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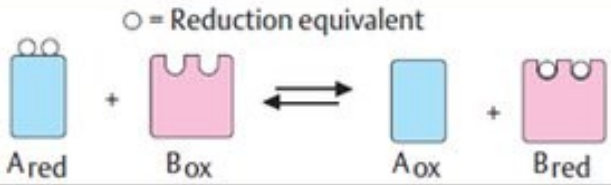
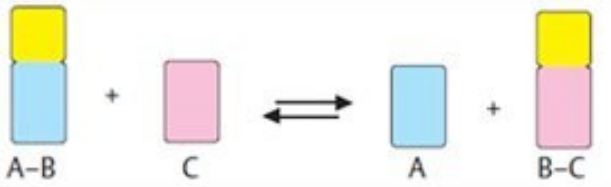
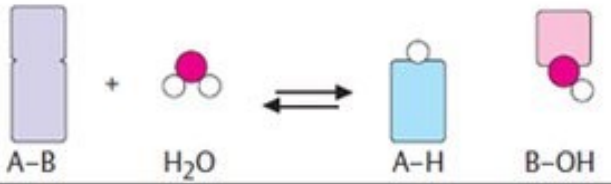
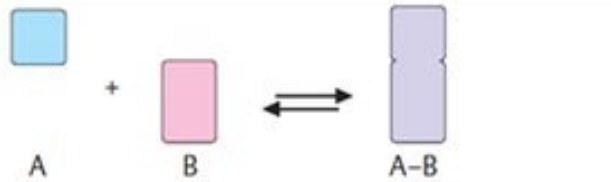
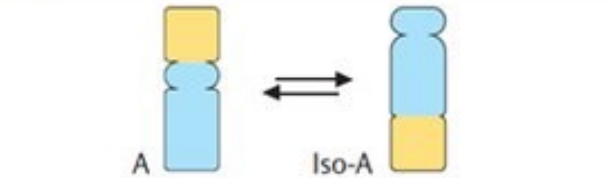
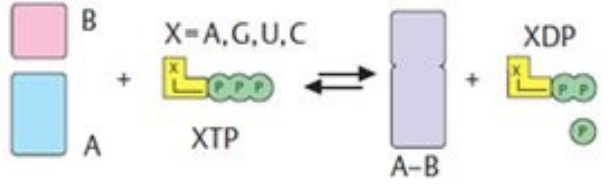
Different types enzymes

Class	Reaction type	Important subclasses
1 Oxidoreductases		Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases		C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases		Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")		C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases		Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")		C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

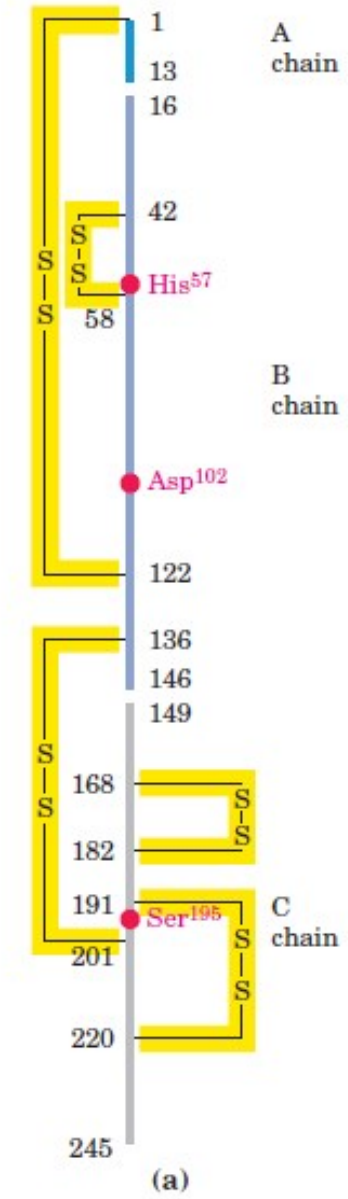
Structure and activity

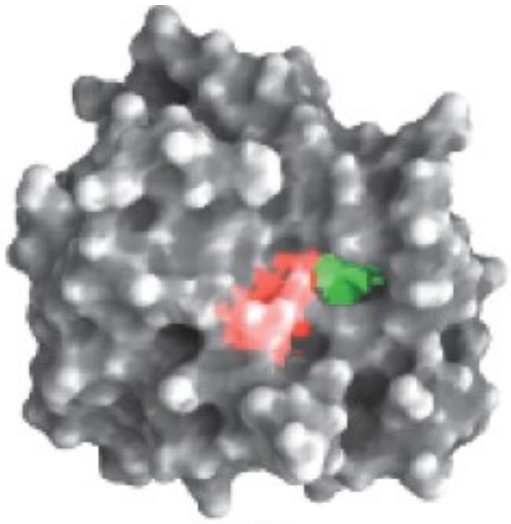
The **activities of enzymes** are determined by their **three-dimensional structures**.

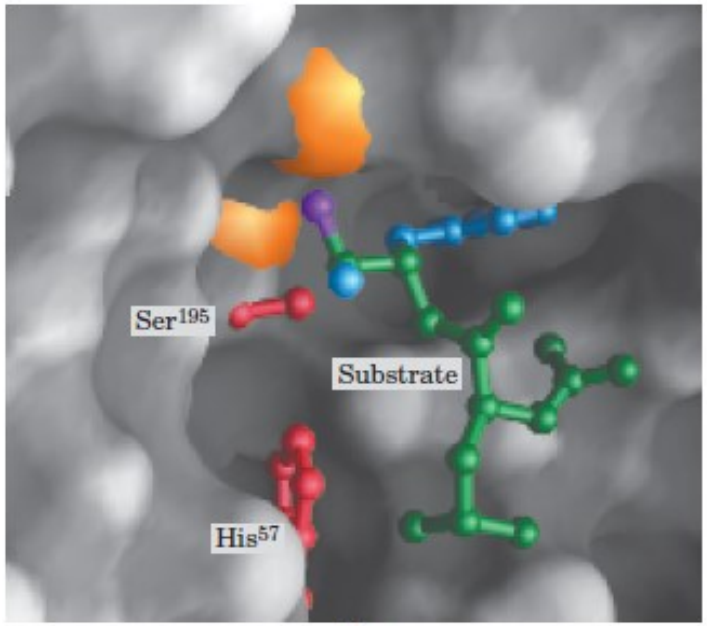
Most enzymes are much larger than the substrates they act on, and only a very small portion of the enzyme (around 3–4 amino acids) is directly involved in catalysis. **The region that contains these catalytic residues and binds the substrate and then carries out the reaction is known as the active site**.

Some enzymes also contain sites that bind **cofactors**, which are needed for catalysis.

Active site of Chymotrypsin







Some enzymes require cofactors

- The functional groups of proteins can participate in **acid-base reactions**, and **charge-charge interactions**,

- They are less suitable for **oxidation-reduction reactions** and many types of **group-transfer reactions**. Proteins require **Cofactors** that act as the enzymes' **chemical teeth**.
- Cofactors can be metal ions (Cu^{2+}, Fe^{3+} or Zn^{2+}.) or organic molecules, that either transiently associate with the enzyme and function as **cosubstance**, or permanently associated with the enzyme **called prosthetic groups (or coenzymes)**.

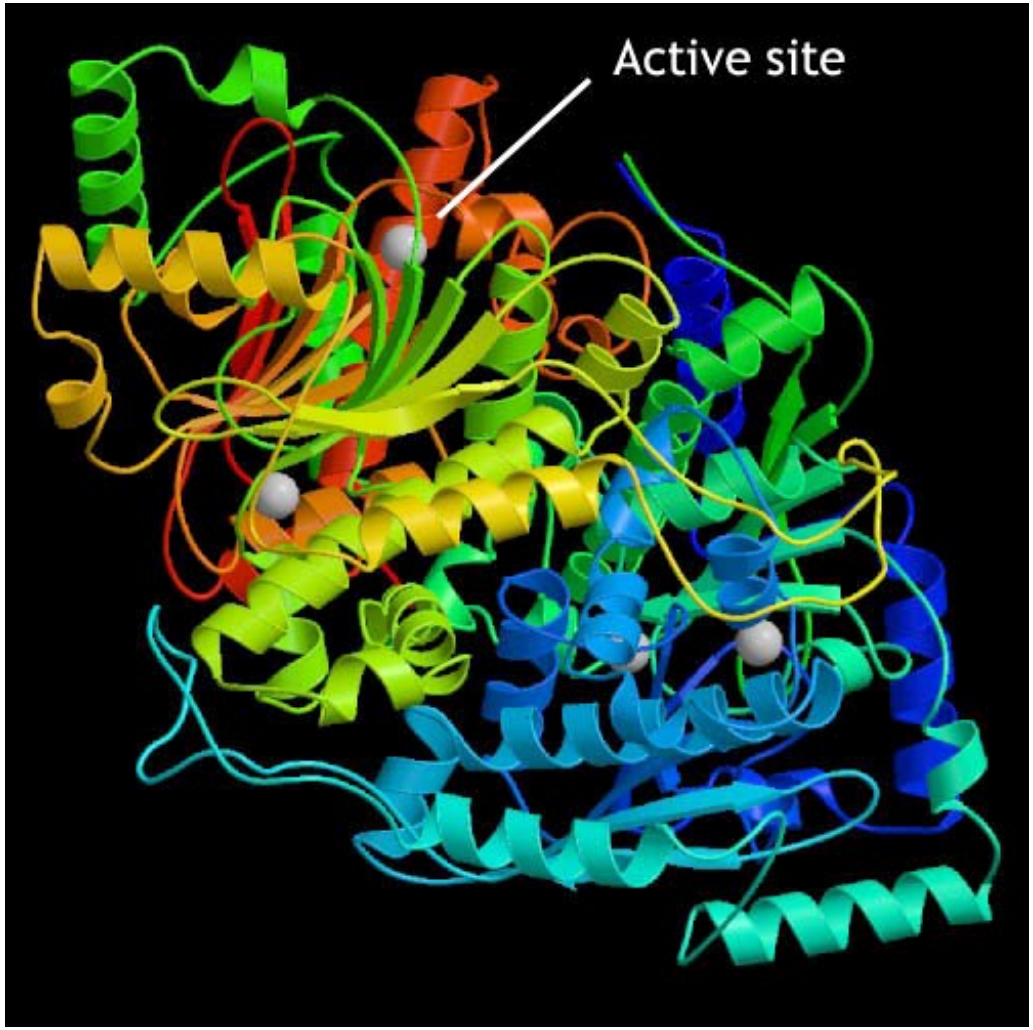
- **Many coenzymes are made of vitamins.**

Metal ion cofactors

- Nearly one-third of all known enzymes require the presence of metal ions for catalytic activity.
- This group of enzymes includes the **metalloenzymes**, which contain tightly bound metal ion cofactors, most commonly **transition metal ions** such as **Fe^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, or Co^{2+}.**
- Metal ions participate in the catalytic process in three major ways:

1) by **binding to substrates to orient them properly for reaction.**
2) by **mediating oxidation-reduction reactions through reversible changes in the metal ion's oxidation state**.
3) by **electrostatically stabilizing or shielding negative charges.** (act the same way as H^+, but have more charges and more abundant at neutral pH.

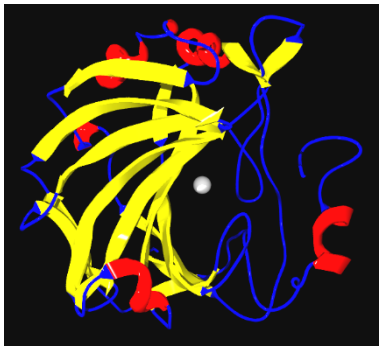
Structure and activity



Ribbon-diagram showing the active sites of Carbonic anhydrase.

The grey spheres are the Zn^{2+} in the four active sites of enzyme and are held within two protein chains.

Metal ion cofactors



Carbonic anhydrase: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$

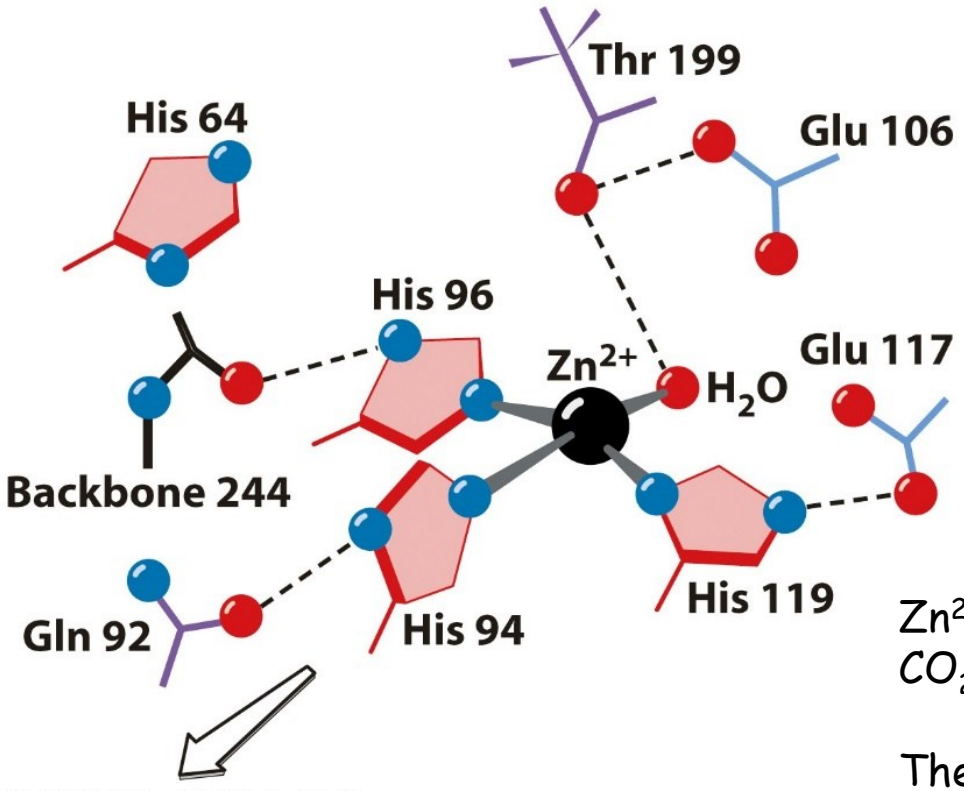


Figure 11-13 Fundamentals of Biochemistry, 2/e
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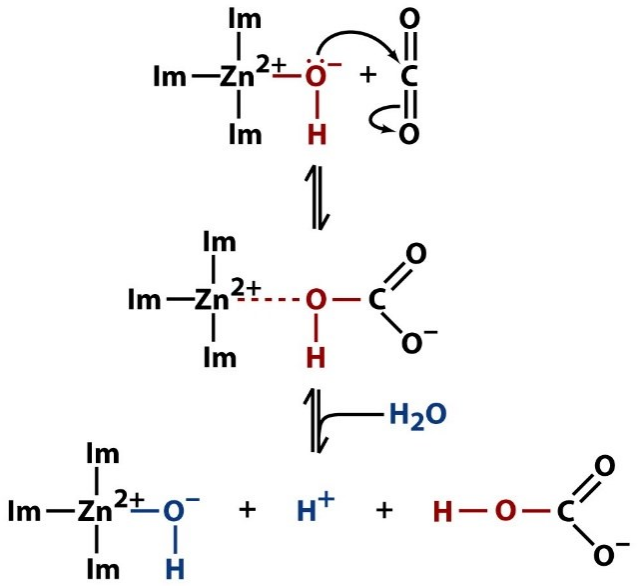

Im = imidazole

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Zn^{2+} polarized H_2O form OH^-, which attaches CO_2 to form HCO_3^-.

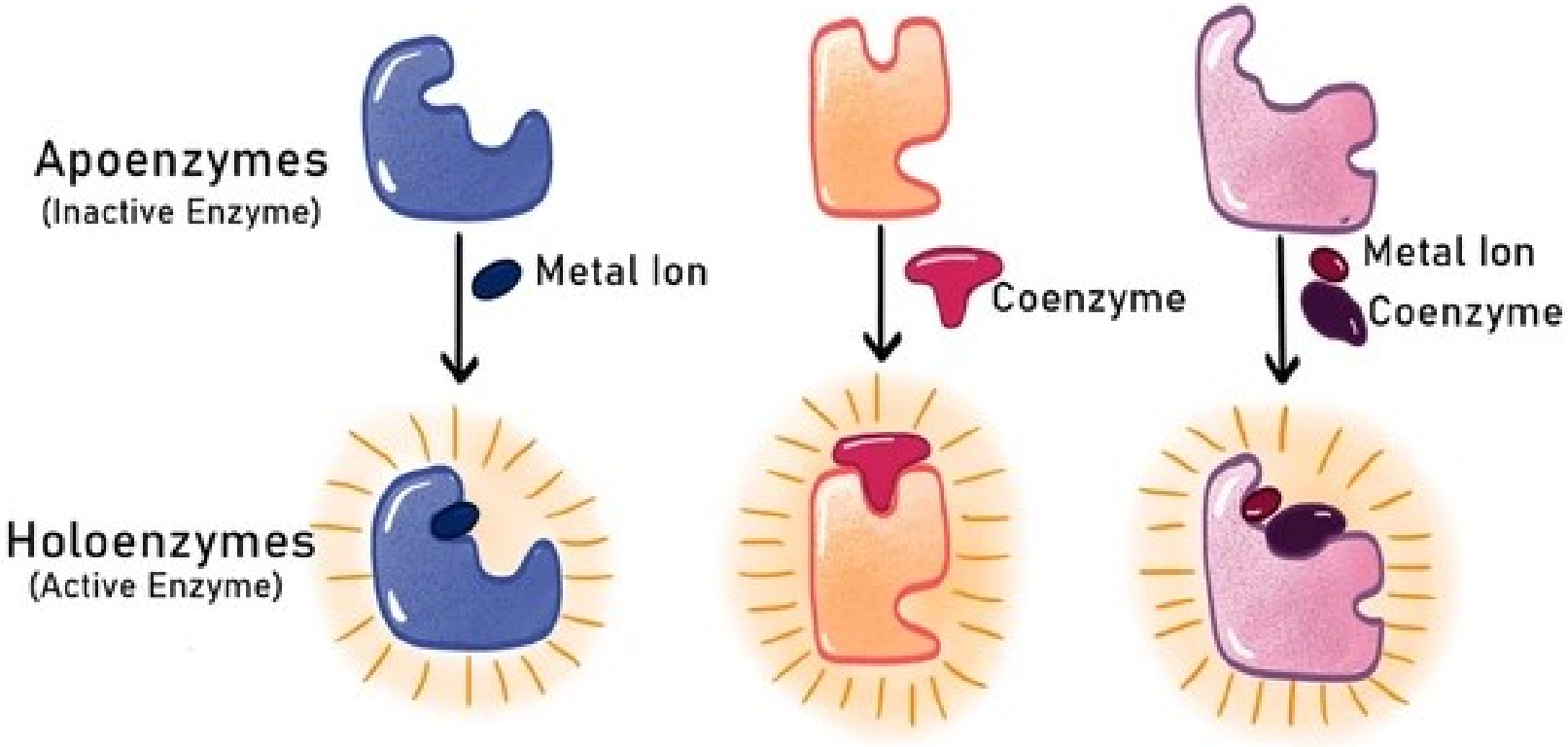
The enzyme is regenerated by binding another water.

The H^+ is shuffled out.

Some enzymes require cofactors

Enzymes that require a cofactor but do not have one bound are called **apoenzymes**. An apoenzyme together with its cofactor(s) is called a **holoenzyme** (*i.e.*, the active form).

Apoenzyme (inactive) + cofactor ↔ holoenzyme (active)

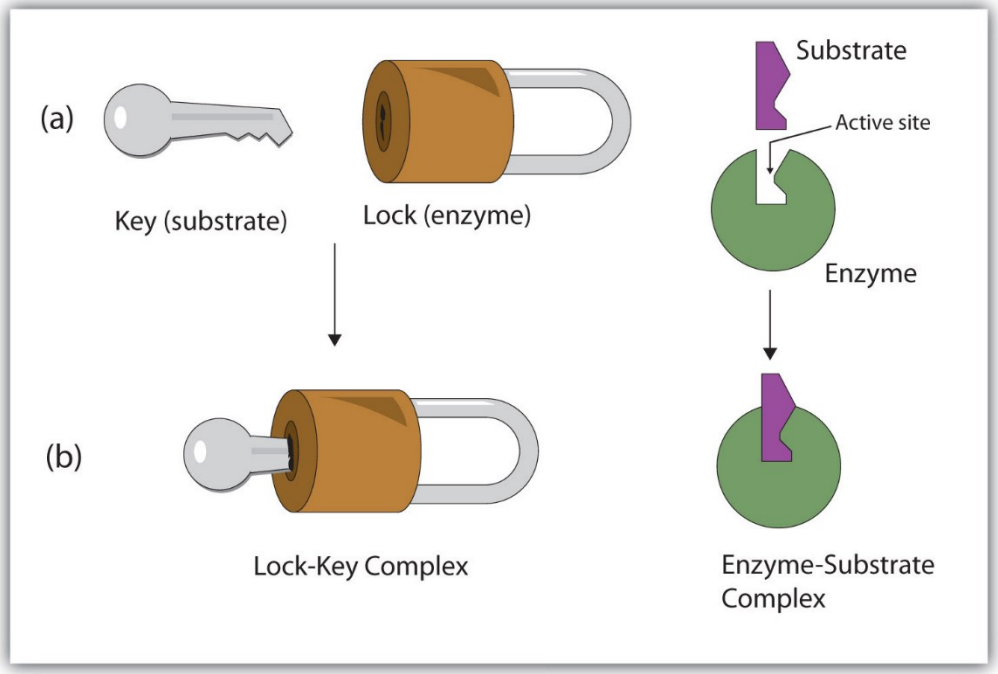


Structure and specificity

Enzymes are usually specific to the **reactions** they catalyze and the **substrates** that are involved in these reactions. **Shape complementarity, charge complementarity, and hydrophilic/hydrophobic** characters of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels of stereospecificity, chemoselectivity.

Structure and specificity

Lock and Key Mechanism



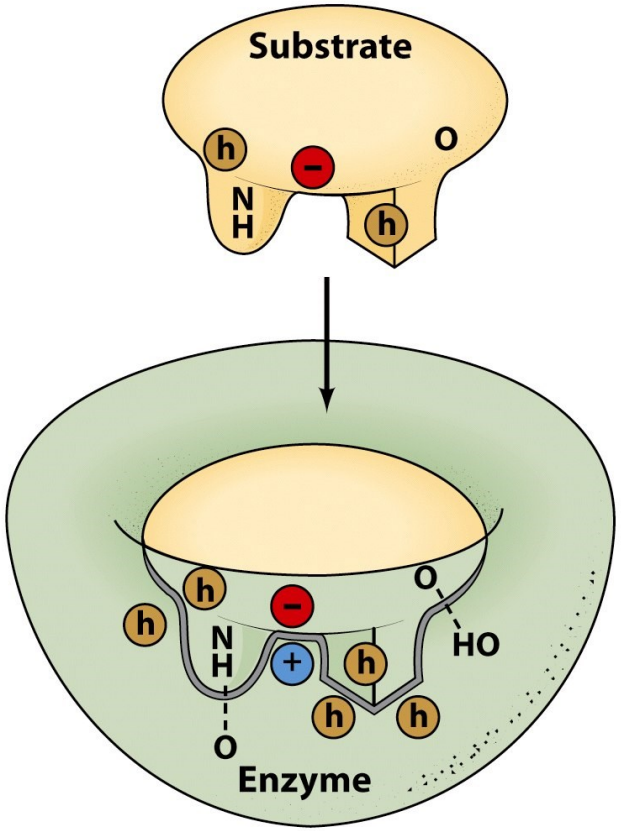


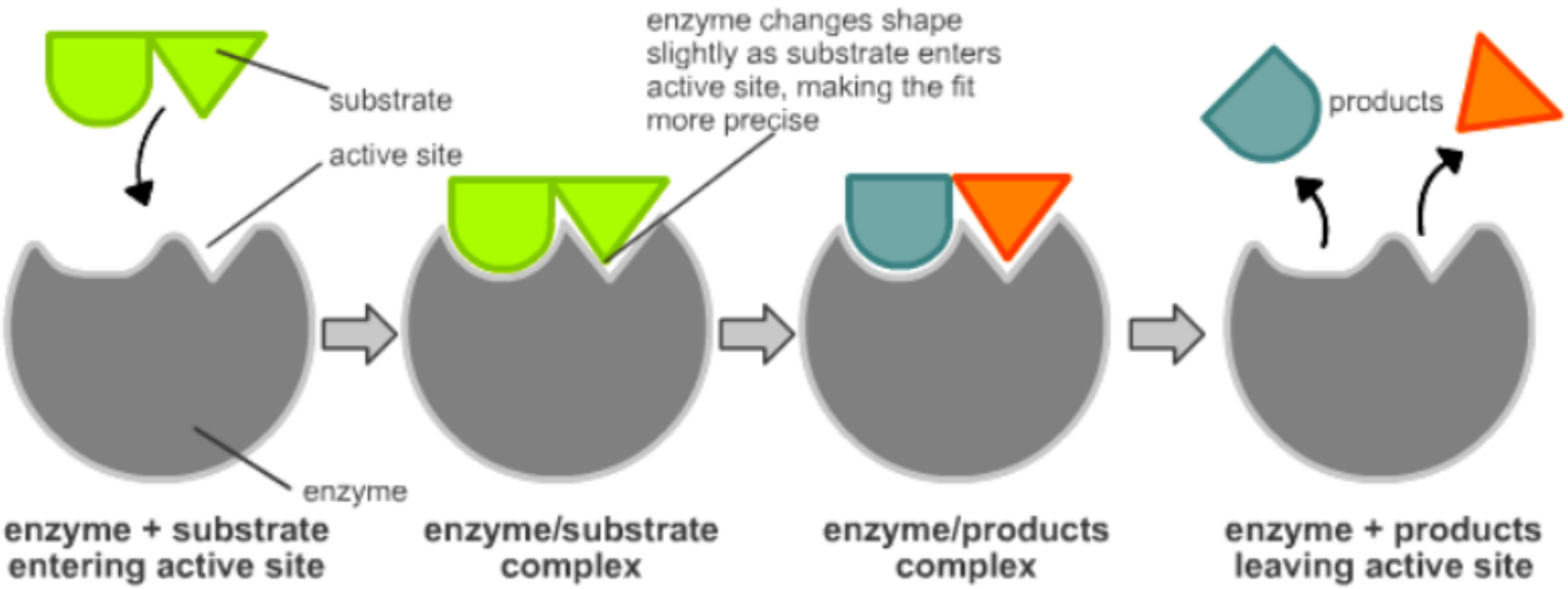
Figure 11-1 Fundamentals of Biochemistry, 2/e

Structure and specificity

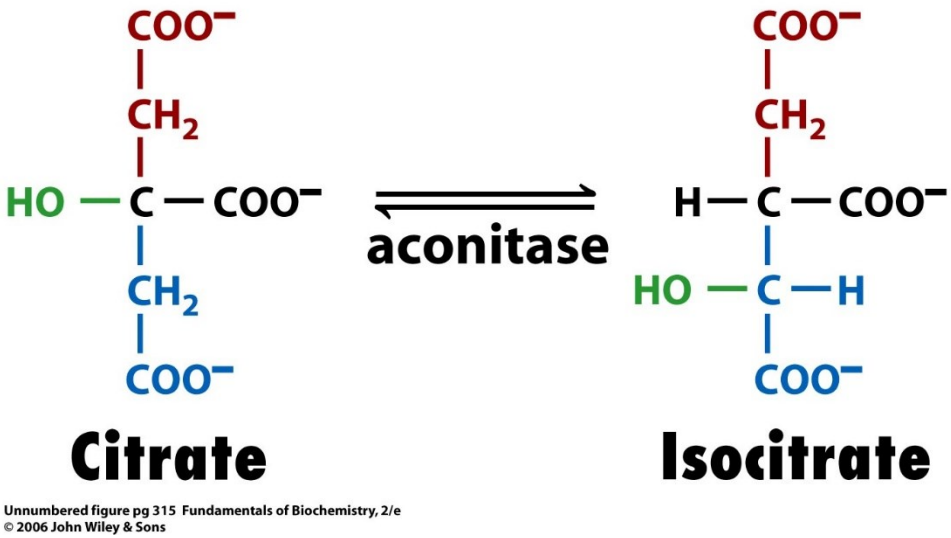
Induced fit model

In 1958 Daniel Koshland suggested a modification to the "lock and key" model. Since **enzymes are rather flexible structures**, the active site can be modified as the substrate interacts with the enzyme.

As a result, **the amino acid side chains making up the active site are molded into a precise shape which enables the enzyme to perform its catalytic function**. In some cases the substrate molecule also changes shape slightly as it enters the active site. This model explains both **enzyme specificity** and the **stabilization of the transition state**.



Enzymes are Stereospecific



prochiral chiral

Nearly all enzymes that participate in chiral reactions are absolutely stereospecific.

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How enzymes enhance reaction rate?

Activation energy and reaction coordinate

Much of our understanding of how enzyme catalyze chemical reactions comes from **transition state theory.** (developed by Henry Eyring, in 1930s)

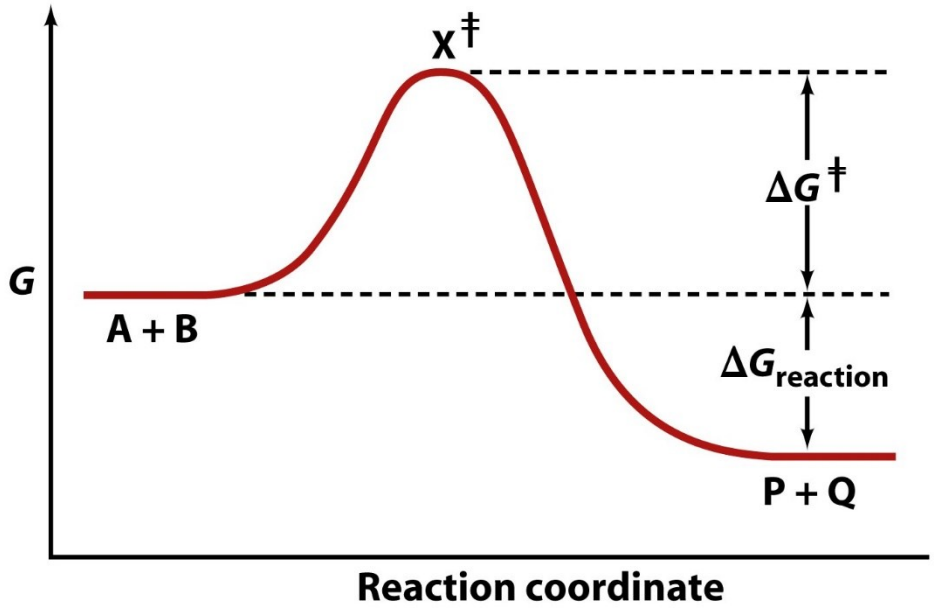

Figure 11-5b Fundamentals of Biochemistry, 2/e
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Reaction coordinate: reactants generally approach one and another **along the path of minimum free energy**.

$\Delta G^{\ddagger}$: the free energy of the transition state less than that of the reactants is known as the activation energy.

Activation energy and reaction coordinate

Much of our understanding of how enzyme catalyze chemical reactions comes from **transition state theory.** (developed by Henry Eyring, in 1930s)

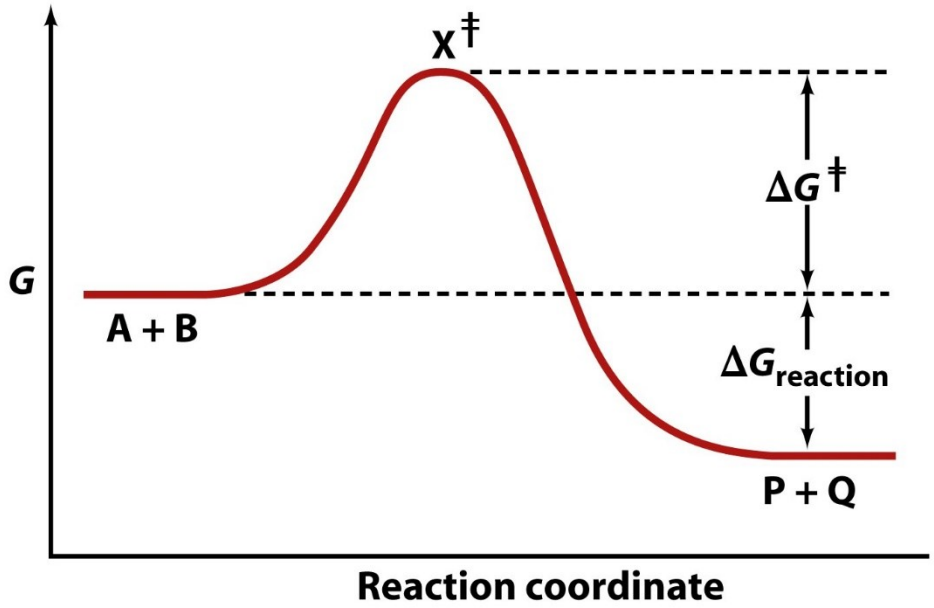


Figure 11-5b Fundamentals of Biochemistry, 2/e

$$rate = Ae^{-\Delta G^{\pm}/RT}$$

$$\ln rate = \ln A - \frac{\Delta G}{RT}$$

Arrhenius plot

Enzyme speed up reactions by lowering activation energy

As with all catalysts, all reactions catalyzed by enzymes must be "spontaneous" (containing a net negative Gibbs free energy). In the presence of an enzyme, a reaction runs in the same direction as it would without the enzyme, just more quickly.

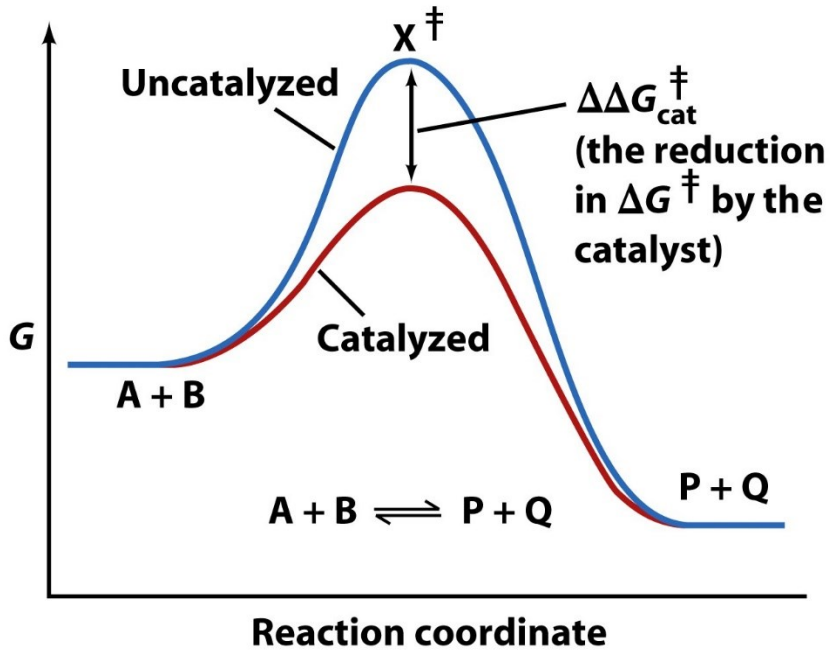


Figure 11-7 Fundamentals of Biochemistry, 2/e

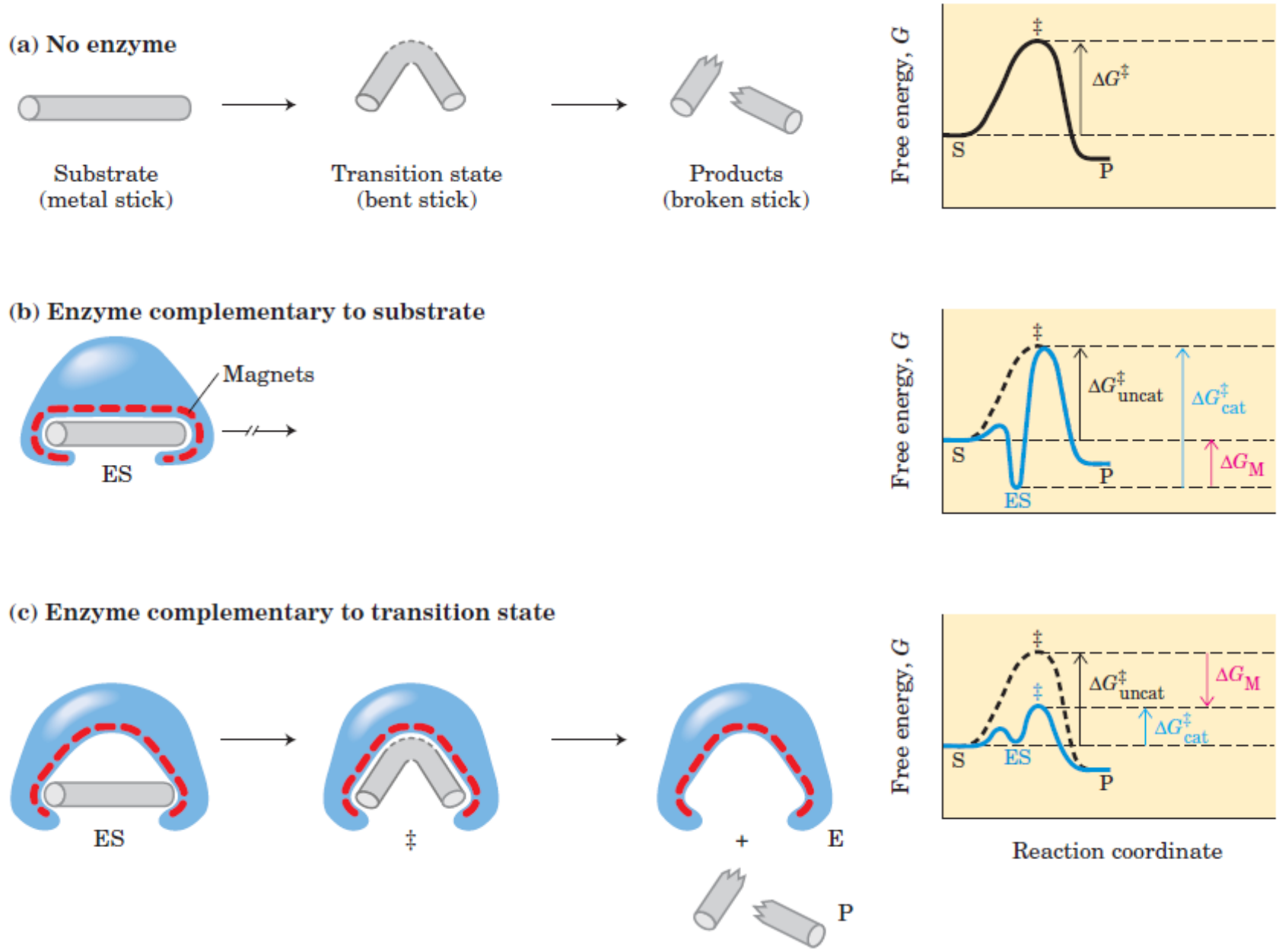
$$\frac{rate_{catalyzed}}{rate_{uncatalyzed}} = e^{\Delta\Delta G_{cat}/RT}$$

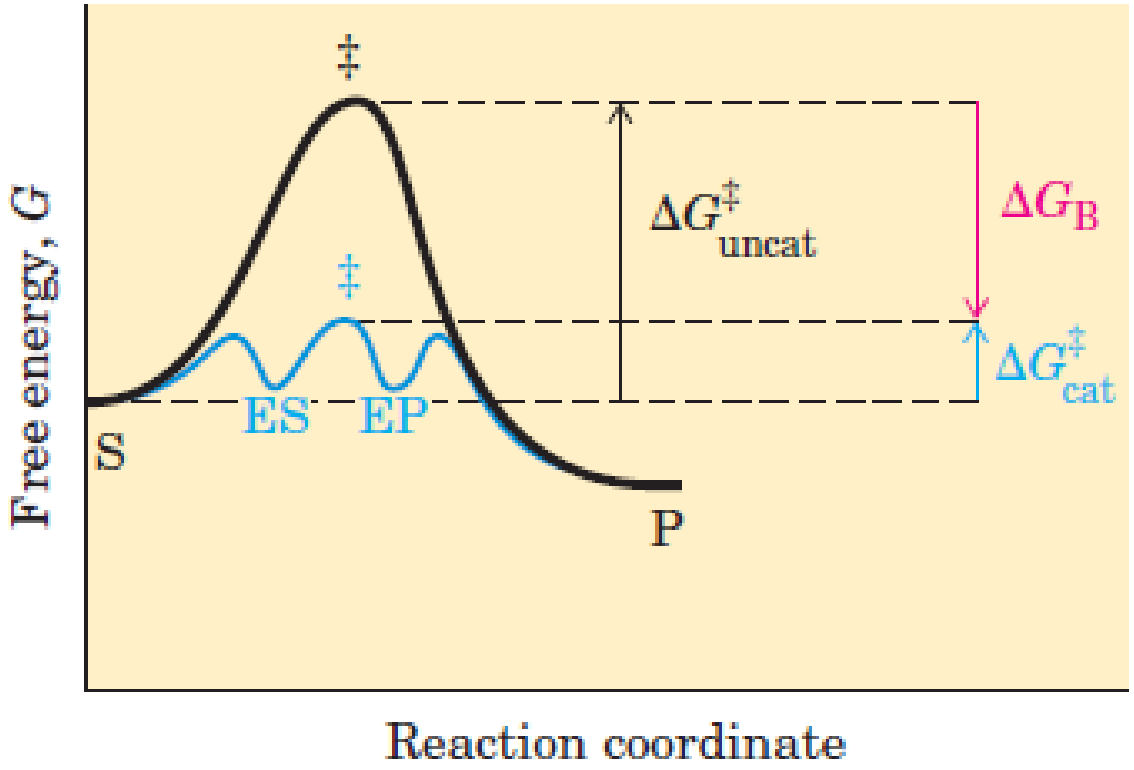
$$K_{eq} = \frac{k_{forward}}{k_{backward}}$$

1RT=~2.5 KJ/mol
25 KJ/mol, 2×10^4 fold faster
40 KJ/mol, ~ 10^7fold faster.

Enzymes catalyze the forward and backward reactions equally. They do not alter the equilibrium itself, but only the speed at which it is reached.

Hypothetical enzyme catalyst





Role of binding energy in catalysis. To lower the activation energy for a reaction, the system must acquire an amount of energy equivalent to the amount by which $\Delta G^{\ddagger}$ is lowered. Much of this energy comes from binding energy (ΔG_B) contributed by formation of weak noncovalent interactions between substrate and enzyme in the transition state. The role of ΔG_B is analogous to that of ΔG_M in the previous figure.

Reaction Kinetics

$A \rightarrow P$ May occur through a sequence of **elementary reactions**

$$A \rightarrow I_1 \rightarrow I_2 \rightarrow P$$

Where I_1 and I_2 symbolize **intermediates.**

Reaction order indicates the number of molecules participating in an **elementary reaction**.

First order reaction: $A \rightarrow P$ (unimolecular reaction)

Second order reaction (must be bimolecular reaction)

Termolecular reaction are unusual. Any higher order of reaction is unknown

Rate equation indicates the progress of a reaction as a function of time.

First order reaction

$$A \xrightarrow{k} P$$ k: the rate constant

v: the reaction rate or reaction velocity can be expressed by the rate of appearance of product or disappearance of reactant.

$$v = \frac{d[P]}{dt} = -\frac{d[A]}{dt} = k[A]$$

$$\frac{d[A]}{[A]} = d\ln[A] = -kt$$

$$\int_{[A]_0}^{[A]} d\ln[A] = -k\int_0^t dt$$

$$\ln[A] = \ln[A]_0 - kt$$

$$[A] = [A]_0 e^{-kt}$$

$$\ln\frac{[A]}{[A]_0} = -kt$$

$$at\ [A] = \frac{[A]_0}{2}, t = t_{1/2}$$

$$-\ln 2 = -kt_{1/2}$$

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

First order reaction

$$A \xrightarrow{k} P$$

k: the rate constant

v: the reaction rate or reaction velocity can be expressed by the rate of appearance of product or disappearance of reactant.

In a first order reaction:
(1) Reaction rate constant has a unit of s^{-1}
(2) Plot ln[A] vs t gives a straight line with slope = -k
(3) $t_{1/2}$ is a constant, independent of the initial reaction concentration.

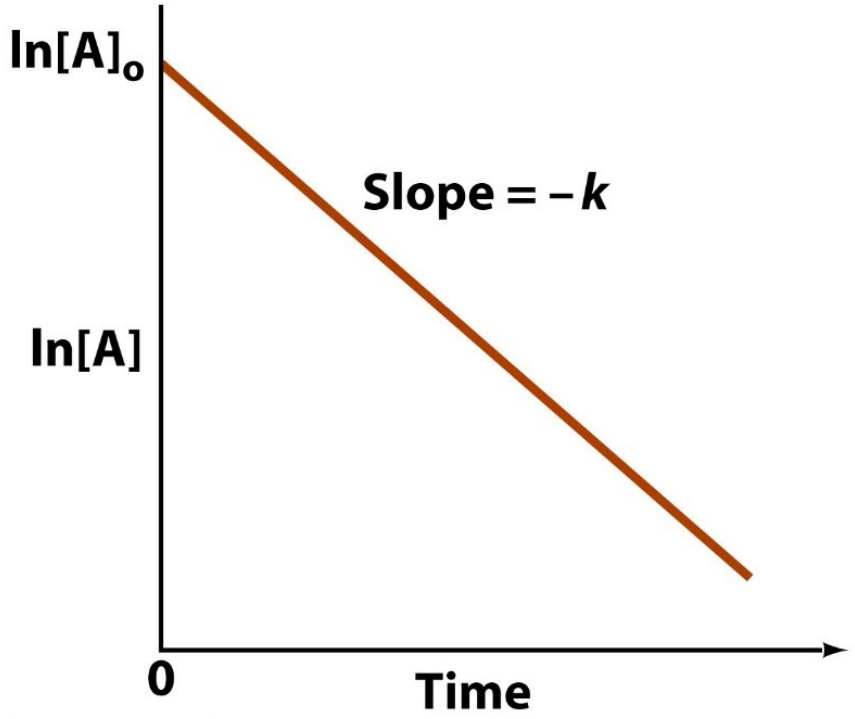


Figure 12-1 Fundamentals of Biochemistry, 2/e

Second order reaction

$2A \rightarrow P$

$$v = -\frac{d[A]}{dt} = k[A]^2$$

$$-\frac{d[A]}{[A]^2} = kdt$$

$$\int_{[A]_0}^{[A]} -\frac{d[A]}{[A]^2} = k\int_0^t dt$$

$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$$

$$at[A] = \frac{[A]_0}{2}, t = t_{1/2}$$

$$\frac{2}{[A]_0} = \frac{1}{[A]_0} + kt_{1/2}$$

$$t_{1/2} = \frac{1}{k[A]_0}$$

Second order reaction

$2A \rightarrow P$

- **In a Second order reaction**
- (1) k has a unit of $M^{-1} S^{-1}$
- (2) plot 1/[A] vs t gives a straight line, with slope = k
- (3) $t_{1/2}$ depends on the initial reactant concentration
- $[A]_0 \uparrow t_{1/2}\downarrow$

(2) $A+B \rightarrow P$

It is often convenient to increase one reaction relative to the other, e.g [B]>>[A], so [B] does not change much through the reaction. k'=k[B]

The second order reaction is reduced to a

pseudo-first order reaction.

Enzyme Kinetics (single substrate reactions)

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

$$v = \frac{d[P]}{dt} = k_2[ES] \qquad K = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

Initial conditions:

(1) [S]>>[E];

(2) [P]→0

(3) **E+S⇌ES reach equilibrium quickly**

Dissociation constant of the **enzyme-substrate complex**

Enzyme Kinetics (single substrate reactions)

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

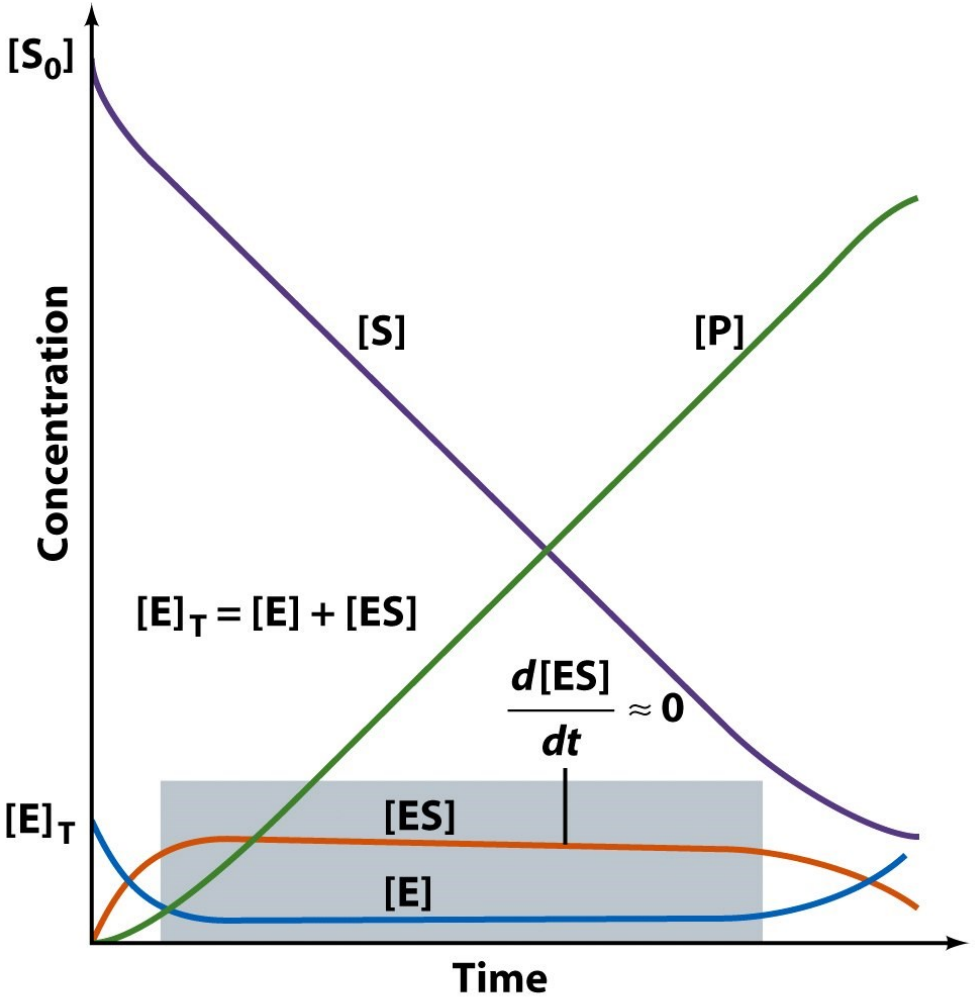


Figure 12-2 Fundamentals of Biochemistry, 2/e

Enzyme Kinetics (single substrate reactions)

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

A steady state assumption:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$$

$$[E] = [E]_T - [ES]$$

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

$$\frac{([E]_T - [ES])[S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_M$$

$$K_M[ES] = ([E]_T - [ES])[S]$$

$$[ES] = \frac{[E]_T[S]}{K_M + [S]}$$

$$v_0 = \left(\frac{d[P]}{dt}\right)_{t\to 0} = k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]}$$

Michaelis-Menten equation (initial velocity)

$$v_0 = \left(\frac{d[P]}{dt}\right)_{t\to 0} = k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]}$$

(1) As [S]>>K_M, when enzyme is saturated $v_0 \to v_{\max} = k_2[E]_T$ (pseudo-first order reaction)

(2) K_M =[S], at which v_0=v_{max}/2,

Michaelis constant has a simple operational definition: the substrate concentration at which the reaction velocity is half-maximal, unit of concentration.

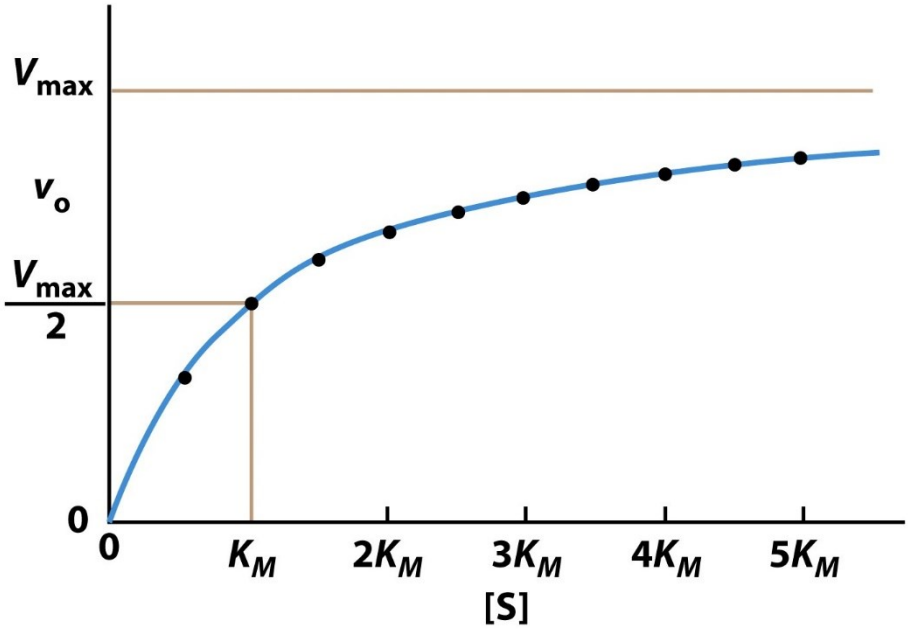


Figure 12-3 Fundamentals of Biochemistry, 2/e
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$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$

K_M is unique for each enzyme-substrate pair

Table 12-1 The Values of K_M, k_{cat}, and k_{cat}/K_M for Some Enzymes and Substrates

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Accetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

Table 12-1 Fundamentals of Biochemistry, 2/e