Chapter 8

Enzymes: basic concept and kinetics

Learning objectives: mechanism of enzymatic catalysis
Michaelis -Menton Model
Inhibition
Single Molecule of Enzymatic Reaction

- Enzymes: catalysis chemical reactions as well as reactions that involve energy converiosn
- Highly efficient and specific
- Reaction takes place at active site
- Majority of enzymes are proteins (except RNA molecules)
- Enzymes specifically binds diverse molecules and catalyze reaction by stabilizing transition state

TABLE 8.1 Rate enhancement by selected enzymes

Enzyme	Nonenzymatic half-life		Uncatalyzed rate (k_{un} s^{-1})	Catalyzed rate (k_{cat} s^{-1})	Rate enhancement (k_{cat} s^{-1}/k_{un} s^{-1})
OMP decarboxylase	78,000,000	years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000	years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000	years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3	years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7	weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9	days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4	hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5	seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wolfenden. *Science* 267:90–93, 1995.

Table 8.1
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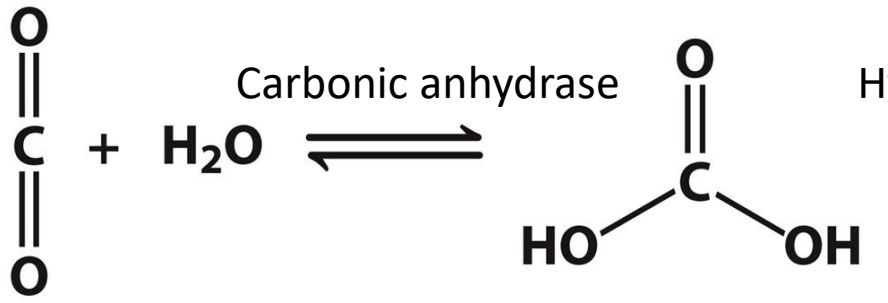

Hydrates 10^6 molecules of CO_2 per seconds

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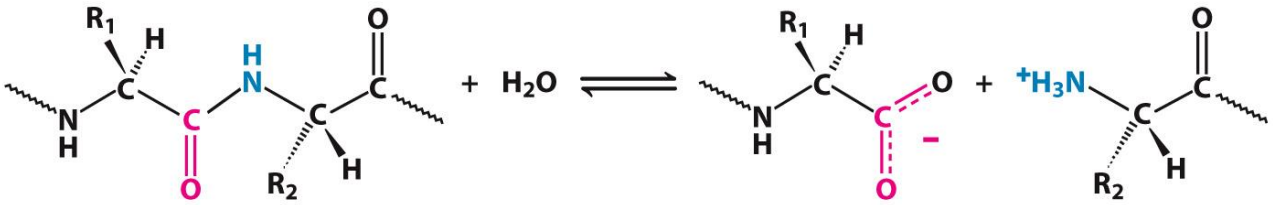
Substrate specificity:

some enzymes are highly specific, some catalysis reactions that are related.

Proteolytic enzymes: catalysis peptide bond hydrolysis



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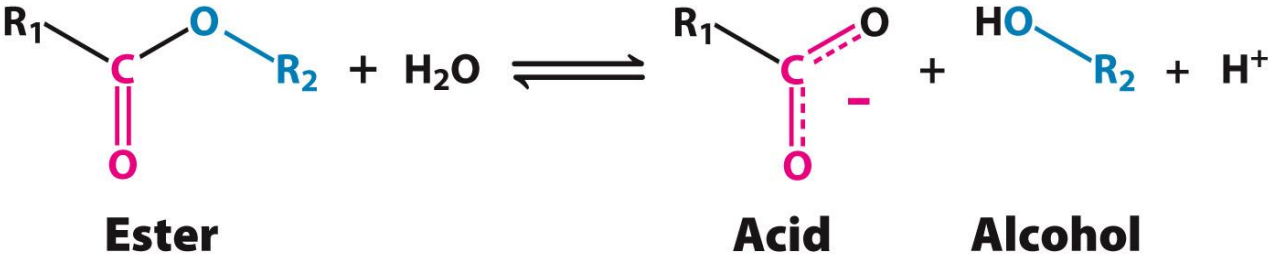
As well as hydrolysis of the ester bond



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- **Papain:** cleaves any peptide bond
- **Trypsin:** cleaves peptide bond at carboxyl side of Lys or Arg
- **Thrombin:** cleaves bond between Arg and Gly at the specific sequence

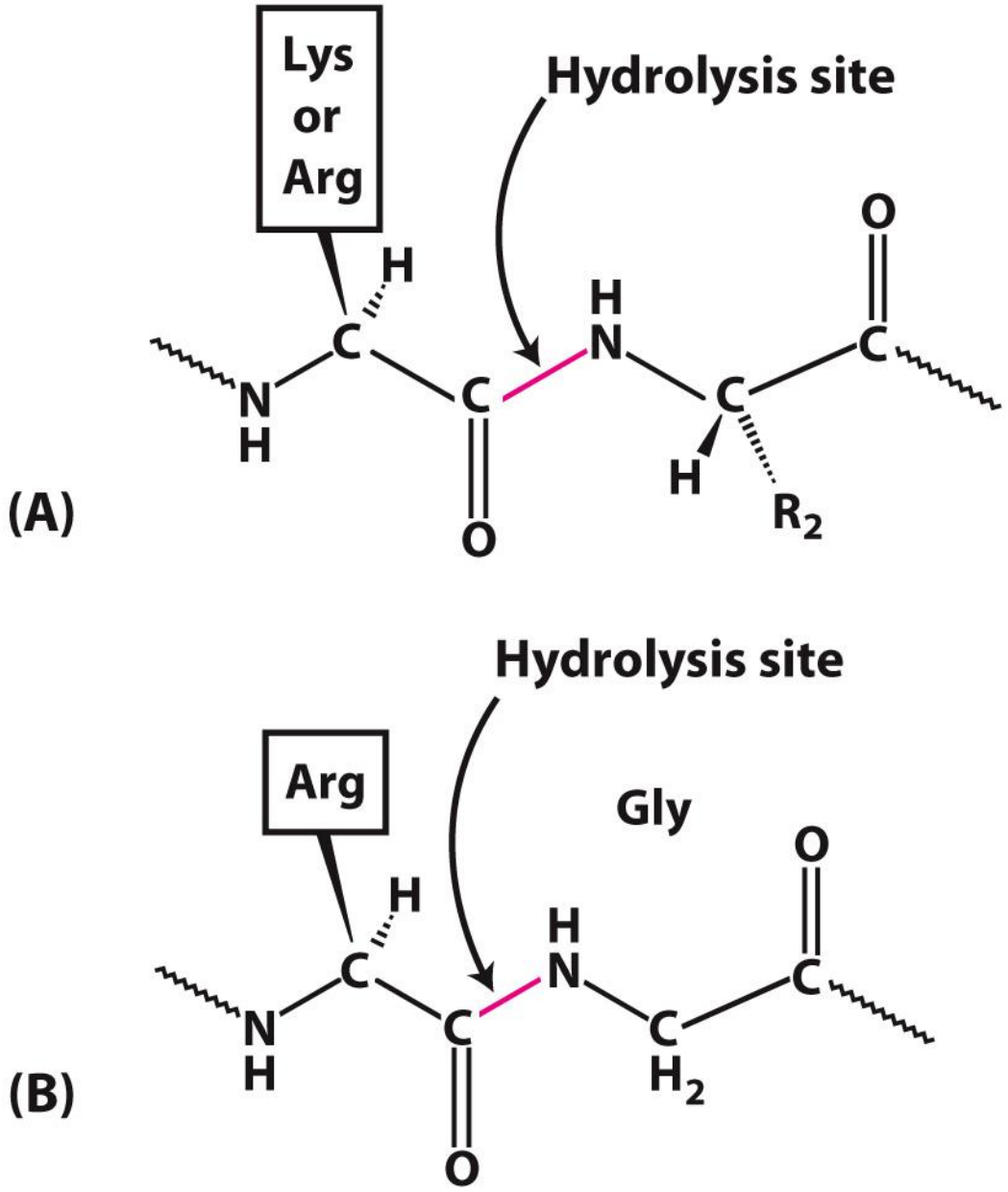

Figure 8.1
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Co-factors

Cofactors are small molecules that some enzymes require for activity. The two main classes of cofactors are coenzymes (**organic molecules** derived from vitamins) and **metals**.

Tightly bound coenzymes are called **prosthetic groups.**

An enzyme with its cofactor is a **holoenzyme**. Without the cofactor, the enzyme is called an **apoenzyme**.

TABLE 8.2 Enzyme cofactors

Cofactor	Enzyme
Coenzyme	
Thiamine pyrophosphate	Pyruvate dehydrogenase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrogenase
Se	Glutathione peroxidase
Mn	Superoxide dismutase
K^{+}	Acetyl CoA thiolase

Table 8.2
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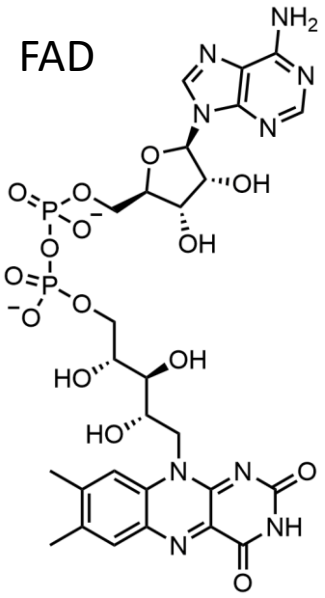
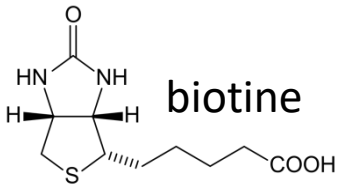
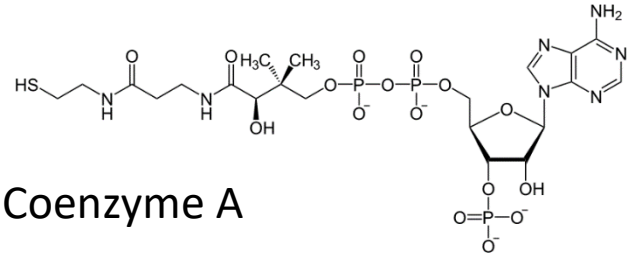
FAD

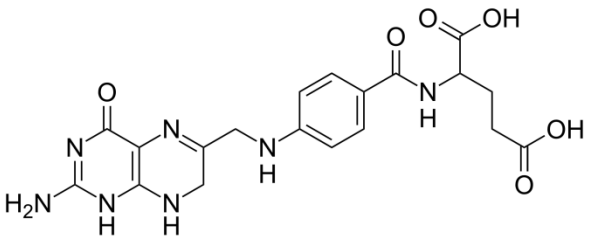


biotine





Coenzyme A



tetrahydrofolate

Thermodynamic of enzymatic reaction

A reaction will occur without the input of energy, or spontaneously, only if ΔG is negative. Such reactions are called **exergonic reactions**.

If a reaction is at equilibrium, there is no net change in the amount of reactant or product. **At equilibrium, ΔG =0.**

A reaction will not occur spontaneously if the ΔG is positive. These reactions are called **endergonic reactions**.

The ΔG of a reaction depends only on the free energy difference between reactants and products and is **independent of how the reaction occurs**.

The ΔG of a reaction provides no information about the rate of the reaction.

The standard free-energy change of a reaction is related to the equilibrium constant

For the reaction

$$A + B \rightleftharpoons C + D$$

The free energy change is given by

$$\Delta G = \Delta G^{\circ} + RT \ln \frac{[C][D]}{[A][B]}$$

Where ΔG^{o} is the standard free-energy change for the reaction under standard conditions (concentration of reactants and product is 1 M), R is the gas constant, T is 298 kelvins and the brackets denote concentration in moles.

$\Delta G^{o\prime}$ symbolizes the standard free energy change at pH 7.

At equilibrium, $\Delta G = 0$, so for the reaction in question

$$0 = \Delta G^{\circ\prime} + RT \ln \frac{[C][D]}{[A][B]}$$

or

$$\Delta G^{\circ\prime} = -RT \ln \frac{[C][D]}{[A][B]}$$

The equilibrium constant for the reaction under standard conditions is

$$K'_{eq} = \frac{[C][D]}{[A][B]}$$

Thus

$$\Delta G^{o\prime} = -RT \ln K'_{eq}$$

Which can be arranged to give

$$K'_{eq} = e^{-\Delta G^{o\prime}/RT}$$

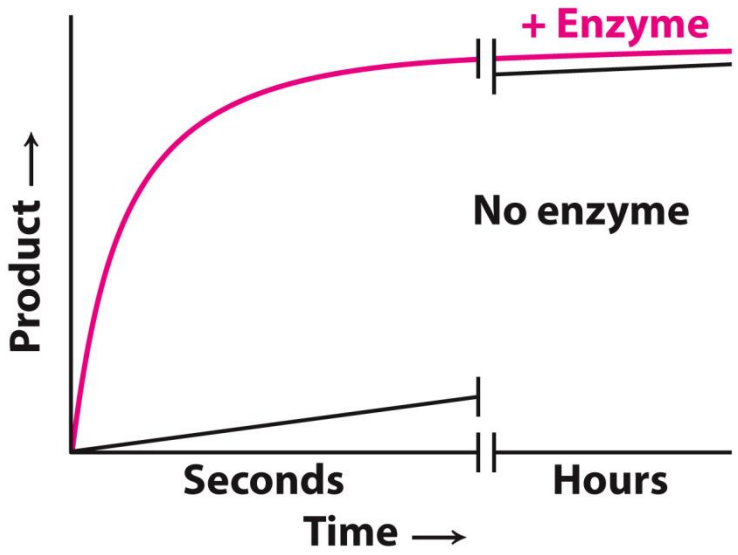
or

$$K'_{eq} = e^{-\Delta G^{o\prime}/2.47}$$

The criterion of spontaneity is ΔG and not $\Delta G^{o'}$

Whether ΔG is larger, smaller of same at $\Delta G^{o'}$
depends on the concentration of reactants and products . Reactions that are not spontaneous based on $\Delta G^{o'}$ may happen at certain concentrations of reactants and products

- The reaction equilibrium is determined only by the free-energy difference between the products and reactants. Enzymes cannot alter this difference.
- Enzymes affect only the rate of the reaction, not the equilibrium



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$$S \underset{k_R}{\overset{k_F}{\rightleftharpoons}} P$$

$$K_{eq} = \frac{[P]}{[S]} = \frac{k_F}{k_R}$$

Enzymes accelerate chemical reactions by facilitating the formation of the transition state

A chemical reaction proceeds through a transition state, a molecular form that is no longer substrate but not yet product.

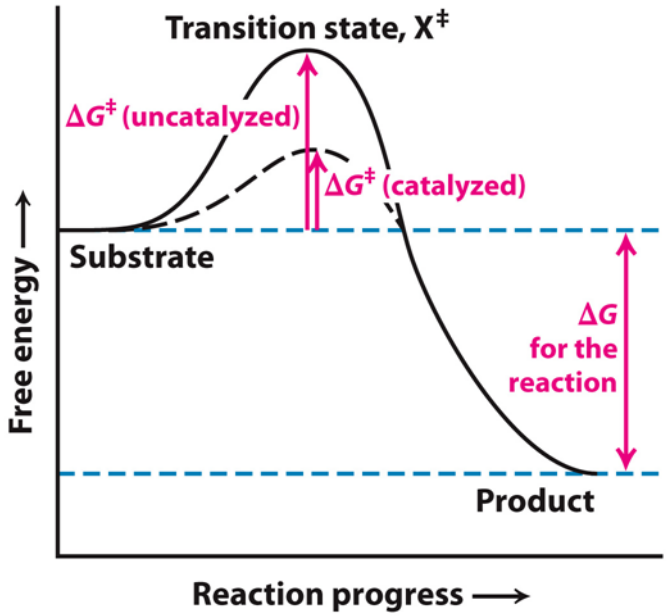
$$S \rightleftharpoons X^{\ddagger} \rightarrow P$$

The transition state is designated by the double dagger.

The energy required to form the transition state from the substrate is called the activation energy, symbolized by

$$\Delta G^{\ddagger} = G_X{}^{\ddagger} - G_S$$

Enzymes facilitate the formation of the transition state.



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To understand the rate increase achieved by enzymes, assume that the transition state is in equilibrium with the substrate.

$$\mathrm{S} \overset{K^{\ddagger}}{\rightleftharpoons} \mathrm{X}^{\ddagger} \xrightarrow{v} \mathrm{P}$$

The rate of reaction is proportional to the concentration of $\mathrm{X}^{\ddagger}$

$$v \propto [\mathrm{X}^{\ddagger}]$$

because only $\mathrm{X}^{\ddagger}$ can be converted into product.

The concentration of $\mathrm{X}^{\ddagger}$ depends on the energy

difference between $\mathrm{X}^{\ddagger}$ and S, the activation energy.

The overall rate of the reaction V depends on $\Delta G^{\ddagger}$.

$$V = v[\mathrm{X}^{\ddagger}] = \frac{kT}{h}[\mathrm{S}]e^{-\Delta G^{\ddagger}/RT}$$

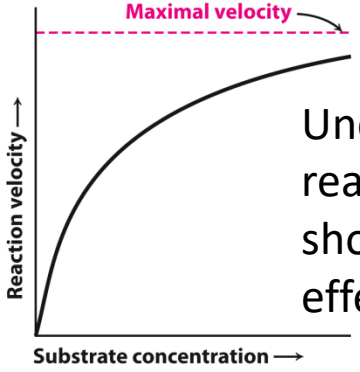
In this equation, *k* is Boltzmann's constant and *h* is Planck's constant.

The formation of an enzyme–substrate complex is the first step in enzymatic catalysis

Enzymes bring substrates together to form an enzyme-substrate complex on a particular region of the enzyme called the active site.

The interaction between enzyme and substrate was established by

1. The observation that a fixed amount of enzyme displays a maximal velocity.
2. The observation that spectroscopic properties of enzyme and substrates change upon interaction.
3. Examination by X-ray crystallography of enzymes bound to substrates or substrate analogs.



Uncatalyzed reaction does not show saturation effect

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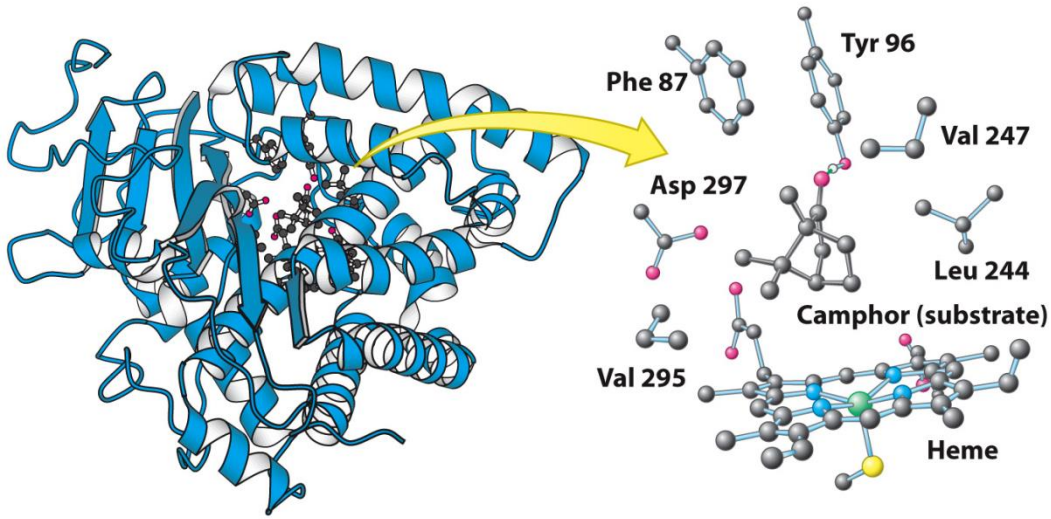

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P450 in complex with camphor

Properties of the active site:

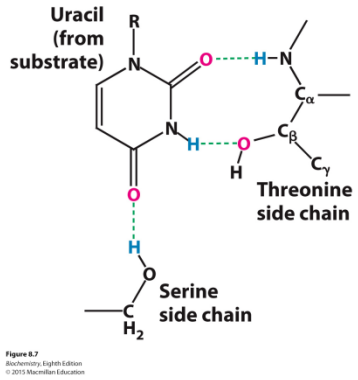
1.The active site is a three-dimensional cleft or crevice created by amino acids from different parts of the primary structure.

2.The active site constitutes a small portion of the enzyme volume.

3.Active sites create unique microenvironments.

4.The interaction of the enzyme and substrate at the active site involves multiple weak interactions.

5.Enzyme specificity depends on the molecular architecture at the active site.



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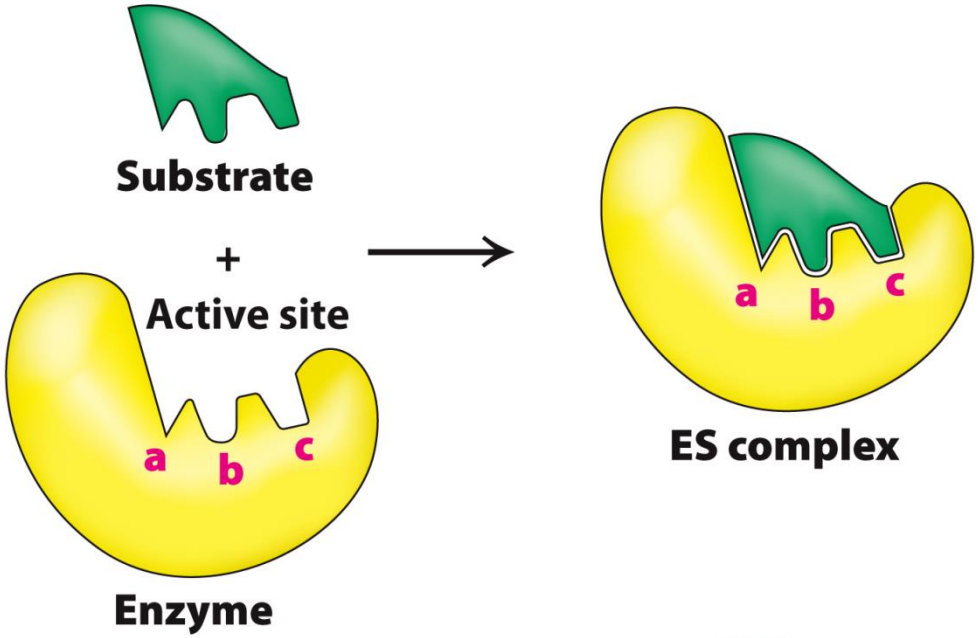
Interactions between ribonuclease and uracil

Enzymes do not interact with their substrates like a lock and key.

Rather, the enzyme changes shape upon substrate binding, a phenomenon called **induced fit**.



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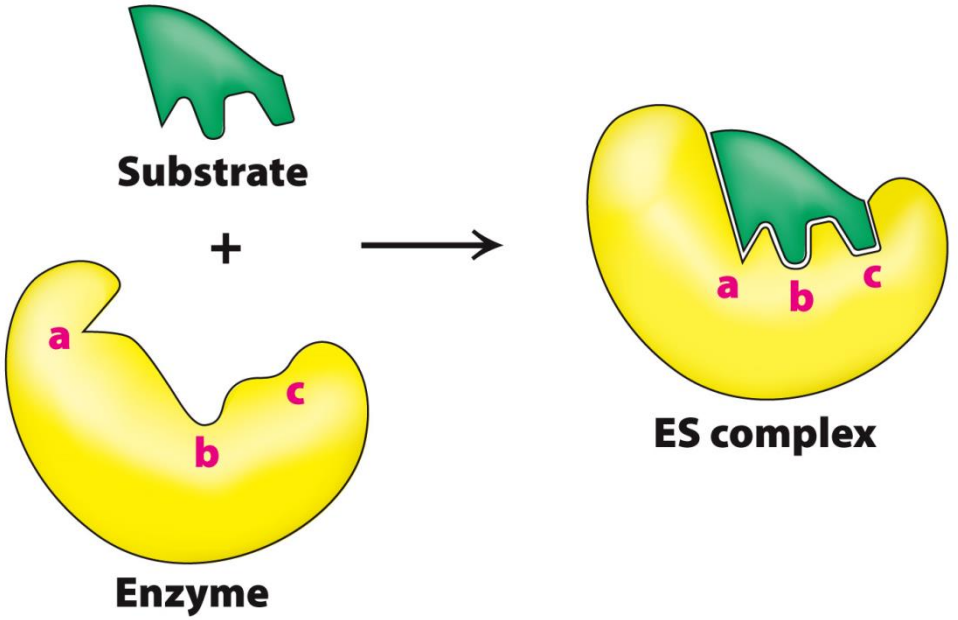

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Michaelis Menton Model

Consider a simple reaction

$$\mathrm{A} \rightarrow \mathrm{P}$$

The velocity or rate of the reaction is determined by measuring how much A disappears as a function of time or how much P appears as a function of time.

$$V = -\Delta \mathrm{A}/\Delta T = \Delta \mathrm{P}/\Delta T$$

Suppose that we can readily measure the disappearance of A. The velocity of the reaction is given by the formula below, where k is a proportionality constant.

$$V = k[\mathrm{A}]$$

When the velocity of a reaction is directly proportional to reactant concentration, the reaction is called a first-order reaction and the proportionality constant has the units s^{-1}.

Many important biochemical reactions are bimolecular or second-order reactions.

$2\,A \rightarrow P$ or $A + B \rightarrow P$

The rate equations for these reactions are

$V = k[A]^2$ and $V = k[A][B]$

The proportionality constant for second-order reactions has the units $M^{-1}s^{-1}$.

The steady-state assumption facilitates a description of enzyme kinetics

A common means of investigating enzyme kinetics is measuring velocity as a function of substrate concentration with a fixed amount of enzyme.

Consider a simple reaction in which the enzyme E catalyzes the conversion of S→P.

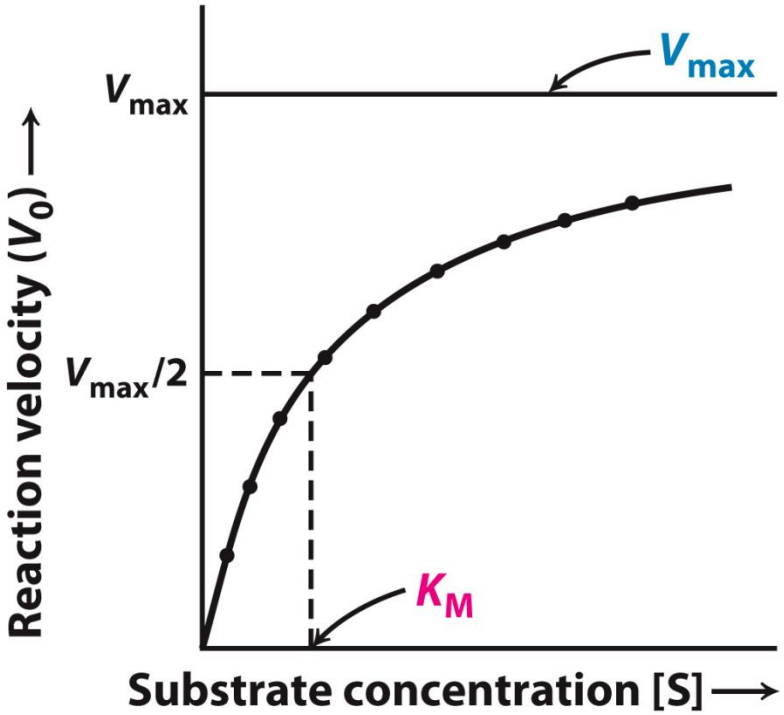
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

with k_1, k_2 k_{-1} and k_{-2} being the rate constants for the indicated reaction steps. So as to ignore the reverse reaction of P→S, we measure activity when [P] ≈ 0.

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

Under these conditions, the velocity is called the initial velocity or V_o.

Initial velocity as a function of substrate concentration



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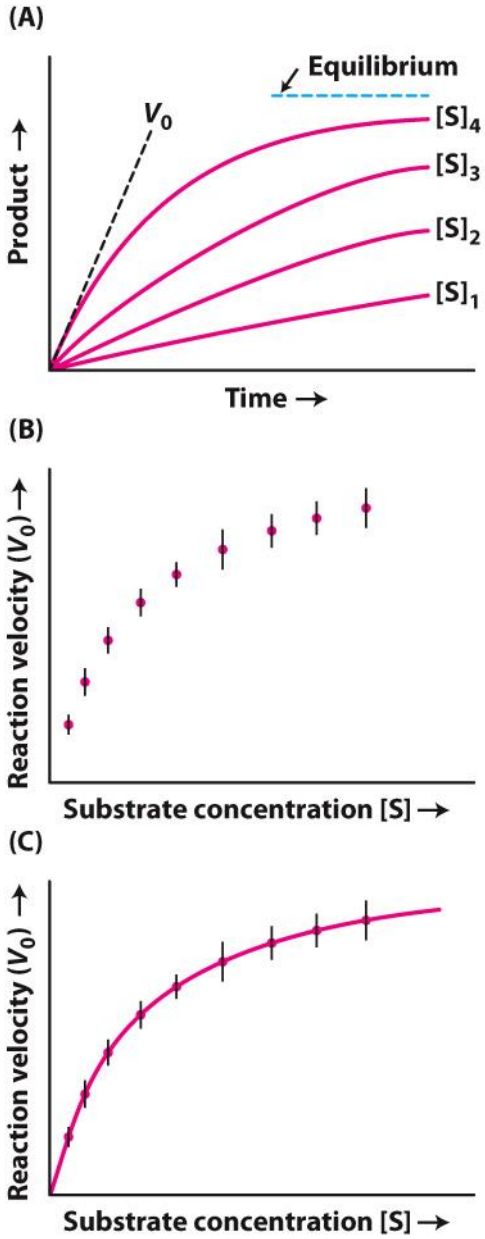
The curve approaches so called maximum velocity (v_{max}) and KM is the concentration of substrate at $v_{max}/2$



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In order to quantitatively understand enzymes kinetics, we need an equation that relates rate of catalysis to substrate concentration, enzyme concentration and the rates of the individual steps.

We start by recognizing that $V_0 = k_2[\mathrm{ES}]$

We will use the steady-state assumption that the rate of formation of ES is equal to the rate of breakdown of ES. Thus

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

This equation can be rearranged to $[\mathrm{E}][\mathrm{S}]/[\mathrm{ES}] = (k_{-1} + k_2)/k_1$

We can define a new constant, K_M, called the Michaelis constant.

$$K_\mathrm{M} = \frac{k_{-1} + k_2}{k_1}$$

It has unit of concentration and it is independent of the concentration of substrate of product

We can then express concentration of enzyme substrate complex

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

$$[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{M}}$$

The amount of free enzyme is equal to the total amount of enzyme present minus any enzyme bound to substrate.

$$[\mathrm{E}] = [\mathrm{E}]_\mathrm{T} - [\mathrm{ES}]$$

Substituting this equation for [E] in $[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{M}}$

yields $$[\mathrm{ES}] = \frac{([\mathrm{E}]_\mathrm{T} - [\mathrm{ES}])[\mathrm{S}]}{K_\mathrm{M}}$$

$$[\mathrm{ES}] = \frac{([\mathrm{E}]_\mathrm{T} - [\mathrm{ES}])[\mathrm{S}]}{K_\mathrm{M}}$$

This equation can be rearranged to yield $$[\mathrm{ES}] = [\mathrm{E}]_\mathrm{T}\frac{[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{M}}$$

Recall that $$V_0 = k_2[\mathrm{ES}]$$

Thus, $$V_0 = k_2[\mathrm{E}]_\mathrm{T}\frac{[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{M}}$$

The maximal rate of catalysis, V_{max}, occurs when all of the enzyme is bound to substrate

$[ES] = [E]_T$ $$V_\mathrm{max} = k_2[\mathrm{E}]_\mathrm{T}$$

Substituting the equation for V_{max} into $$V_0 = k_2[\mathrm{E}]_\mathrm{T}\frac{[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{M}}$$

yields the Michaelis-Menten equation

$$V_0 = V_\mathrm{max}\frac{[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{M}}$$

The Michaelis-Menten equation describes the initial reaction velocity as a function of substrate concentration.

$$V_0 = V_{\max} \frac{[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{M}}$$

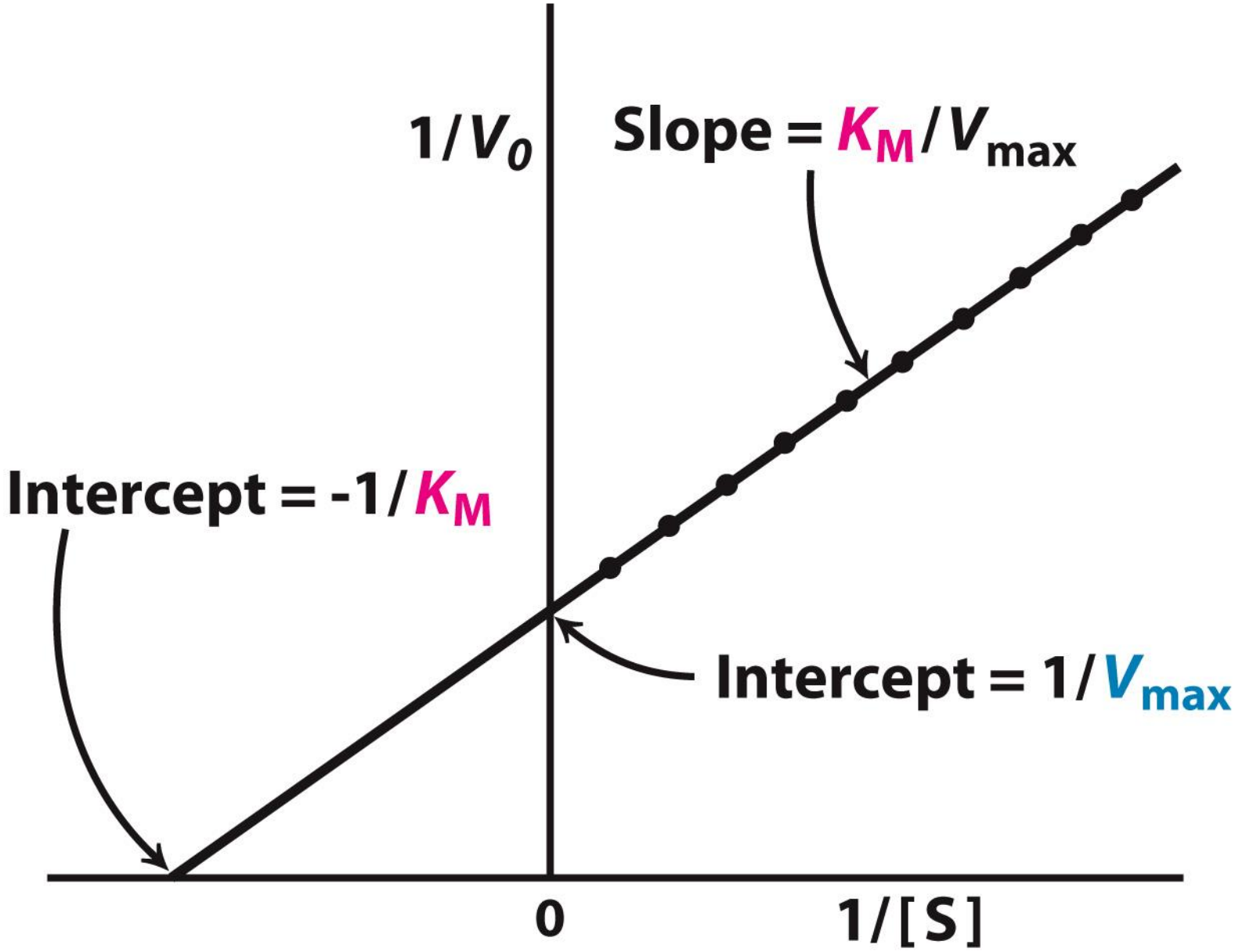
When v_o = ½ V_{max}, K_M =[S]. Thus, K_M is the substrate concentration that yields ½ V_{max}.

K_M and V_{max} values can be determined by several means

The Michaelis-Menten equation can be manipulated into one that yields a straight line plot.

$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}}$$

This double-reciprocal equation is called the Lineweaver-Burk equation.



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K_M and V_{max} values are important enzyme characteristics

K_M values for enzymes vary widely and evidence suggests that the K_M value is approximately the substrate concentration of the enzyme in vivo.

K_M approximates the dissociation constant of the ES complex only if $k_{-1} >> k_2$.

$$K_M \approx \frac{k_{-1}}{k_1} \qquad K_{ES} = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1}$$

TABLE 8.4 K_M values of some enzymes

Enzyme	Substrate	K_M (μM)
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

Table 8.4
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If the enzyme concentration, $[E]_T$, is known, then

$$V_{max} = k_2[E]_T$$

and

$$k_2 = V_{max}/[E]_T$$

K_2, also called K_{cat}, is the turnover number of the enzyme, which is the number of substrate molecules converted into product per second.

K_M and V_{max} also allow the determination of f_{ES}, the fraction of enzymes bound to substrate.

$$f_{ES} = \frac{V}{V_{max}} = \frac{[S]}{[S] + K_M}$$

TABLE 8.5 Turnover numbers of some enzymes

Enzyme	Turnover number (per second)
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Table 8.5
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Most biochemical reactions include multiple substrates

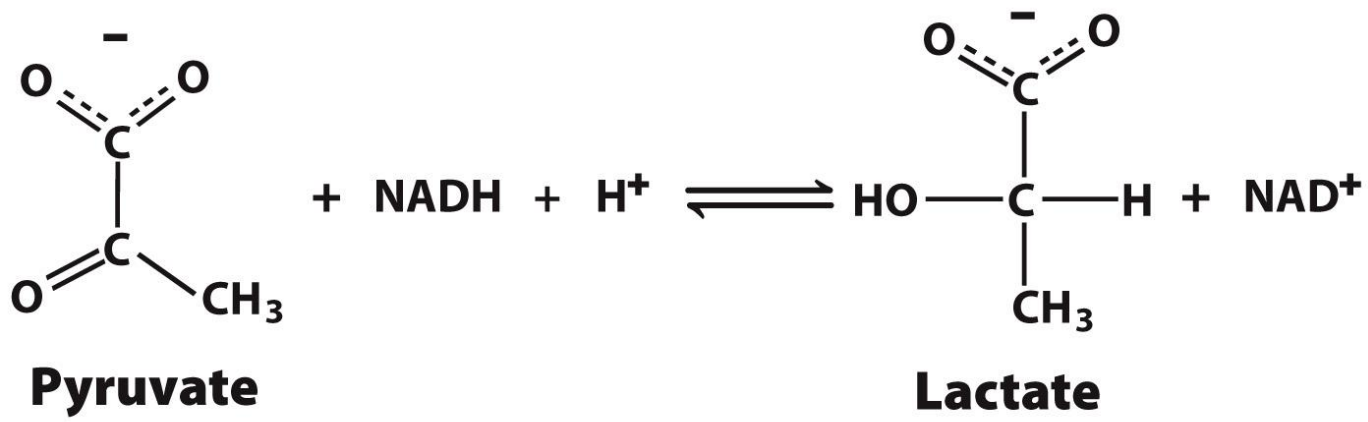
$$A + B \overset{E}{\rightleftharpoons} P + Q$$

There are two classes of multiple substrate reactions: sequential and double-displacement reactions.

Sequential reactions, which may be random or ordered, are characterized by the formation of a ternary complex consisting of the enzyme and both substrates.

Double-displacement reactions are characterized by the formation of a substituted enzyme intermediate.

A sequential ordered reaction.



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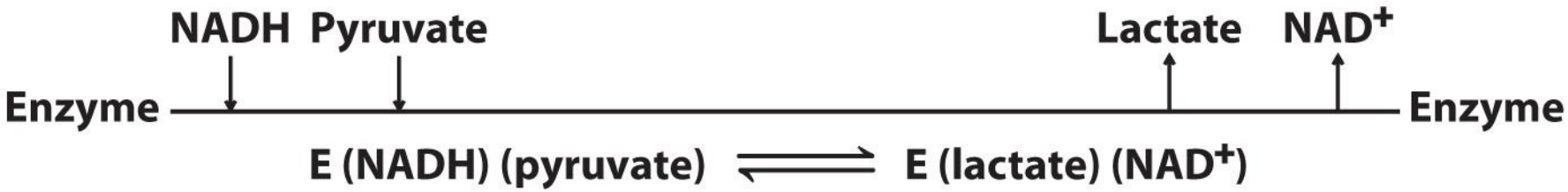

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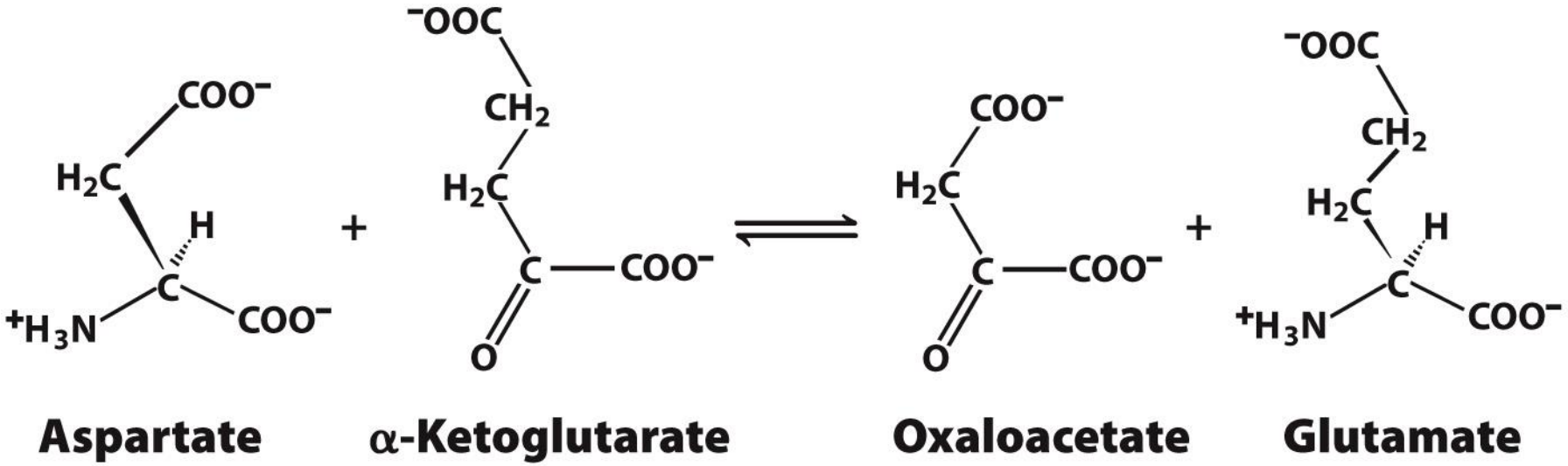
Double-displacement reaction (ping-pong) reaction

Existence of substituted enzyme intermediate
Aspartate amino transferase transfers amino group



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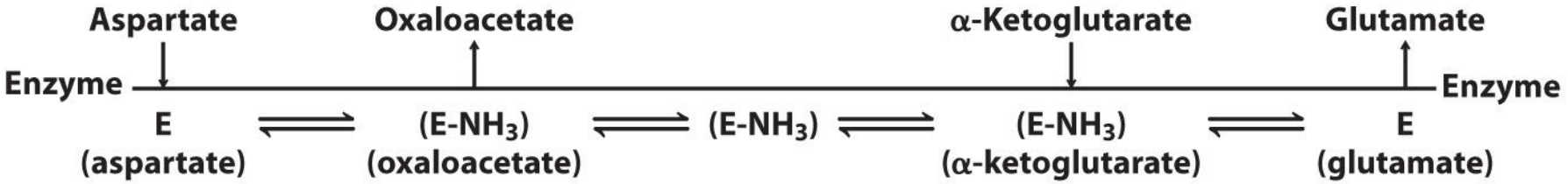

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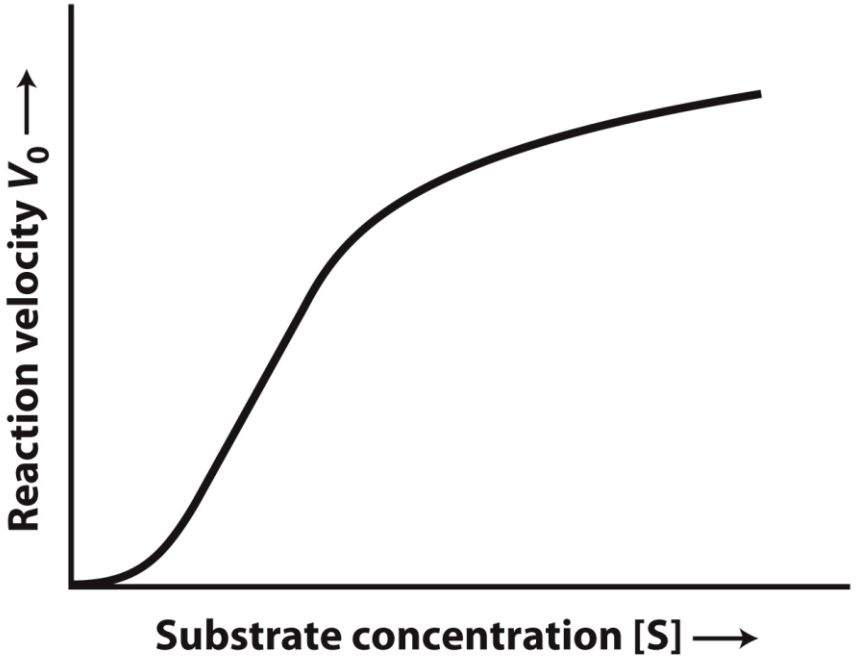
Allosteric enzymes

Allosteric enzymes do not obey Michaelis–Menten kinetics

Allosteric enzymes display cooperative substrate binding which is evident as a sigmoidal reaction velocity curve.

Allosteric enzymes are also regulated.



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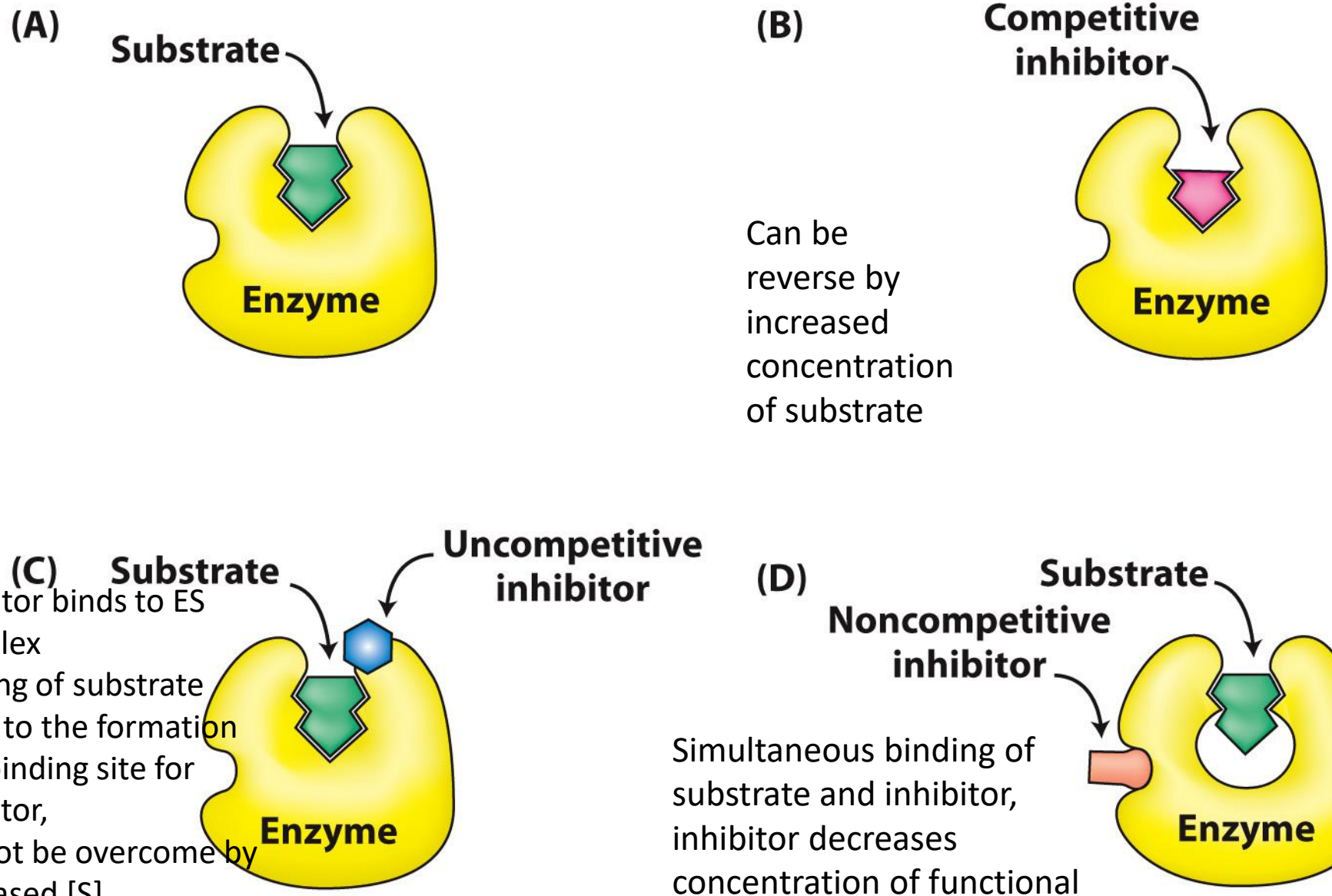
Enzymes inhibition

Irreversible enzyme inhibitors bind covalently or noncovalently to the enzyme, but with a negligible dissociation constant.

Reversible inhibition is characterized by a rapid dissociation of the enzyme-inhibitor complex.

There are three common types of reversible inhibition:

1. Competitive inhibition: The inhibitor is structurally similar to the substrate and can bind to the active site, preventing the actual substrate from binding.
2. Uncompetitive inhibition: The inhibitor binds only to the enzyme-substrate complex in what is essentially substrate-dependent inhibition.
3. Noncompetitive inhibition: The inhibitor binds either the enzyme or enzyme-substrate complex.



Can be reverse by increased concentration of substrate

Inhibitor binds to ES complex
Binding of substrate leads to the formation of a binding site for inhibitor,
Cannot be overcome by increased [S]

Simultaneous binding of substrate and inhibitor, inhibitor decreases concentration of functional enzyme, decrease in the turnover number

Figure 8.14
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Competitive inhibition

In competitive inhibition, the inhibitor competes with the substrate for the active site. The dissociation constant of the inhibitor is given by

$$K_i = [E][I]/[EI]$$

In competitive inhibition, V_{max} of the enzyme is unchanged because the inhibition can be overcome by a sufficiently high concentration of substrate.

However, K_M in the presence of inhibitor, called K_M^{app}, is increased.

$$K_M^{app} = K_M(1 + [I]/K_i)$$

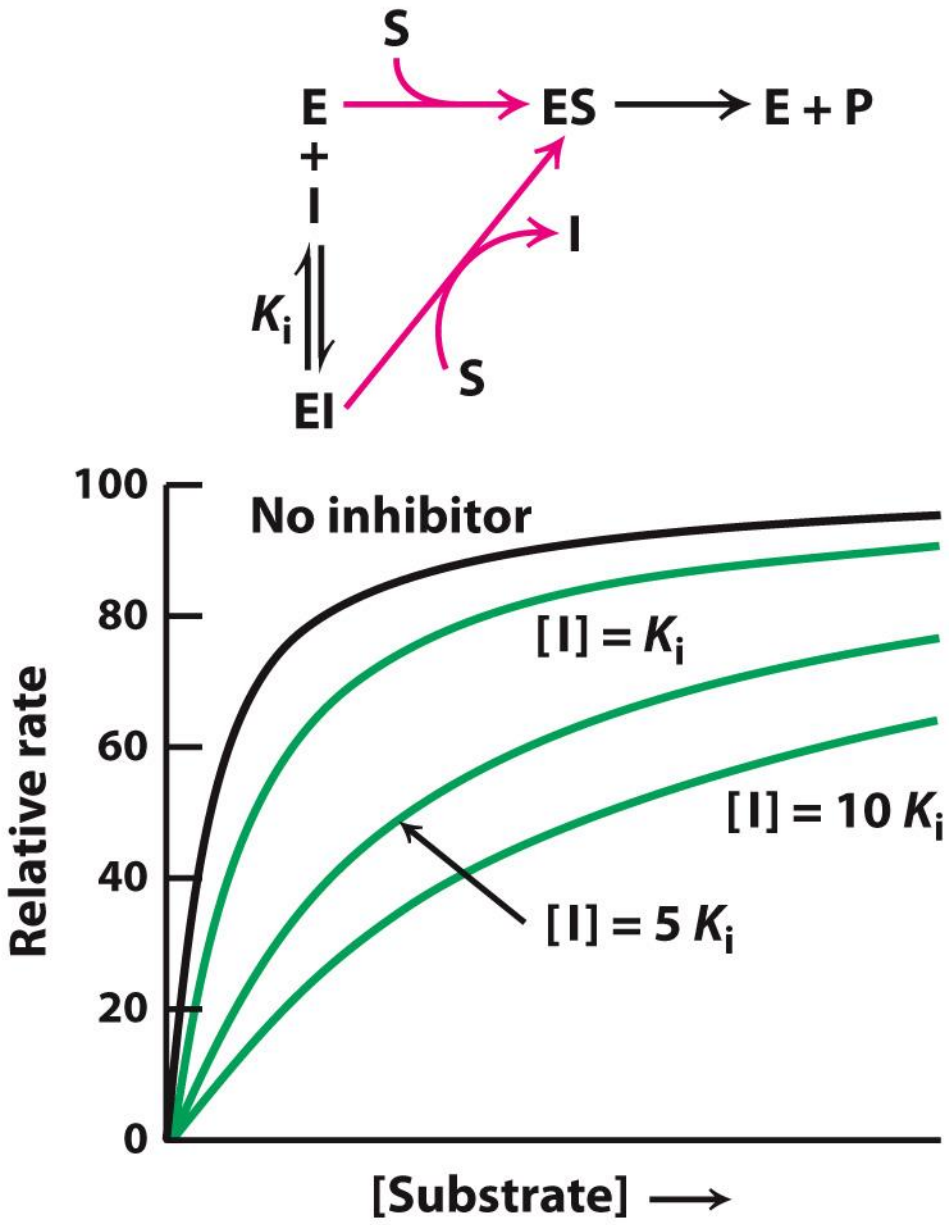

Figure 8.16
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Uncompetitive inhibition

In uncompetitive inhibition, the enzyme-inhibitor-substrate complex does not form product. The apparent $V_{max,}$ called V_{max}^{app} , is lower in the presence of inhibitor.

The K_M is also lower since lower concentration of substrate is necessary to form half of the maximum concentration of ES.

Uncompetitive inhibition cannot be overcome by the addition of excess substrate.

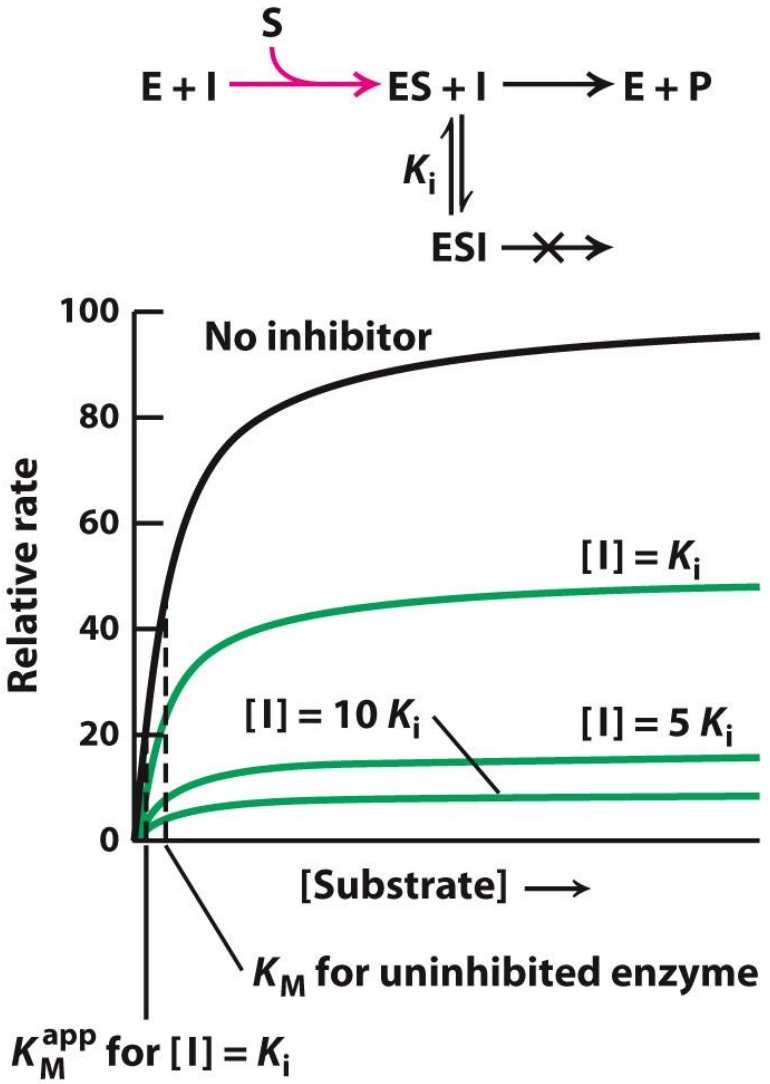

Figure 8.17
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Noncompetitive inhibitor

- the inhibitor binds both to free enzyme and to an enzyme–substrate complex. Consequently, as with uncompetitive competition, V_{max} cannot be attained. In pure noncompetitive inhibition, K_M remains unchanged.

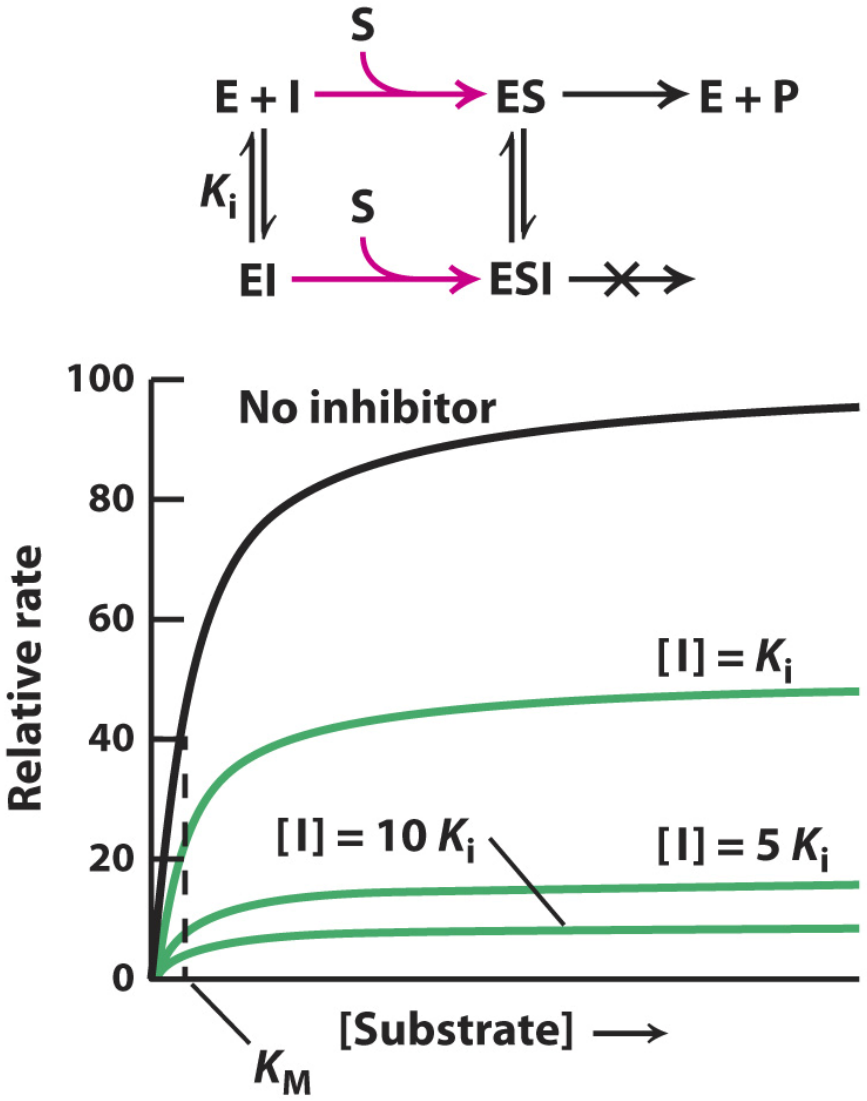

Figure 8.18
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Competitive inhibition

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\left(\frac{1}{[S]}\right)$$

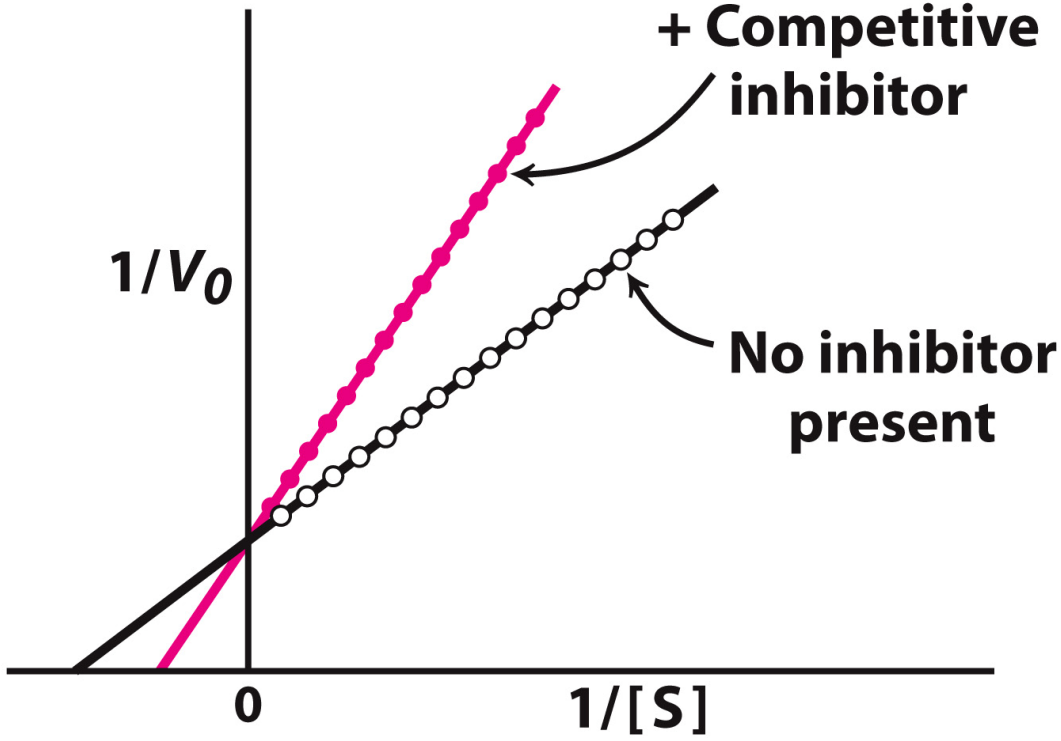

Figure 8.19
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Uncompetitive inhibitor

$$\frac{1}{V_0} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$

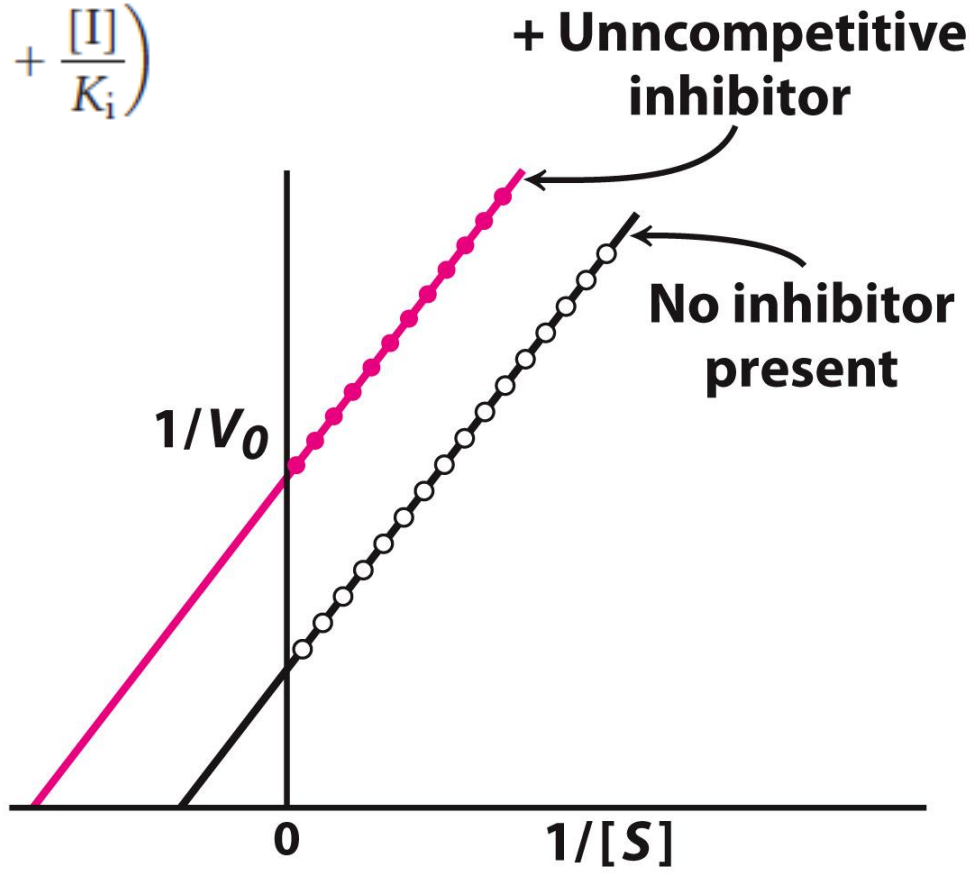

Figure 8.20
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Noncompetive inhibitor

$$V_{max}^{app} = \frac{V_{max}}{1 + \frac{I}{K_I}}$$

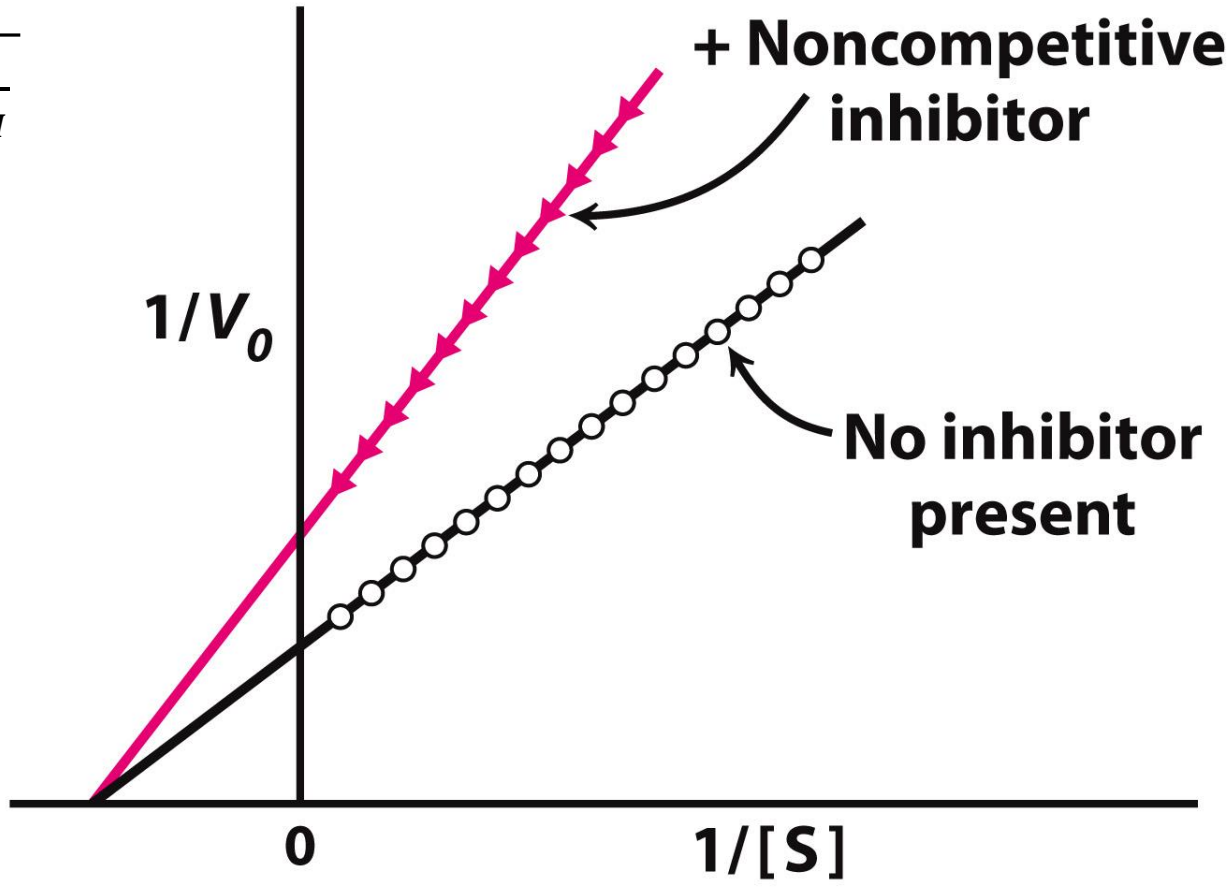

Figure 8.21
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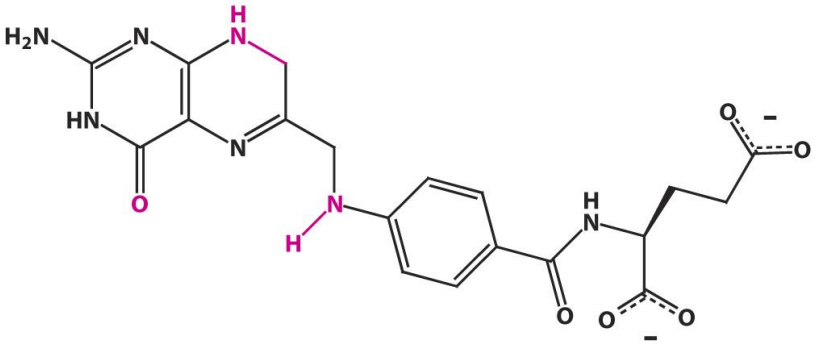
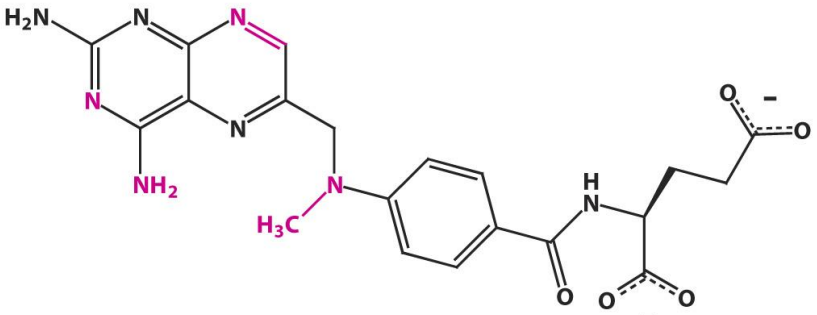
Examples of inhibitors

- Dihydrofolate reductase is involved in purine and pyrimidine synthesis



Dihydrofolate



Methotrexate

Figure 8.15
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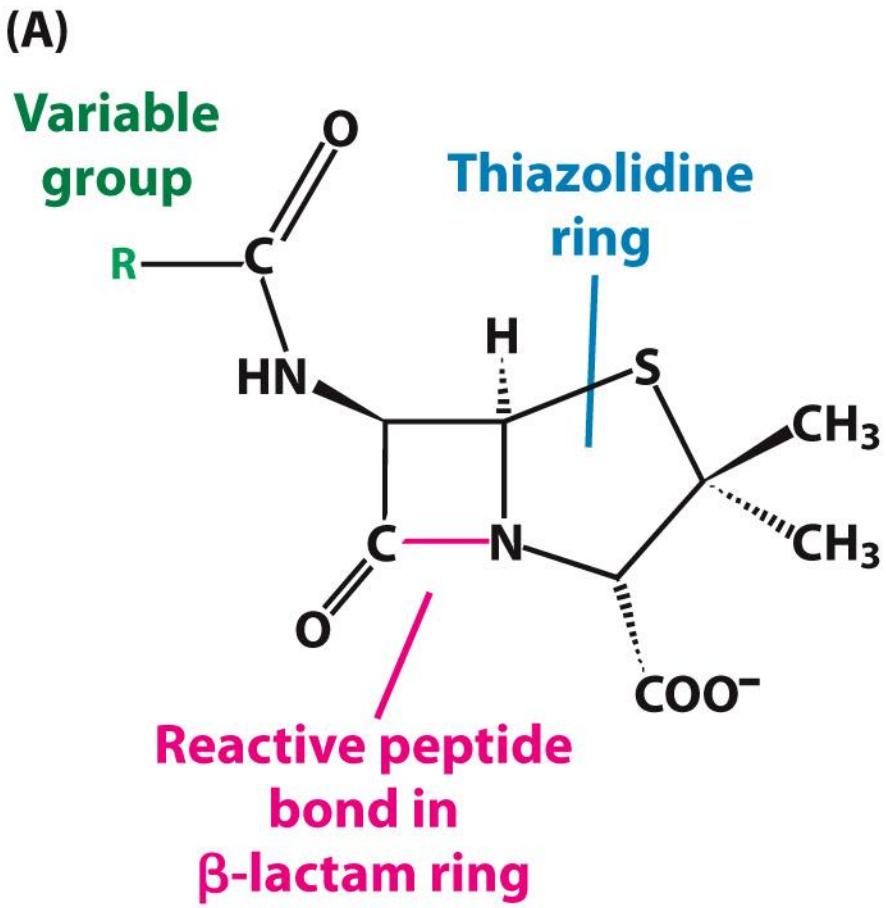
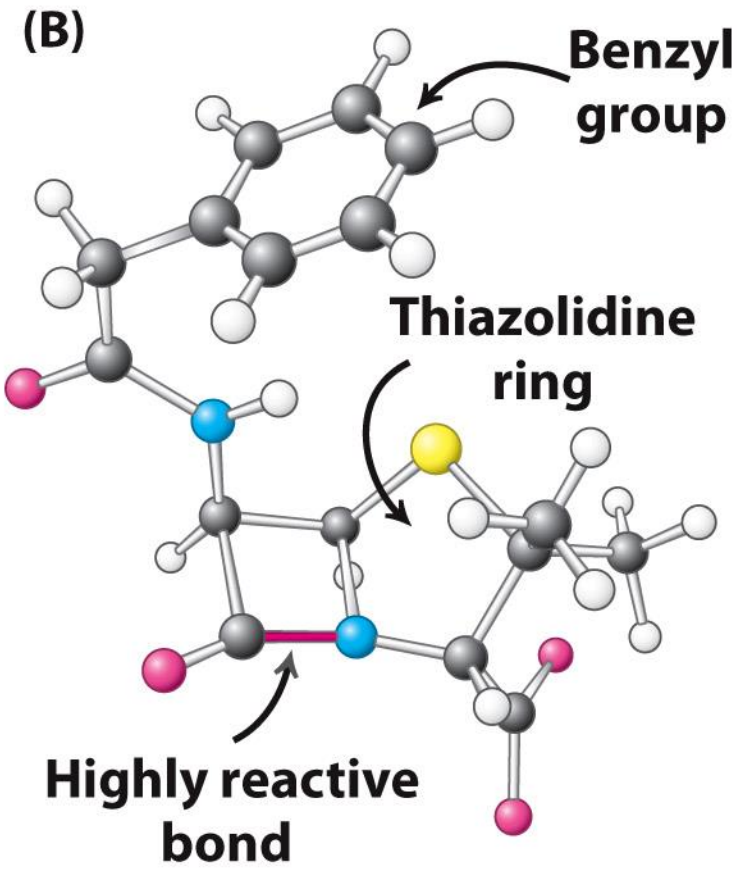
Competitive inhibitor
Use to treat cancer

- penicillin





Benzyl penicilin

Figure 8.26
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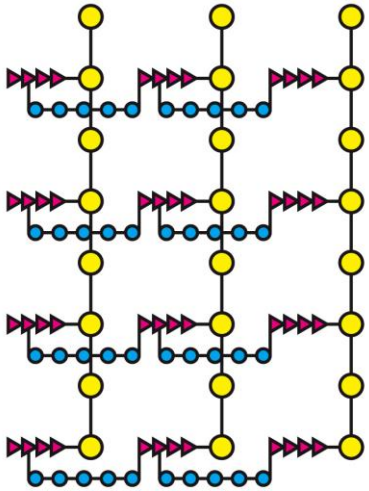
Bacterial wall is formed by peptidoglycan polysaccharide linked by short peptides



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Sugar in yellow
Pentaglycine bridge in blue
Tetrapeptides in red

Formation of the crosslink between pentaglycine and tetrapeptide is catalyzed by **glycopeptide transferase**

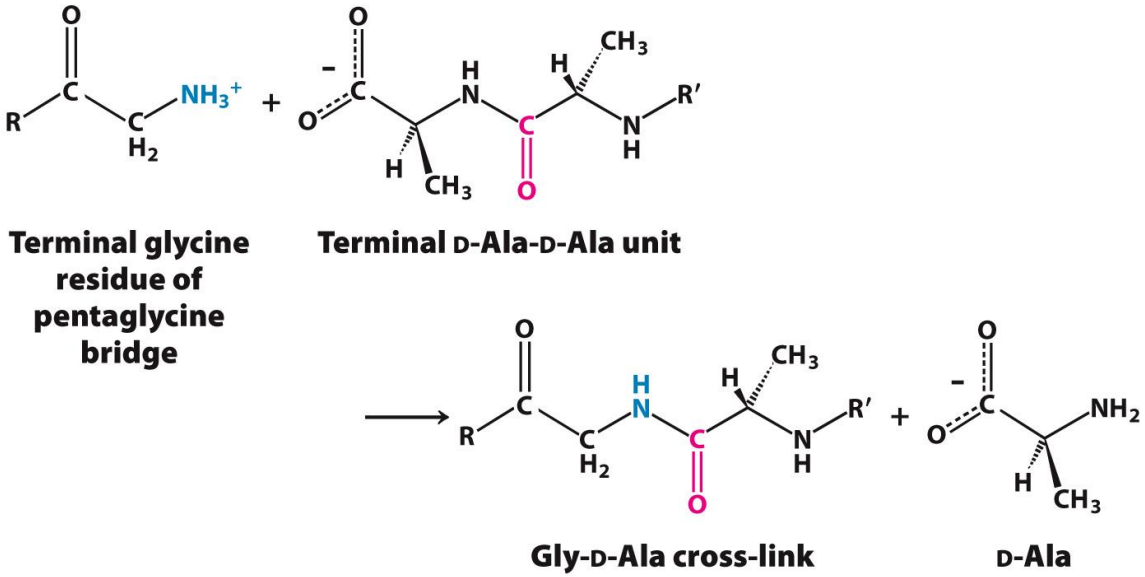

Figure 8.28
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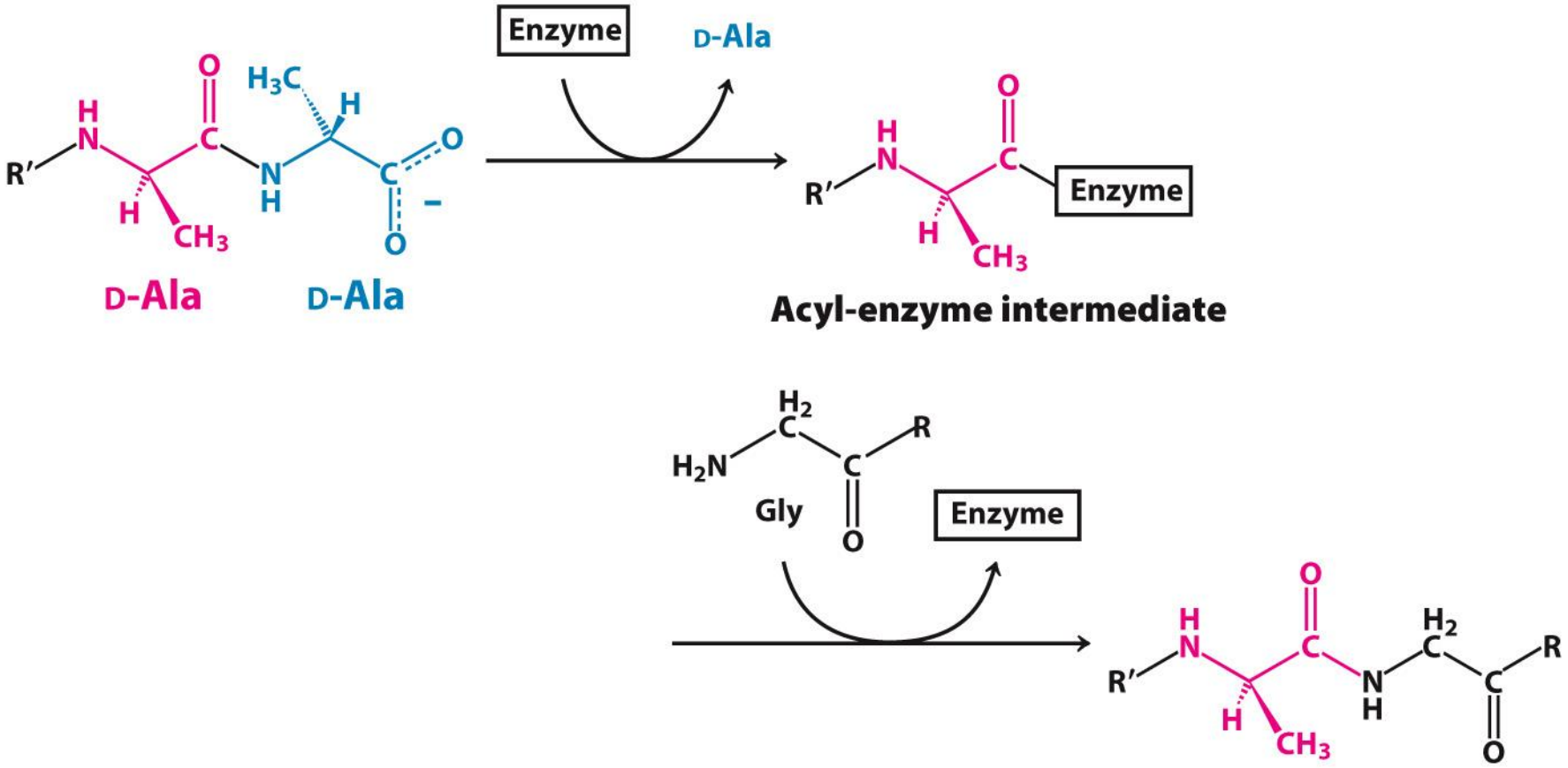

Figure 8.29
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- Penicillin mimics D-Ala D-Ala substrate
- Forms a covalent bond with Ser residue in the transpeptidase enzyme
- Blocks the active site, example of irreversible inhibition