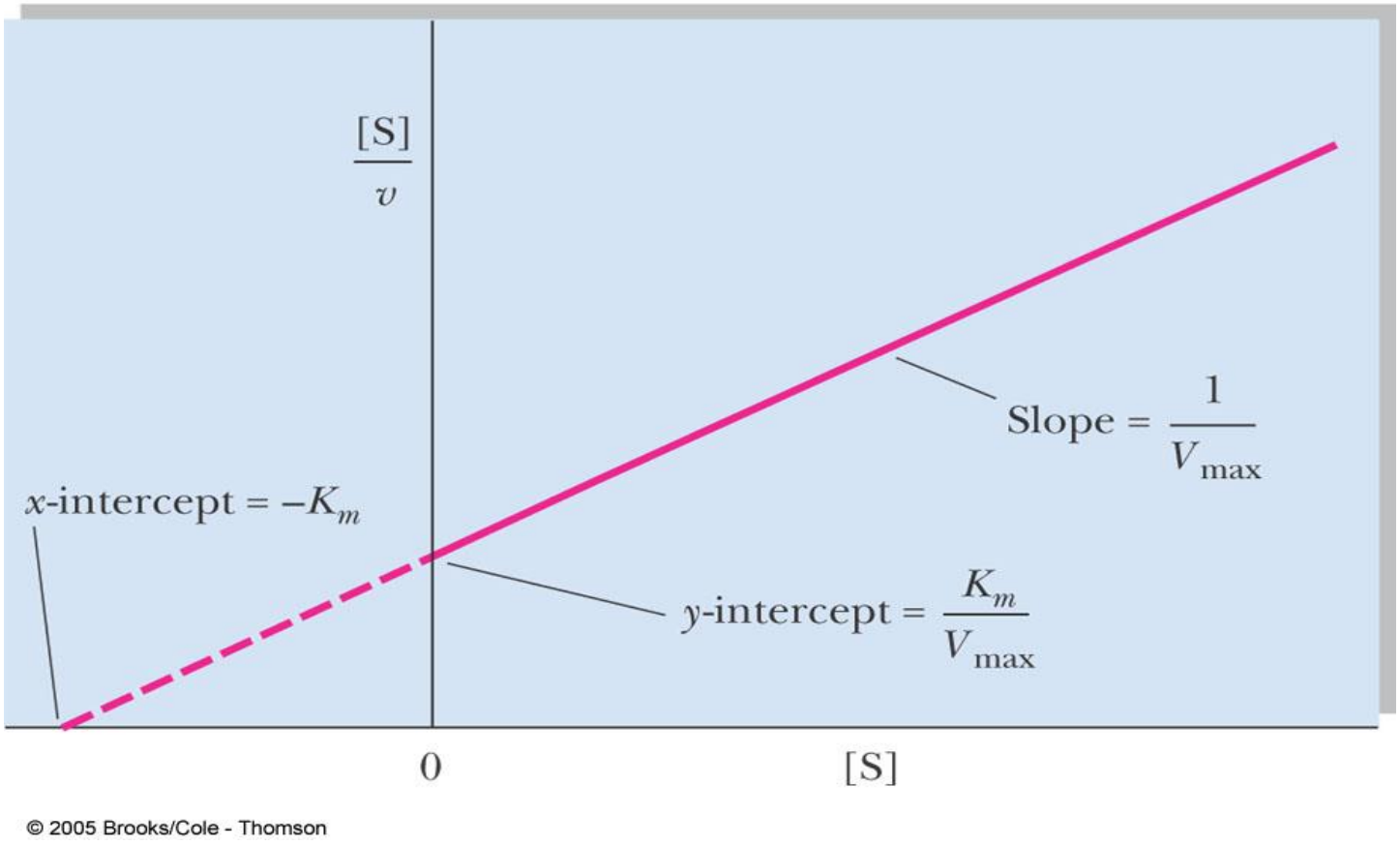

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Figure 13.10

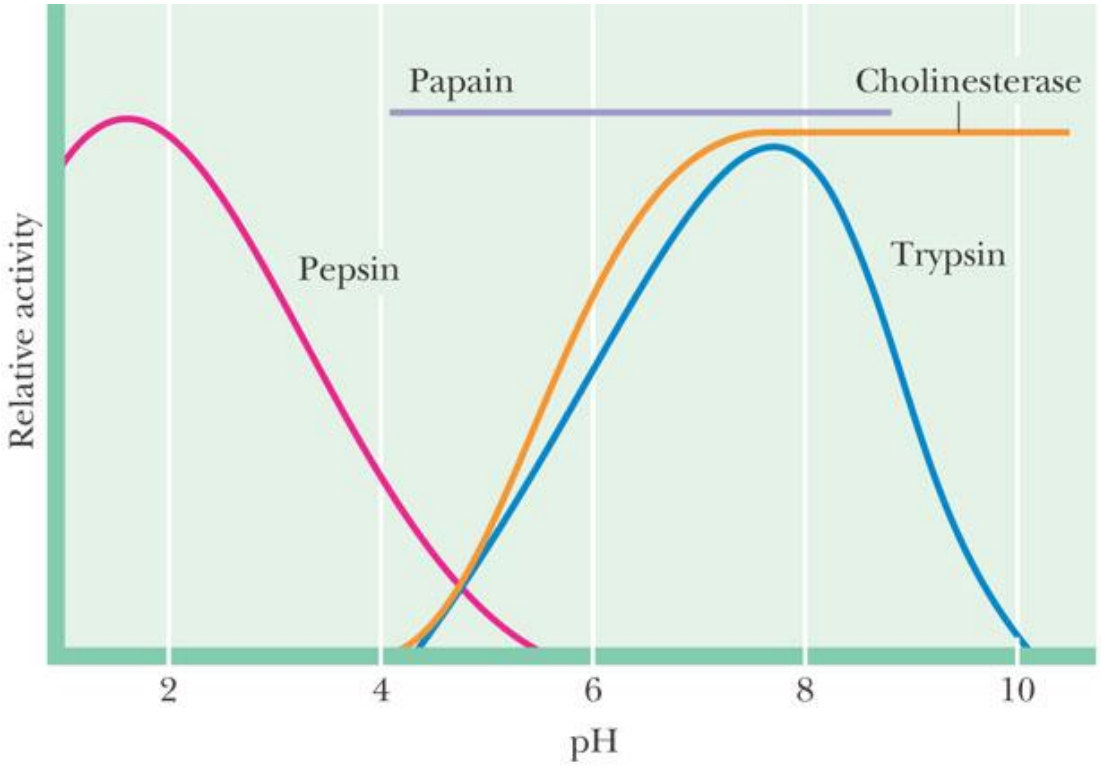
A Hanes-Woolf plot of **[S]/*v* versus [S],** another straight-line rearrangement of the Michalelis-Menten equation.

操作酵素實驗態度

- 照顧Enzyme reaction　如寵物照顧（細心耐心專心）
- 複習Ch13-15 溫故知新
- 實驗設計

  目的方法, 樣品數, 正負控制組, 濃度觀念, 三重複,　反應順序,
  新鮮酵素材料 (native/stable), 同一批反應儲存液,
  設備, 齊全正確
- 規劃時間 (8 hrs/day; 2,1,2/週)
- 實驗區域, 乾淨衛生
- Everything ready體力充沛,謹慎進行, 操作正確,一氣呵成
- 再現性, 點線面 --> 拚圖

酵素的最適pH不同



Optimum pH of Some Enzymes	
Enzyme	Optimum pH
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

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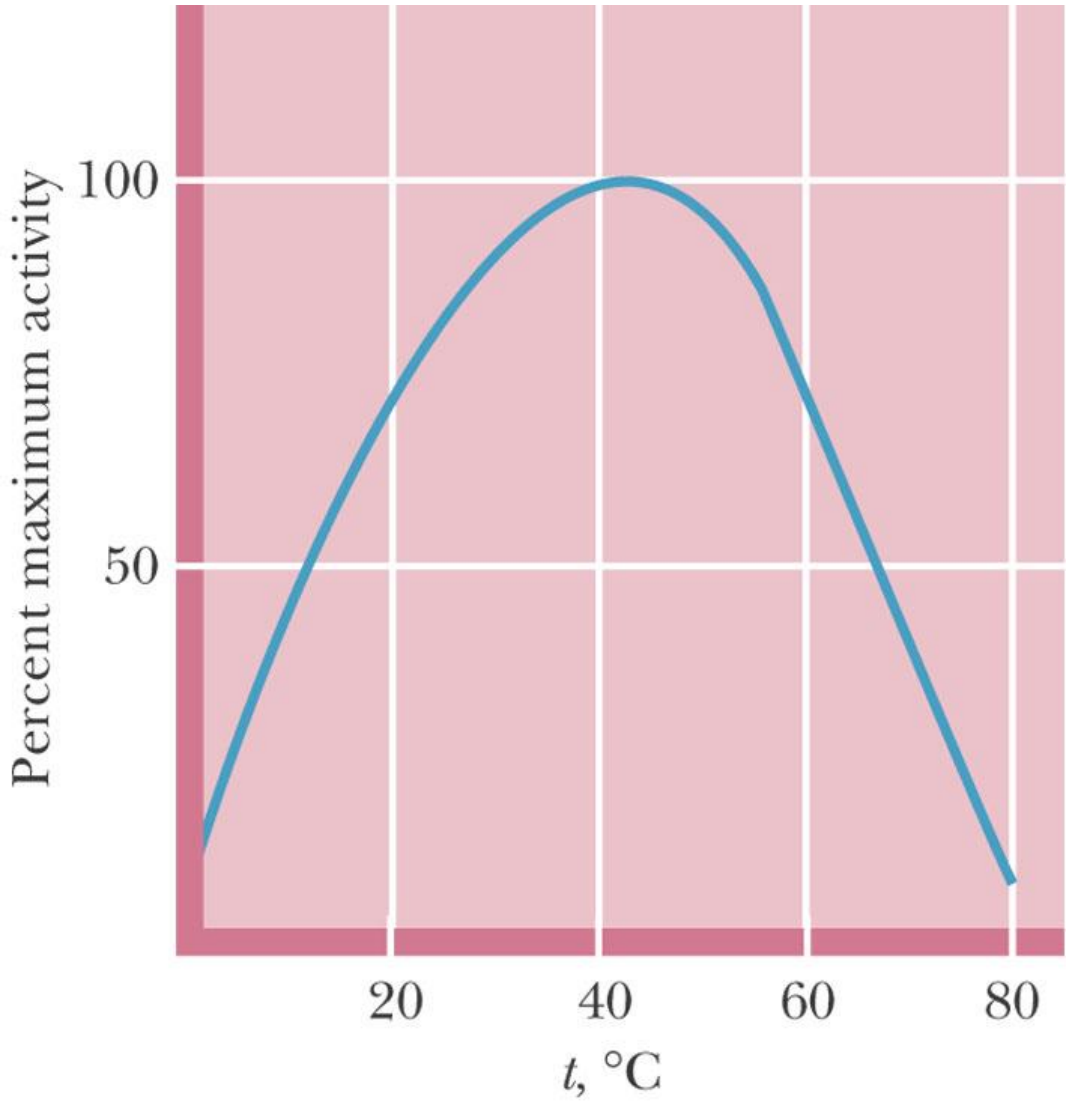
Figure 13.11

The pH activity profiles of four different enzymes. *Trypsin*, an intestinal protease, has slightly alkaline pH optimum, whereas *pepsin*, a gastric protease, acts in the acidic confines of the stomach and has a pH optimum near 2. *Papain*, a protease found in papaya, is relatively insensitive to pHs between 4 and 8. *Cholinesterase* activity is pH sensitive below pH 7 but not between pH 7 and 10. The cholinesterase pH activity profile suggests that an ionizable group with pK' near 6 is essential to its activity. Might it be a histidine residue within the active site?

典型之最適溫度曲線

Figure 13.12
The effect of temperature on enzyme activity. The relative activity of an enzymatic reaction as a function of temperature. The decrease in the activity above 50°C is due to thermal denaturation.



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Q_{10}

- The ration of activities at two temperatures 10 ℃ apart

- Most enzymatic rxn double in rate for every 10 ℃ rise in temperature, i.e. Q_{10} =2, as long as the enzyme is active and fully active

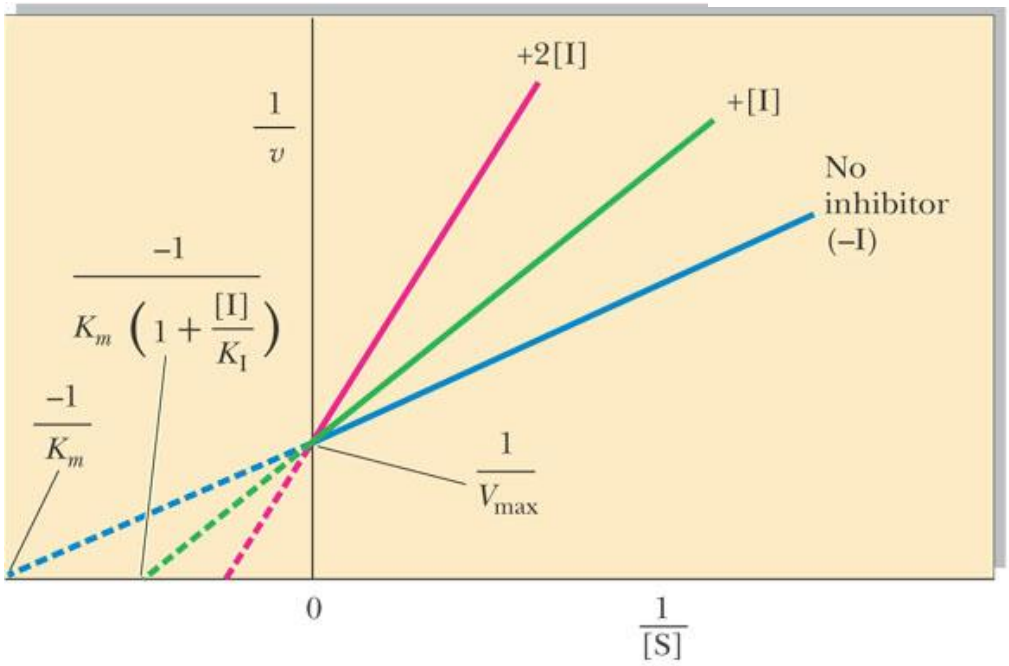
- Above 50°C: Typically enzyme denatures

13.4 – What Can Be Learned from the Inhibition of Enzyme Activity?

- Enzymes may be inhibited reversibly (可逆型) or irreversibly (不可逆型)
- •Reversible inhibitors may bind at the active site or at some other site (allosteric regulation; 異位調節)

  可逆型分為: 競爭型, 非競爭型, 混合型, 不競爭型
- Enzymes may also be inhibited in an irreversible manner
  - Penicillin is an irreversible suicide inhibitor

競爭型抑制



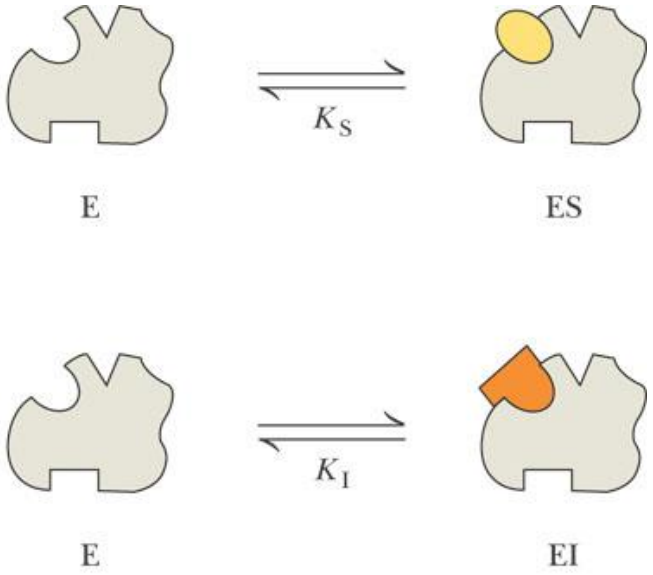


Figure 13.13
Lineweaver-Burk plot of competitive inhibition, showing lines for no I, [I], and 2[I].

Note that when [S] is infinitely large (1/[S] = 0),
V_{max} is the same, whether I is present of not.
In the presence of I, Km increase.

Structural Analog-competitive inhibitor

結構相似物為競爭型抑制劑

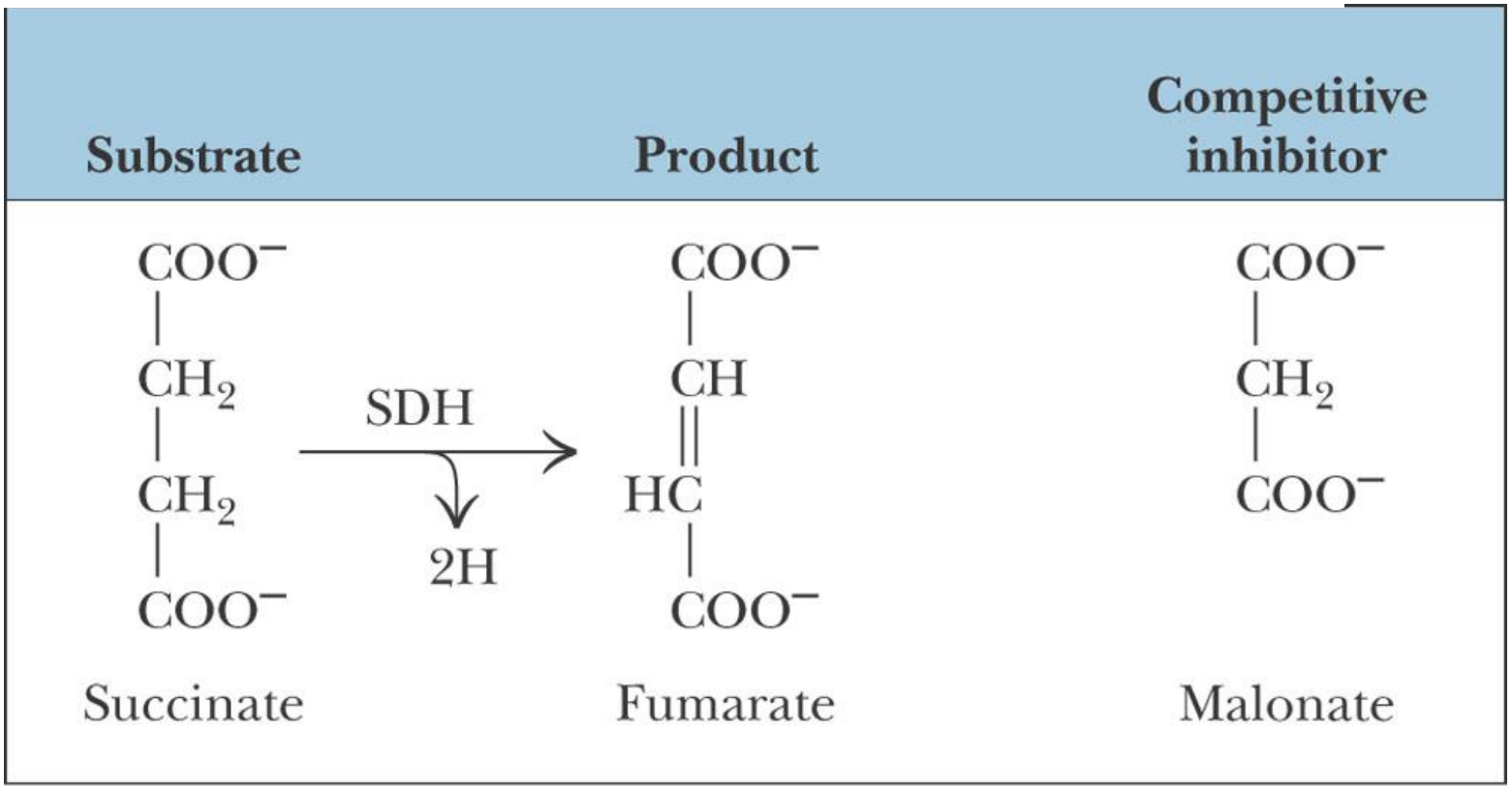


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Figure 13.14
Structures of succinate, the substrate of succinate dehydrogenase (SDH), and malonate, the competitive inhibitor. Fumarate (the product of SDH action on succinate) is also shown.

非競爭型抑制

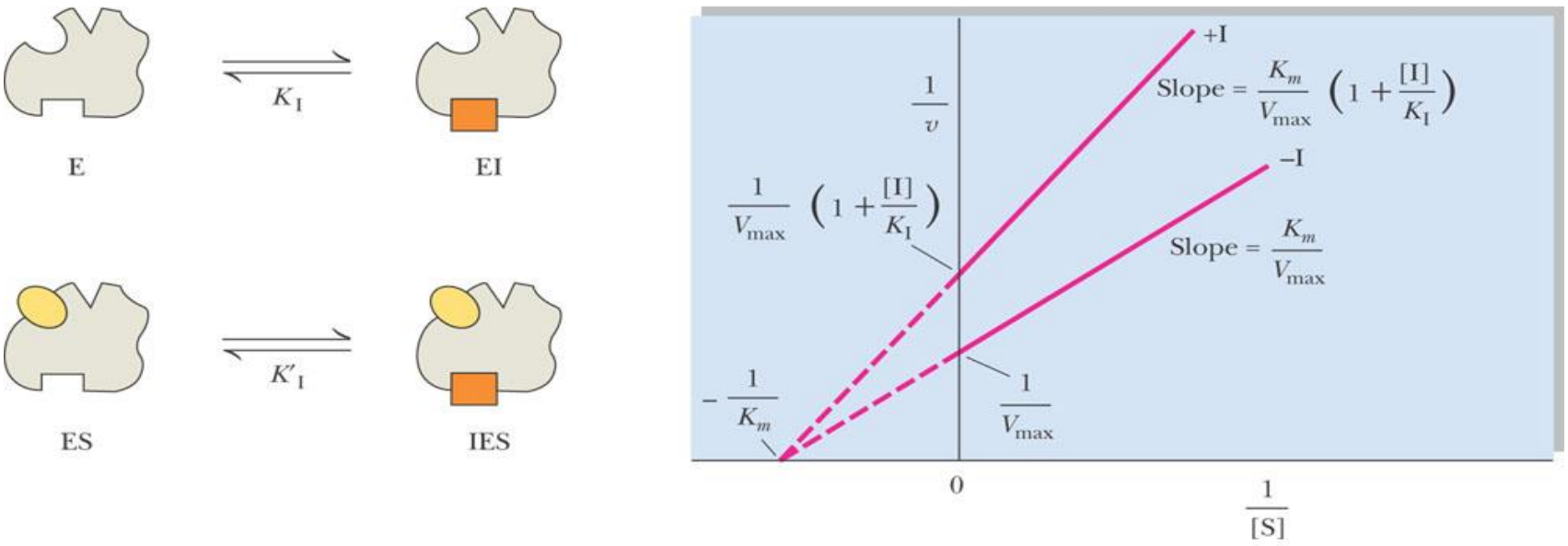

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Figure 13.15
Lineweaver-Burk plot of pure noncompetitive inhibition.

Note that I does not alter K_m but that it decreases $V_{max.}$ In the presence of I, the *y*-intercept is equal to $(1/V_{max})(1 + I/K_I)$.

混合式非競爭型抑制

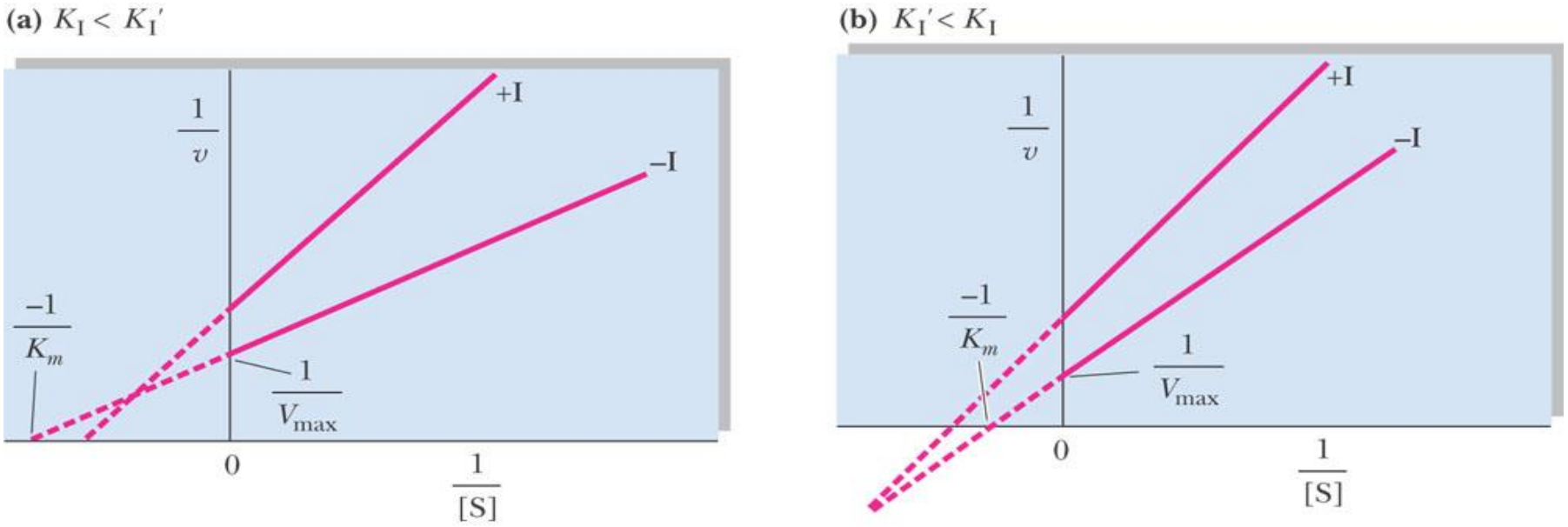

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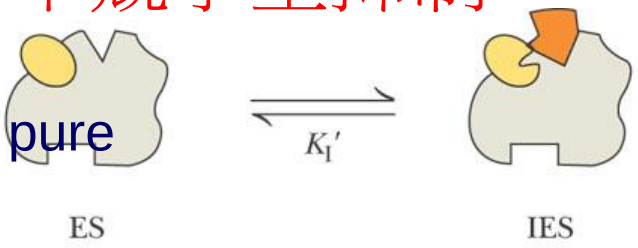
Figure 13.16
Lineweaver-Burk plot of mixed noncompetitive inhibition.

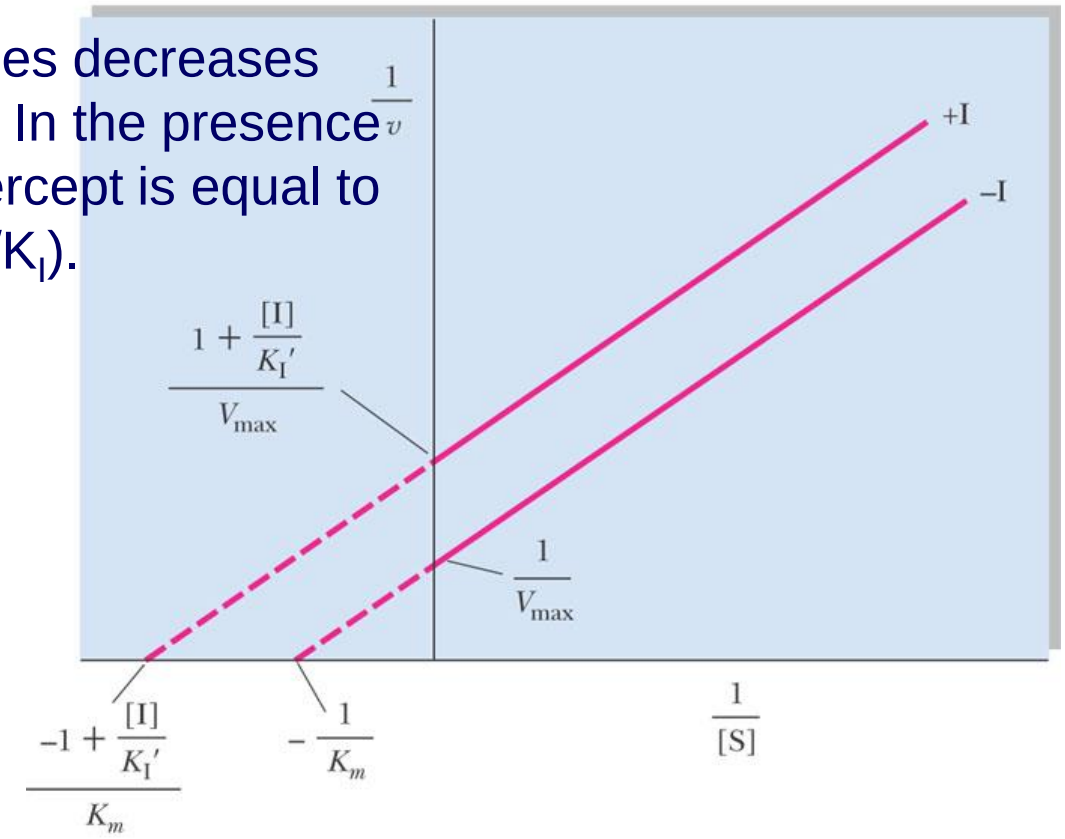
Note that both intercepts and the slope change in the presence of I. **(a)** When K_I is less than K_I'; **(b)** when K_I is greater than K_I'.

不競爭型抑制

Figure 13.17
Lineweaver-Burk plot of pure uncompetitive inhibition.



Note that I does decreases K_m and $V_{max.}$ In the presence of I, the *y*-intercept is equal to $(1/V_{max})(1 + I/K_I)$.



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Irreversible inhibition
(不可逆型抑制常用於藥物開發)

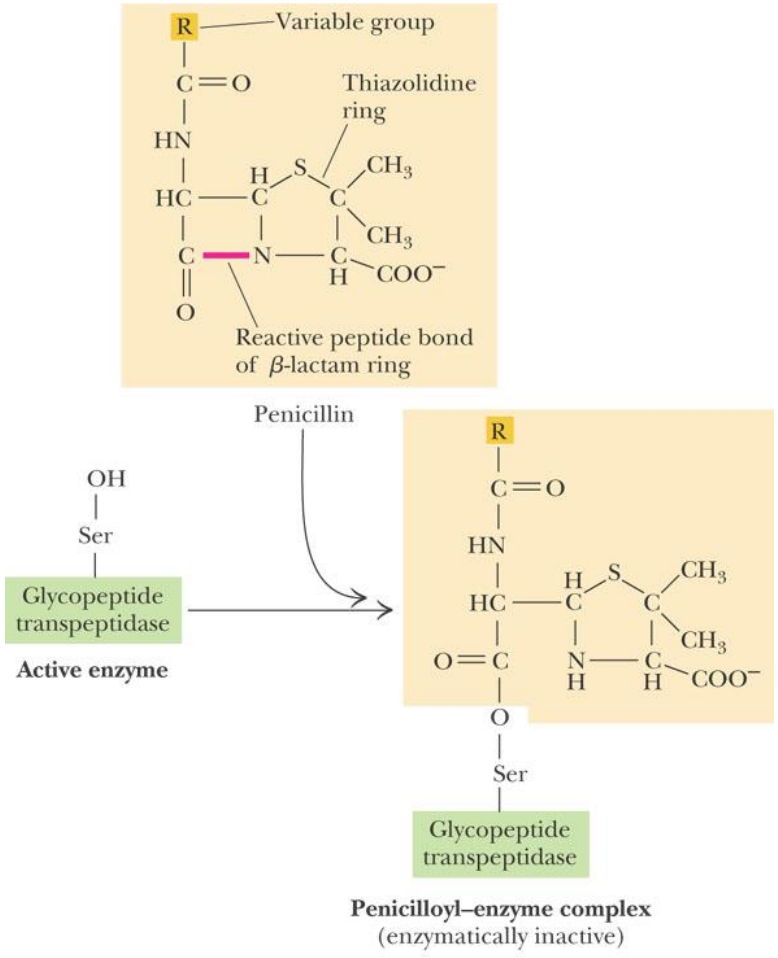
– : Covalent bonding
  - Suicide substrate or
  - Trojan Horse substrate
  - Useful in Site-specific affinity label

Figure 13.18

Penicillin is an irreversible inhibitor of the enzyme *glycoprotein peptidase*, which catalyzes an essential step in bacterial cell wall synthesis.

Penicillin consists of a thiazolidine ring fused to a β-lactam ring to which a variable group R is attached. A reactive peptide bond in the β-lactam ring covalently attaches to a serine residue in the active site of the glycopeptide transpeptidase. (The conformation of penicillin around its reactive peptide bond resembles the transition state of the normal glycoprotein peptidase substrate.)

The penicilloyl-enzyme complex is catalytically inactive. The bond between the enzyme and penicillin is indefinitely stable; that is, penicillin binding is irreversible.



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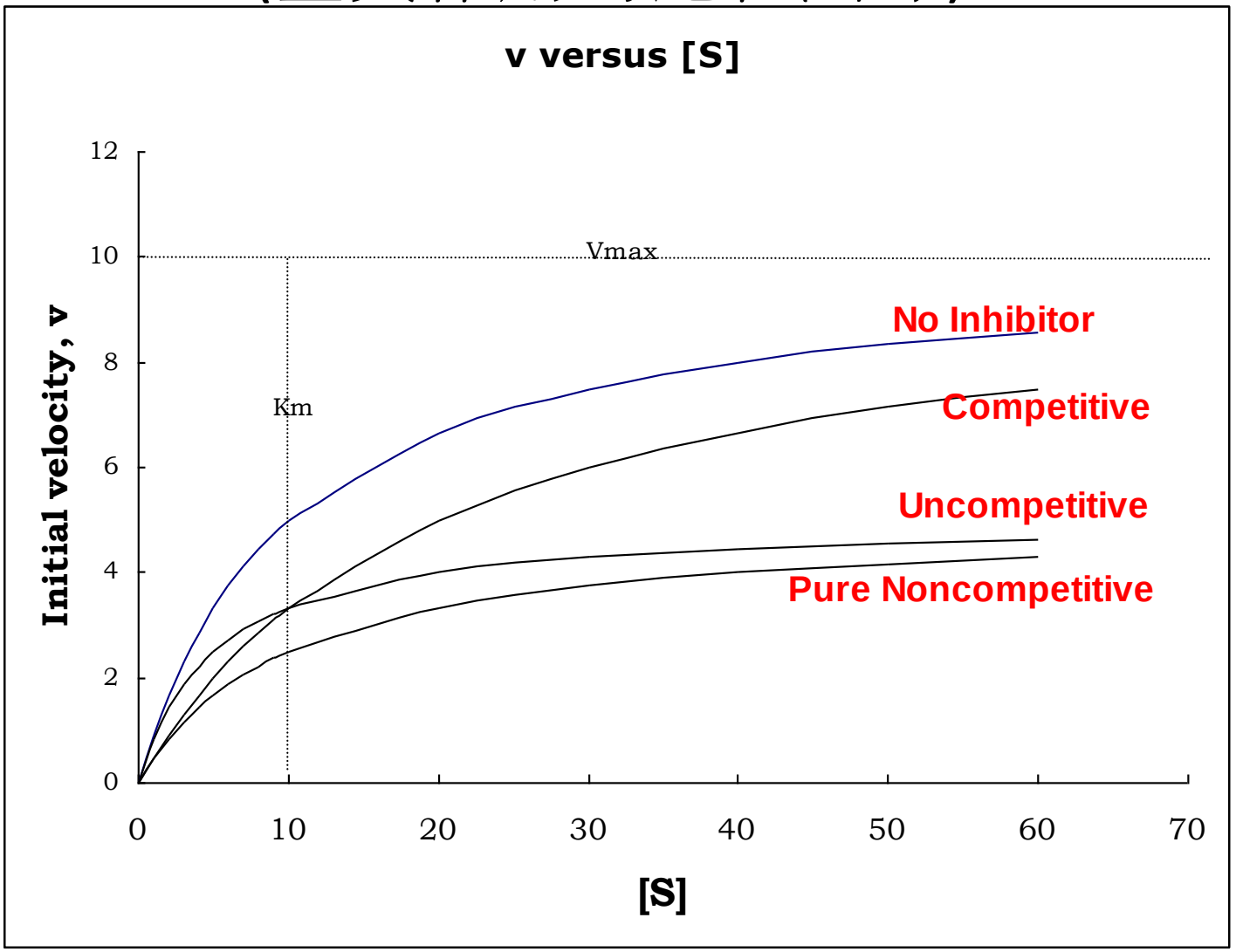
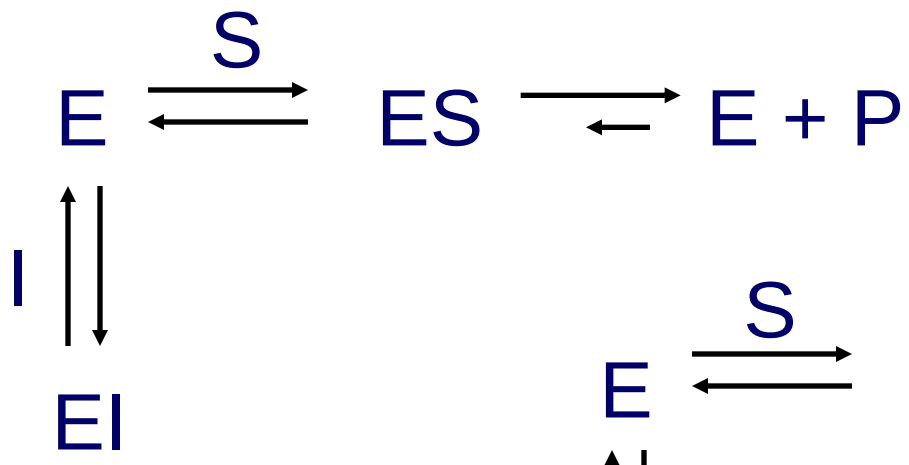
Penicillin抑菌性: 與細菌之醣蛋白轉肽酶active site 的Ser共價結合帶來不可逆型抑制細胞壁合成

Substrate saturation curves
(基質作用的飽和曲線)



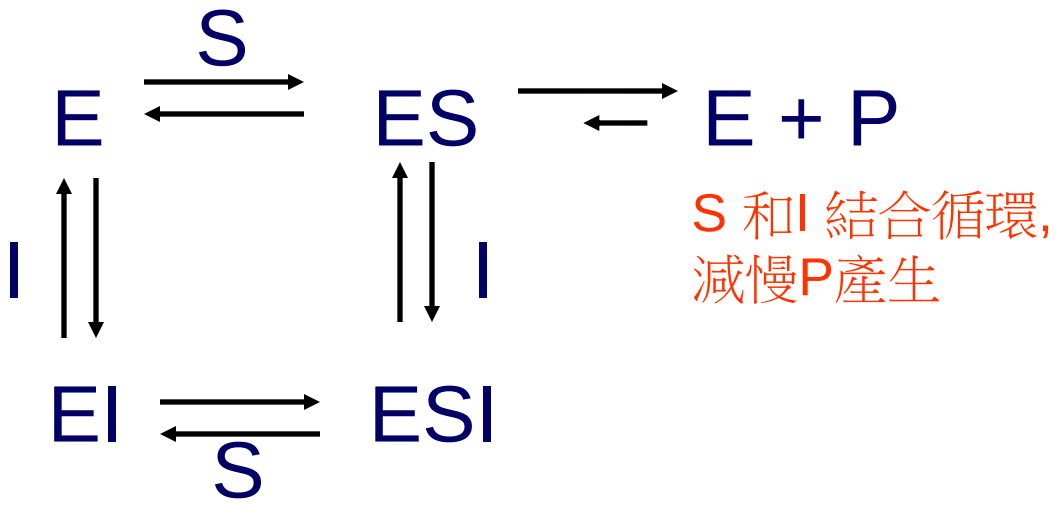


I 和 S 競爭active site,
需較高[S] 達到V_{max}

Competitive

V_{max} no change

K_m ↑

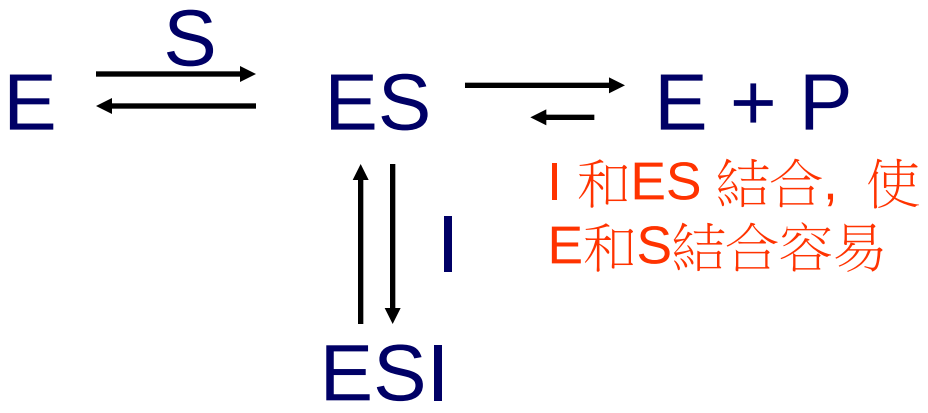


S 和I 結合循環,
減慢P產生

Non-competitive

V_{max} ↓

Km no change

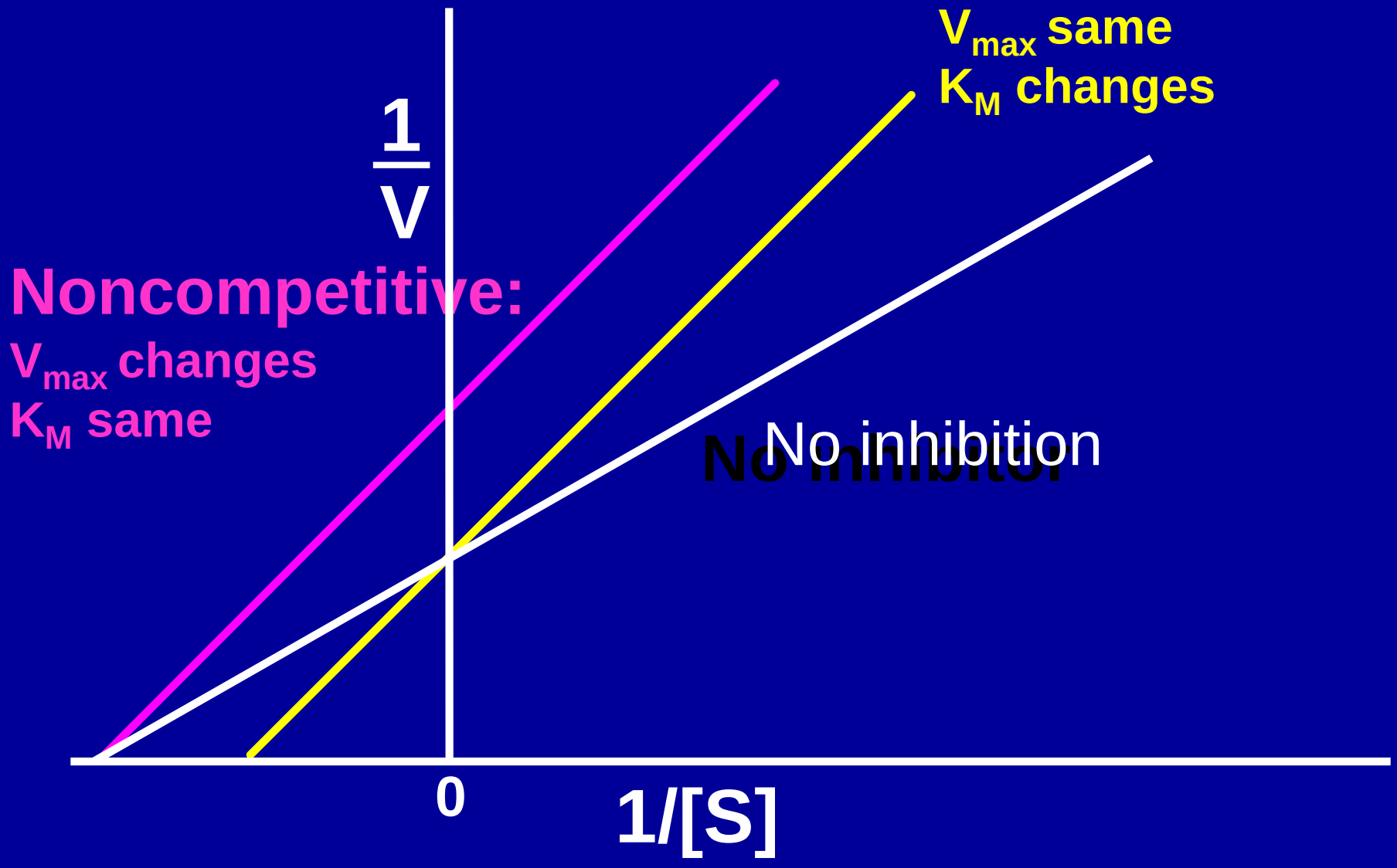


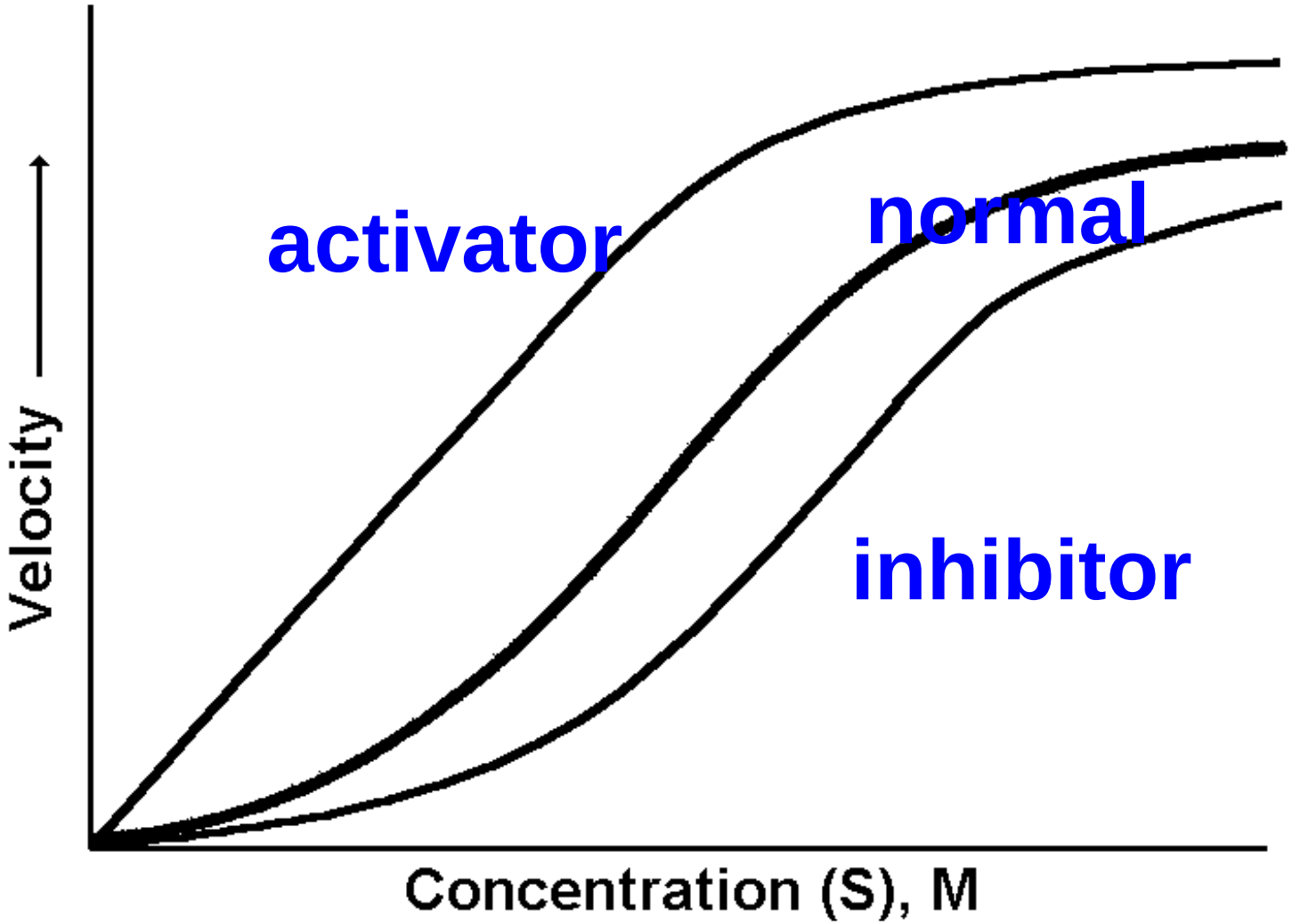
I 和ES 結合，使
E和S結合容易

Uncompetitive

Vmax ↓

Km ↓


Competitive:
V_{max} same
K_M changes
1
V
Noncompetitive:
V_{max} changes
K_M same
No inhibition
0
1/[S]


activator
normal
inhibitor
Velocity
Concentration (S), M

***13.5 - What Is the Kinetic Behavior of Enzymes Catalyzing Bimolecular Reactions?

- Enzymes often use two (or more) substrates
- Reactions may be (I) sequential or single-displacement reactions; (II) Ping-Pong or double-displacement
- And they can be (1) random or (2)ordered

淨反應: A + B --> P + Q

Bisubstrate Reaction (雙基質反應)-1

(1) Sequential (single displacement):

E + A + B → AEB → PEQ → E + P + Q

(2) Ping-Pong (double-displacement):

E + A → EA → E'P → E' → E'B → EQ → E + Q

P B

** Bisubstrate reactions (雙基質反應)-2

- Sequential or single displacement (單置換)
  - Random (任意): Either substrate binds to enzyme, either product is released
  - Ordered (順序): Leading substrate binds first
- Ping-Pong or double-displacement (雙置換) 通常Holoenzyme有Coenzyme參與

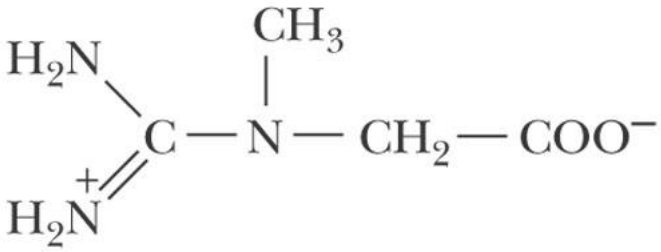
  Leading substrate binds: Enzyme modified: Product released: Second substrate binds: Enzyme unmodified: Second product released

Ping-pong 反應例子: Table 13-2所列舉, Figs. 13-23, 24-3 acetyl CoA carboxylase (biotin as coenzyme), Glutamate:aspartate aminotransferase (pyridoxal phosphate as coenzyme

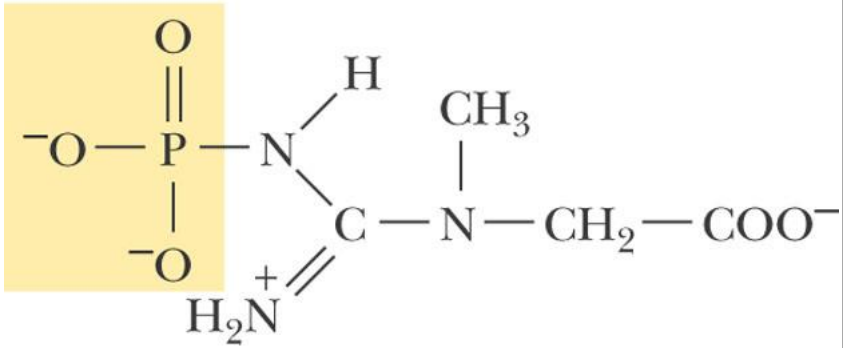
雙基質反應-3

Random, single displacement of creatine kinase

Cr + ATP → Cr-P + ADP



Creatine



Creatine-P

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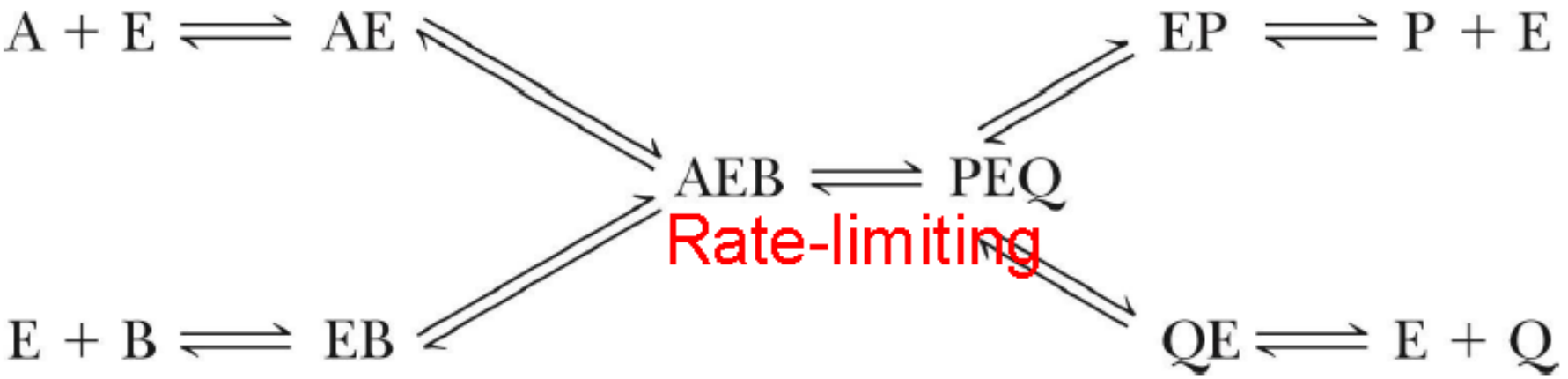
Figure 13.21
The structures of creatine and creatine phosphate, guanidinium compounds that are important in muscle energy metabolism
.

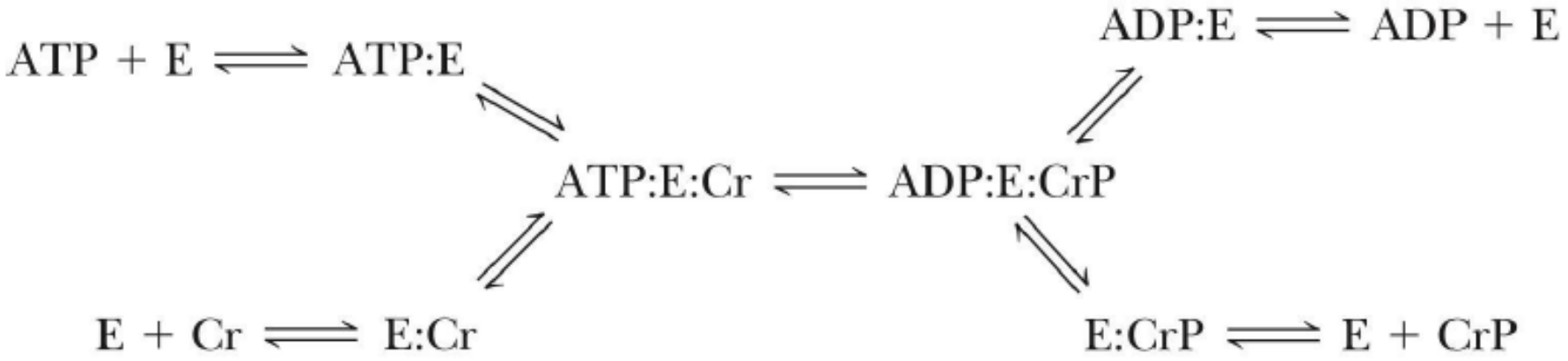
Cr-P是肌肉能量代謝的磷酸庫

雙基質反應-4

Random (single-displacement) 分子作用圖



例子: Cr + ATP → Cr-P + ADP; Cr 是creatine kinase



雙基質反應-5

Double-reciprocal form of the rate equation:

$$\frac{1}{v} = \frac{1}{V_{max}}\left(K_m^A + \frac{K_S^A K_m^B}{[B]}\right)\left(\frac{1}{[A]} + \frac{1}{V_{max}}\left(1 + \frac{K_m^B}{[B]}\right)\right)$$

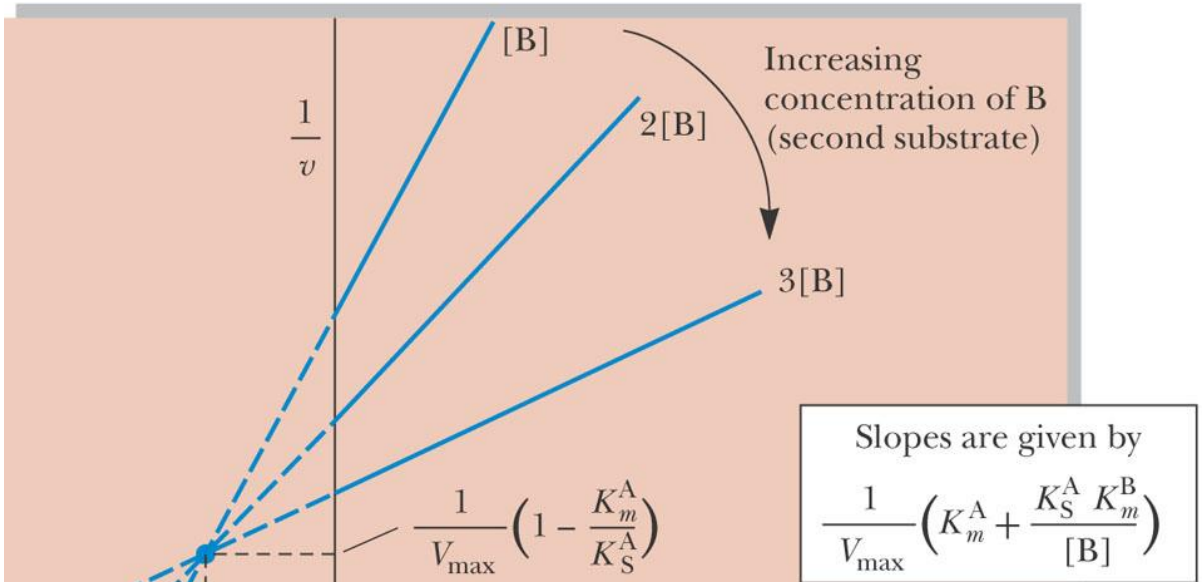


Figure 13.19

Ordered, Single-displacement bisubstrate mechanism. Double-reciprocal plots of the rates observed with different fixed concentrations of one substrate (B here) are graphed versus a series of concentrations of A. Note that, in these Lineweaver-Burk plots for single-displacement bisubstrate mechanisms, the lines intersect to the left of the 1/*v* axis.

雙基質反應-6

加[A]不會影響B與E結合, 反之亦然

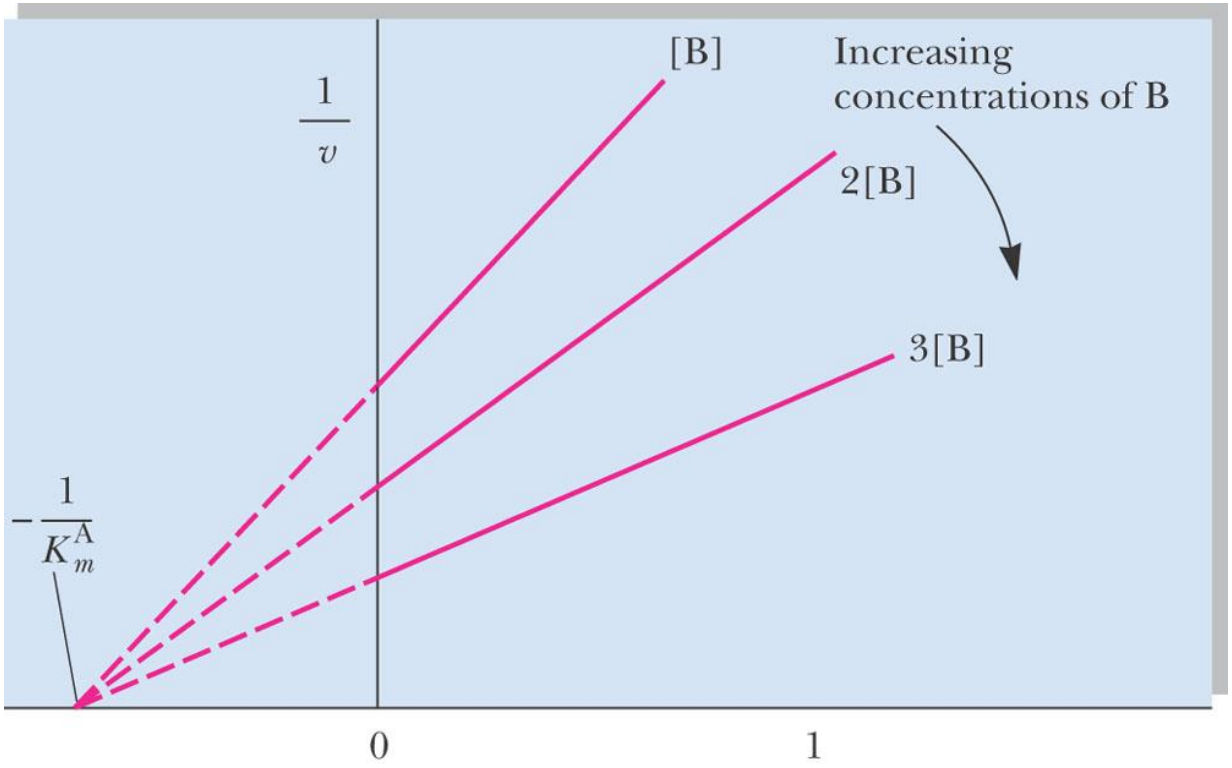
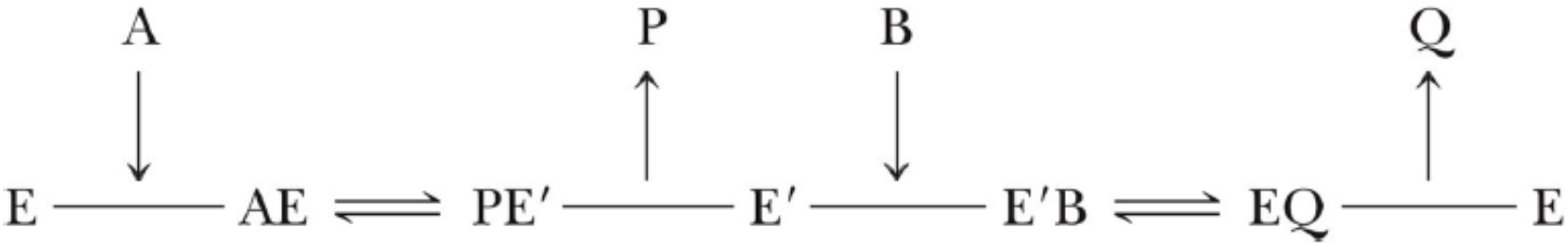


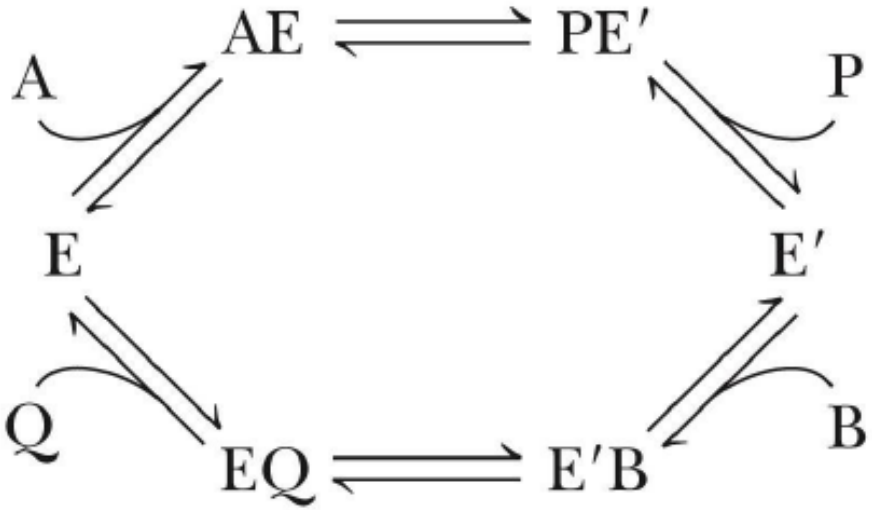
Figure 13.20

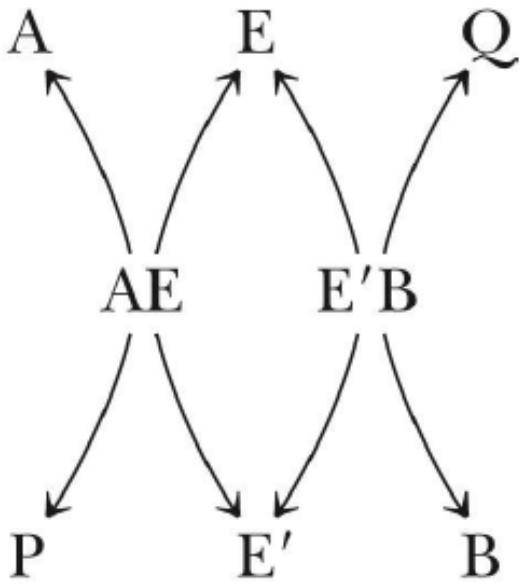
Random, single-displacement bisubstrate mechanisms **where A does not affect B binding**, and vice versa. Note that the lines intersect at the 1/[A] axis. (If [B] were varied in an experiment with several fixed concentrations of A, the lines would intersect at the 1/[B] axis in a 1/*v* versus 1/[B] plot.)

雙基質反應-7

Bisubstrate reactions-Ping-Pong 反應之分子作用圖







*Vit B6 (pyridoxal)*參與雙基質的*ping-pong*反應

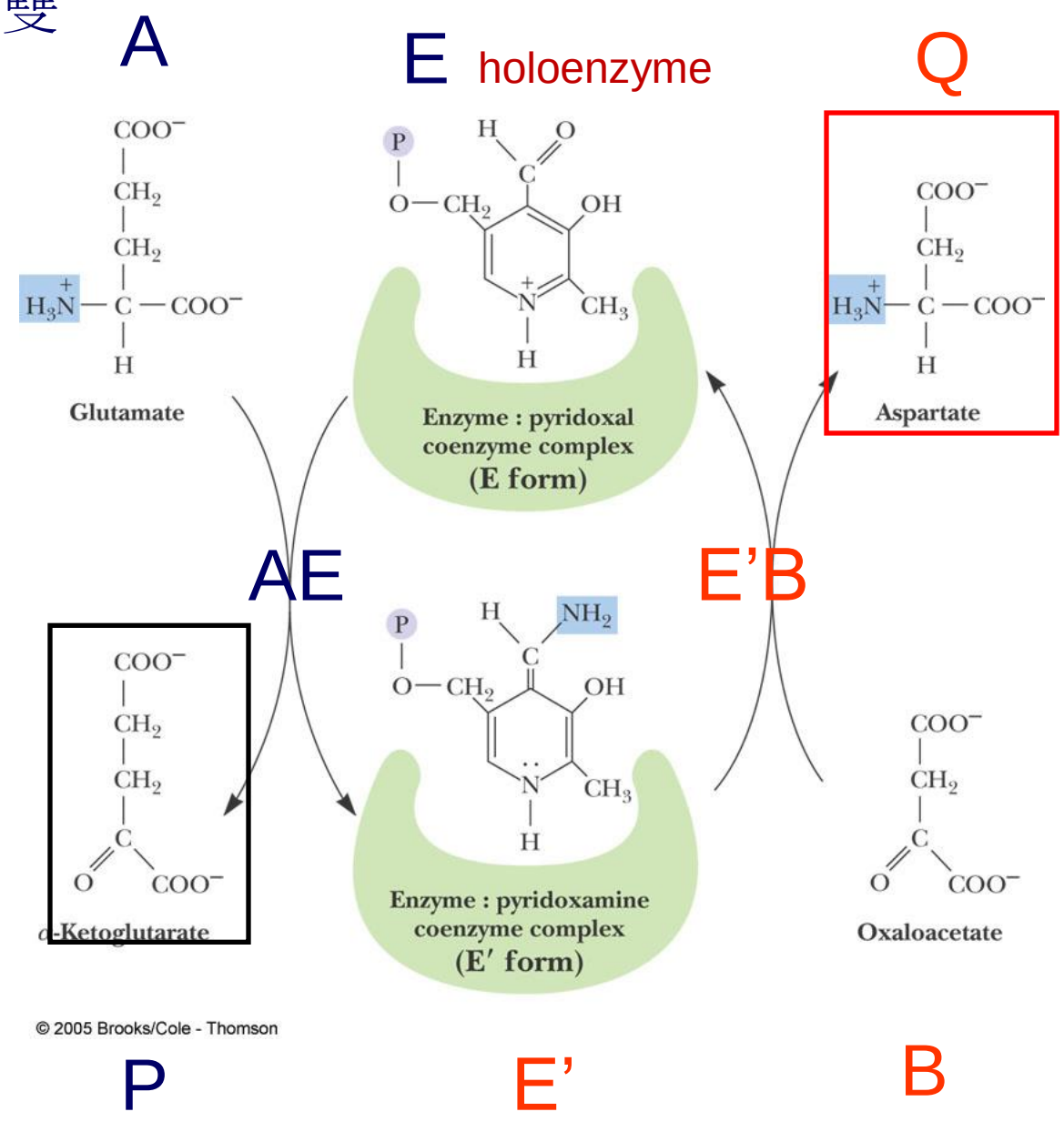


Figure 13.23 *Glutamate:aspartate aminotransferase*, an enzyme conforming to a double-displacement bisubstrate mechanism. Glutamate:aspartate aminotransferase is a pyridoxal phosphate-dependent enzyme. The pyridoxal serves as the -NH2 acceptor from glutamate to form pyridoxamine.

Pyridoxamine is then the amino donor to oxaloacetate to form asparate and regenerate the pyridoxal coenzyme form. (The pyridoxamine: enzyme is the E' form.)

雙基質反應-7

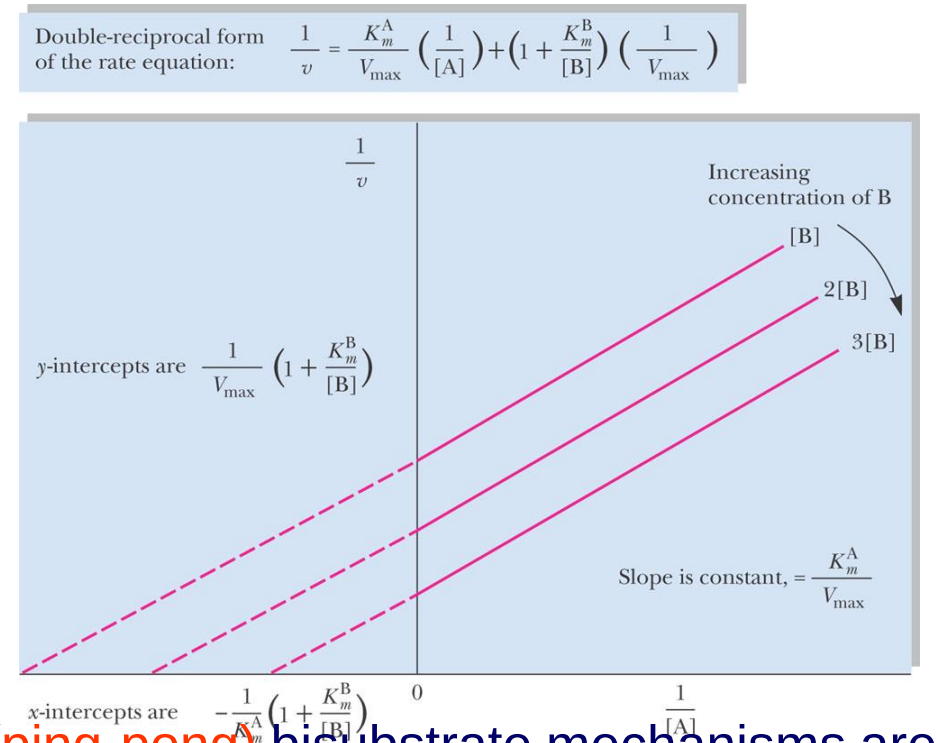


Figure 13.22
Double-displacement (ping-pong) bisubstrate mechanisms are characterized by Lineweaver-Burk plots of parallel lines when double-reciprocal plots of the rates observed with different fixed concentrations of the second substrate, B, are graphed versus a series of concentrations of A.

13.6 – How Can Enzymes Be So Specific?

- "Lock and key" was the first explanation for specificity (舊理論)
- "Induced fit" provides a more accurate description
- Induced fit favors formation of the transition-state intermediate (新理論)
- Specificity of E reaction to be reactive, S in the active site induce conformation change in E, creating the catalytic site

Induced fit favor formation of transition state

- The shape of the enzyme's active site is actually modified upon binding S, in a process of dynamic recognition between E and S.
  - The protein and substrate "fit" each other more precisely; i.e. precise orientation of catalytic residue comprising the active site is necessary for the reaction to occur.
  - Optimally active enzyme:transition state conformation
  - Active site→ Catalytic site to perform catalysis

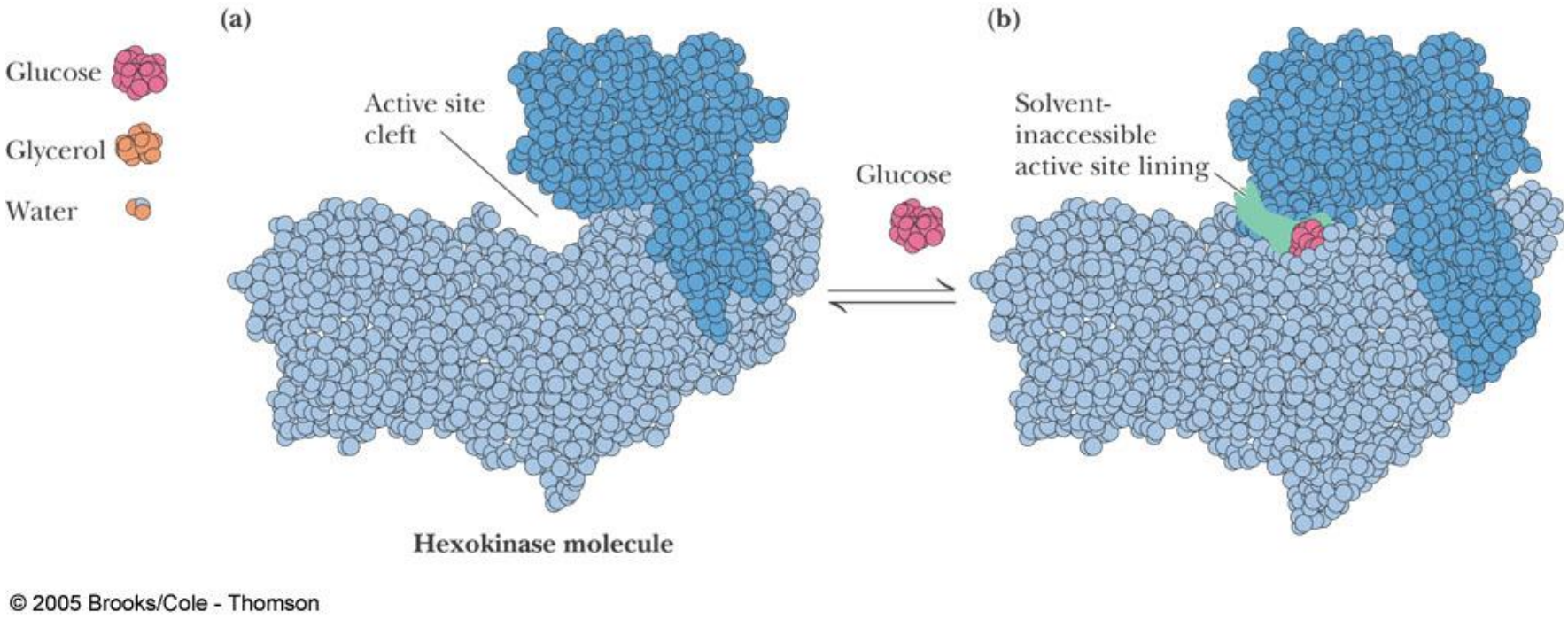


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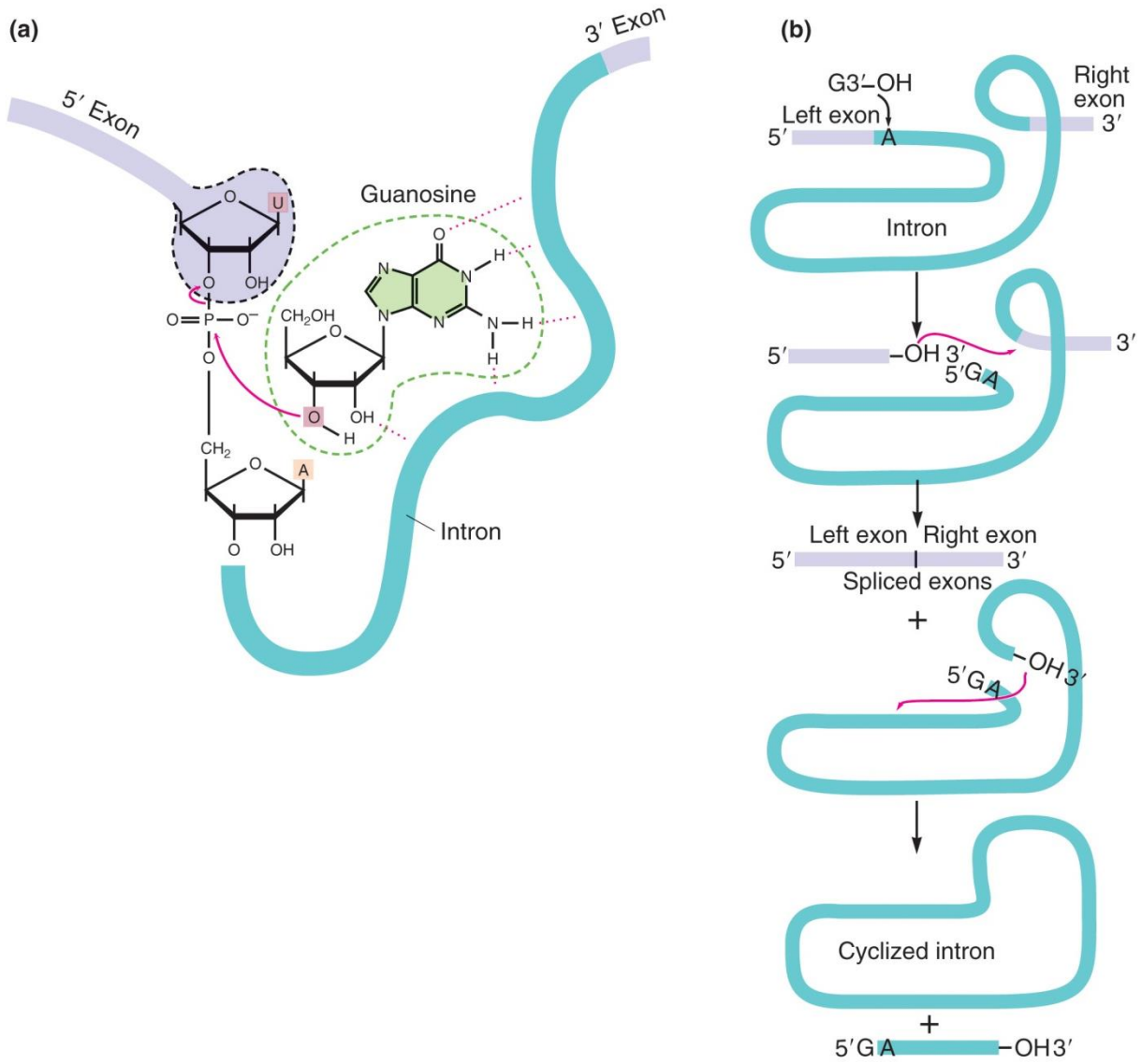
Figure 13.24
A drawing, roughly to scale, of H_2O, glycerol, glucose, and an idealized hexokinase molecule. Note the tow domains of structure in hexokinase **(a)**, between which the active site is located. Binding of glucose induces a conformational change in hexokinase. The two domains close together, creating the catalytic site **(b)**. The shaded area in **(b)** represents solvent inaccessible surface area in the active site cleft that results when the enzyme binds substrate.

13.7– Are All Enzymes Proteins? No.

- Ribozymes - segments of RNA that display enzyme activity in the absence of protein
  - E.g. RNase P and peptidyl transferase
- Abzymes - antibodies raised to bind the transition state of a reaction of interest
  - E.g. Antigen used to create an abzyme with aminotransferase activity.

RNA splicing in Tetrahymena (四膜蟲) rRNA maturation



Nucleophilic attack of the 3’-OH on the phosphodiester bond that is 15 nucleotides from the 5’-GA end of the spliced-out intron.

Cyclization frees a linear 15-mer with a 5’-GA end.

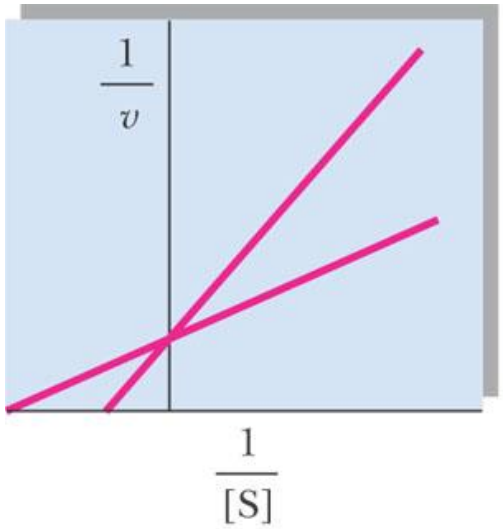
Fig. 13-25, p. 411

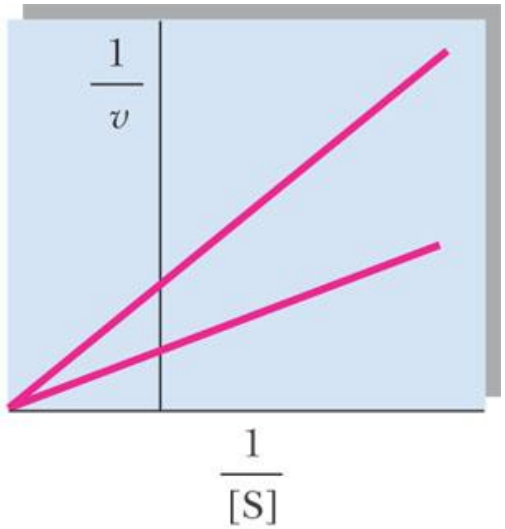
13.8 Is it possible to design an enzyme to catalyze any desirable reaction?

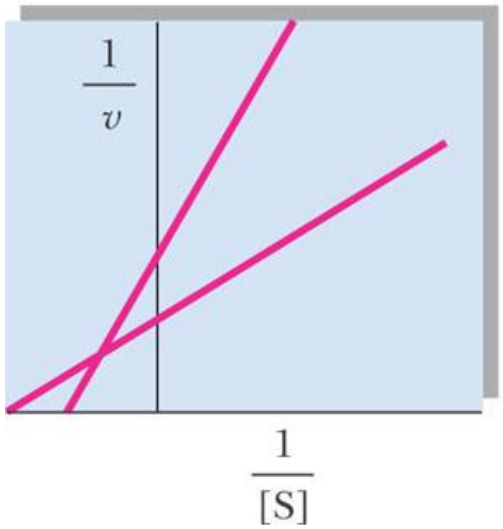
- Designer enzymes
- Create a desired enzyme de novo (from scratch)
  - Begin with a known enzyme and engineer it
  - Computer modeling to design a protein with the desired structure and activity (in silico)
  - Couple natural evolutionary process with rational design

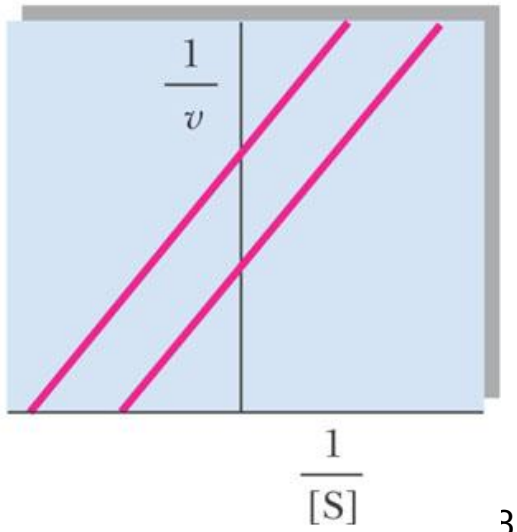
Exercise

Give at least 2 possible interpretations of these plot?









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Match the terms in the first column with the descriptions in the second column.

- a. A → P
- b. v
- c. d[P]/dt = d[S]/dt
- d. k in v = k[A][B]
- e. k-1/k1
- f. (k-1 + k2)/k1
- g. u at [S] = ∞
- h. [S] when v = Vmax/2
- i. kcat

1. Km
2. Vmax
3. First-order reaction
4. Michaelis constant
5. $Vmax/E_{total}$
6. The velocity or rate of a reaction.
7. Enzyme:substrate dissociation constant
8. 1/Vmax
9. Equilibrium

4. The following kinetic data were obtained for an enzyme in the absence of inhibitor (1), and in the presence of two different inhibitors (2) and (3) at 5 mM concentration. Assume $[E_T]$ is the same in each experiment.

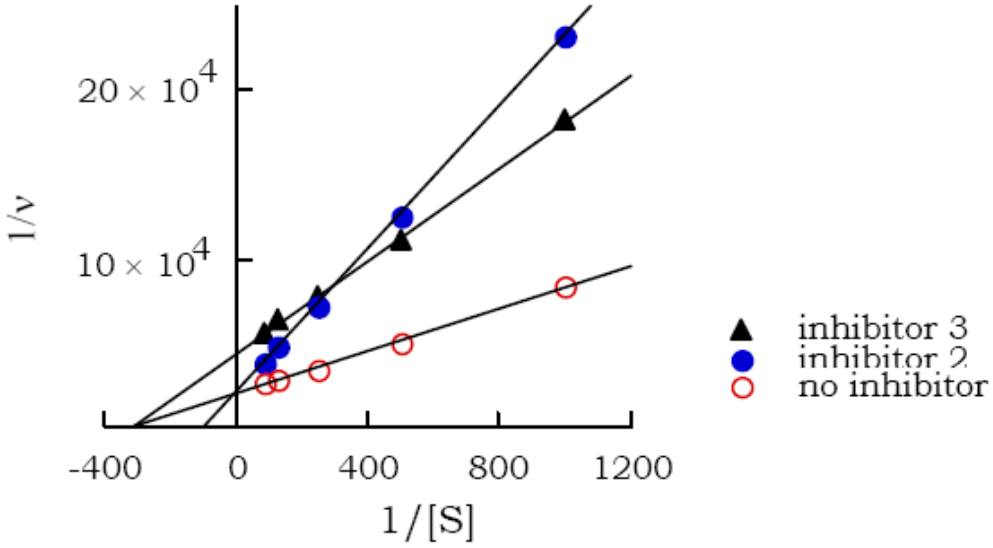
[S] (mM)	(1) υ (μmol/mL·sec	(2) υ (μmol/mL·sec	(3) υ (μmol/mL·sec
1	12	4.3	5.5
2	20	8	9
4	29	14	13
5	35	21	16
12	40	26	18

a. Determine V_{max} and K_m for the enzyme.

b. Determine the type of inhibition and the K_I for each inhibitor.

Answer: The data may be analyzed using double-reciprocal variables. For each [S] and corresponding υ, we will calculate 1/[S] and 1/υ.

[S] (mM)	1/[S] M^{-1}	υ (1) μmol/mL·sec	1/ υ (1) mL·sec/μmol	υ (2) μmol/mL·sec	1/ υ (2) mL·sec/μmol	υ (3) μmol/mL·sec	1/ υ (3) mL·sec/μmo
1	1000	12	8.33×10^4	4.3	2.33×10^5	5.5	1.82×10^5
2	500	20	5.00×10^4	8	1.25×10^5	9	1.11×10^5
4	250	29	3.45×10^4	14	7.14×10^4	13	7.69×10^4
5	235	35	2.86×10^4	21	4.76×10^4	16	6.25×10^4
12	83.3	40	2.52×10^4	26	3.85×10^4	18	5.56×10^4



Plots of 1/υ vs. 1/[S] indicate straight lines given by

(1) $1/\upsilon = 63.1(1/[S]) + 1.96 \times 10^4$
(2) $1/\upsilon = 212.3(1/[S]) + 1.99 \times 10^4$
(3) $1/\upsilon = 137.4(1/[S]) + 4.38 \times 10^4$

In general,

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

Thus, the y-intercept is equal to $1/V_{max}$ and the x-intercept is equal to $-1/K_m$.

(a)

Condition	V_{max} (μmol/mL·sec)	K_m (mM)
No inhibitor	51	3.2
5 m_M inhibitor (2)	50.3	10.7
5 mM inhibitor (3)	22.8	3.1

(b) Inhibitor (2) increases the apparent K_m of the enzyme without affecting V_{max}. This is characteristic of a competitive inhibitor. In this case K_I is calculated as follows

$$K_{m,app} = K_m(1 + \frac{[I]}{K_I}) \text{ or}$$

$$10.7 \text{ mM} = 3.2 \text{ mM}(1 + \frac{5 \text{ mM}}{K_I})$$

Solving for K_I we find

$$K_I = \frac{5 \text{ mM}}{(\frac{10 \text{ mM}}{3.2 \text{ mM}} - 1)} = 2.35 \text{ mM}$$

Inhibitor (3) decreases V_{max} but leaves K_m unchanged, an example of noncompetitive inhibition. In this case K_I is calculated as follows

$$\frac{1}{V_{max,app}} = \frac{1}{V_{max}}(1 + \frac{[I]}{K_I}) \text{ or}$$

$$\frac{1}{22.8} = \frac{1}{51}(1 + \frac{[I]}{K_I})$$

Solving for K_I we find

$$K_I = \frac{5 \text{ mM}}{(\frac{51}{22.8} - 1)} = 4.04 \text{ mM}$$

10. Triose phosphate isomerase catalyzes the conversion of glyceraldehyde-3-phosphate to dihydroxyacetone phosphate.

Glyceraldehyde-3-P ⇆ dihydroxyaceone-P

The K_m of this enzyme for its substrate glyceraldehyde-3-phosphate is 1.8 x 10^{-5} M. When [glyceraldehyde-3-phosphate] = 30 uM, the rate of the reaction, v, was 82.5 umole mL^{-1} sec^{-1}.

a. What is V_{max} for this enzyme?

b. Assuming 3 nanomoles per mL of enzyme was used in this experiment ($[E_{total}]$ = 3 nanomol/mL), what is k_{cat} for this enzyme?

c. What is the catalytic efficiency (k_{cat}/K_m) for triose phosphate isomerase?

d. Does the value of k_{cat}/K_m reveal whether triose phosphate isomerase approaches "catalytic perfection" ?

e. What determines the ultimate speed limit of an enzyme-catalyzed reaction? That is, what is it that imposes the physical limit on kinetic perfection?

Answer:

a. We are given K_m, v and [S] and are asked to determine V_{max}. Starting with the Michaelis-Menten equation, we solve it for V_{max} as follows.

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

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

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

Given $v = 82.5\ \mu mol\ mL^{-1}sec^{-1}$, $Km = 1.8 \times 10^{-5}$ M = 18 μM, [S] = 30 μM

$$V_{max} = \frac{82.5\ \mu mol\ mL^{-1}sec^{-1} \times (18+30)\mu M}{30\ \mu M}$$

$$V_{max} = 132\ \mu mol\ mL^{-1}sec^{-1}$$

b. The turnover number, k_{cat}, is given by $V_{max}/[E_T]$. Thus,

$$k_2 = \frac{V_{max}}{E_{total}}$$

Given $V_{max} = 132\ \mu mol\ mL^{-1}sec^{-1}$ and $E_{total} = 3$ nanomol/mL

$$k_2 = \frac{132 \times 10^{-6}\ mol\ mL^{-1}sec^{-1}}{3 \times 10^{-9}\ mol/mL}$$

$$k_2 = 44{,}000\ sec^{-1}$$

c. The catalytic efficiency is the turnover number divided by K_m, both of which we know.

$$\frac{kcat}{K_m} = \frac{44{,}000 sec^{-1}}{1.8 \times 10^{-5} M}$$

$$\frac{kcat}{K_m} = 2.44 \times 10^{9} M^{-1}sec^{-1}$$

d. The very fastest we could expect the enzyme to function is given by the rate constant for diffusion of a small molecule, which is in the range 10^8 to 10^9 M^{-1} sec^{-1}. The catalytic efficiency of the enzyme actually exceeds this range.

e. The ultimate limit is how fast ES forms. More precisely, it is the rate at which E encounters S. Given that enzymes are typically large molecules relative to their substrates, the limit of the reaction is determined by the rate of diffusion of S.