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Enzymes 2: Inhibition and Control

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Objectives

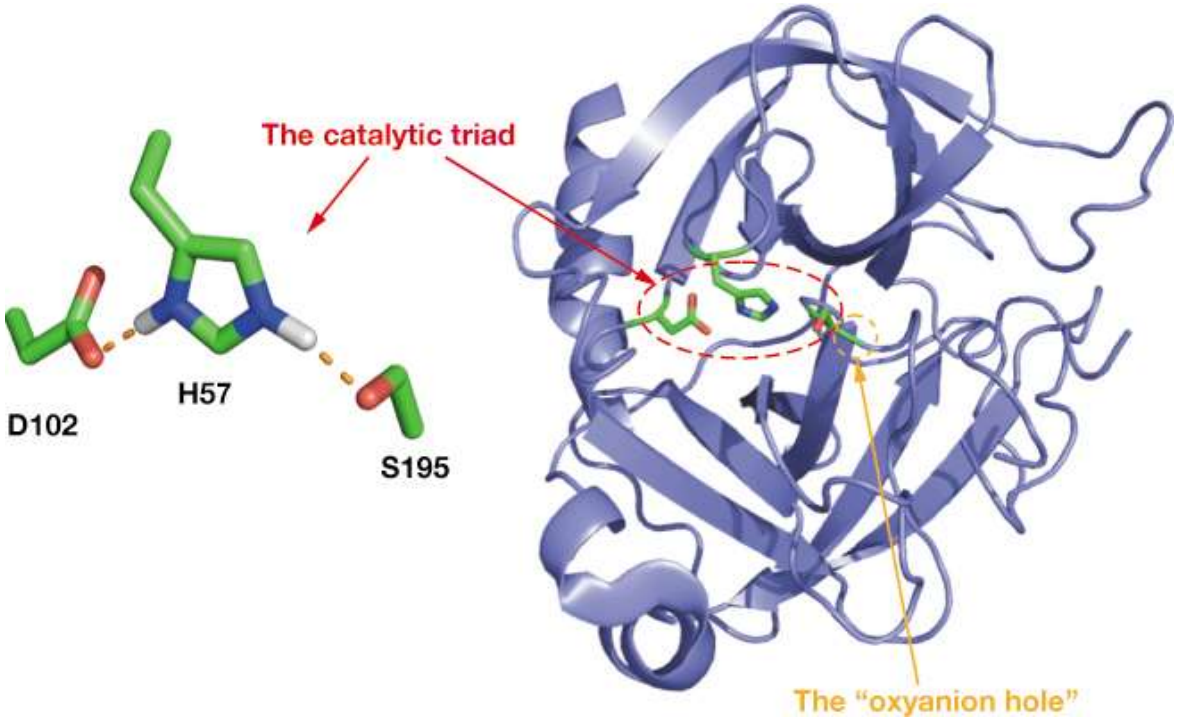
- The active site
- Enzyme inhibition
  - At the active site
  - At another site
- Regulation of enzyme activity
  - Allosteric effectors
  - Chemical modifications

Textbook Chapter 8

The active site

- The **active site** of a protein is where one or more **substrate** molecules bind, for the protein to carry out its function (defined in Chap. 7)
- Comprises a few residues critical for the protein or enzyme's function, usually located far apart in the amino acid sequence, but close in 3D space (e.g. serine protease catalytic triad).
- Mutating these residues either destroys or modifies enzyme function.
- Active site residues are localized to a specific substrate binding pocket by the folding of the enzyme 3D structure.
- Complements the transition state.

Serine proteases: digestive enzymes trypsin, chymotrypsin and elastase



- The three catalytic residues are absolutely essential for function
- H57, D102 and S195: far apart in the sequence
- But spatially localized and held in a specific geometry by the entire 3D structure.
- Histidine (H57) acts as a base under physiological conditions: i.e. neutral pH
- The OH of serine (S195) participates in the reaction
- Aspartate (D102) holds the histidine in place as well as hydrogen bonds with the peptide carbonyl of the substrate, after the recognition residue.

FIGURE 8.16 The structure of chymotrypsin and the serine protease catalytic triad.

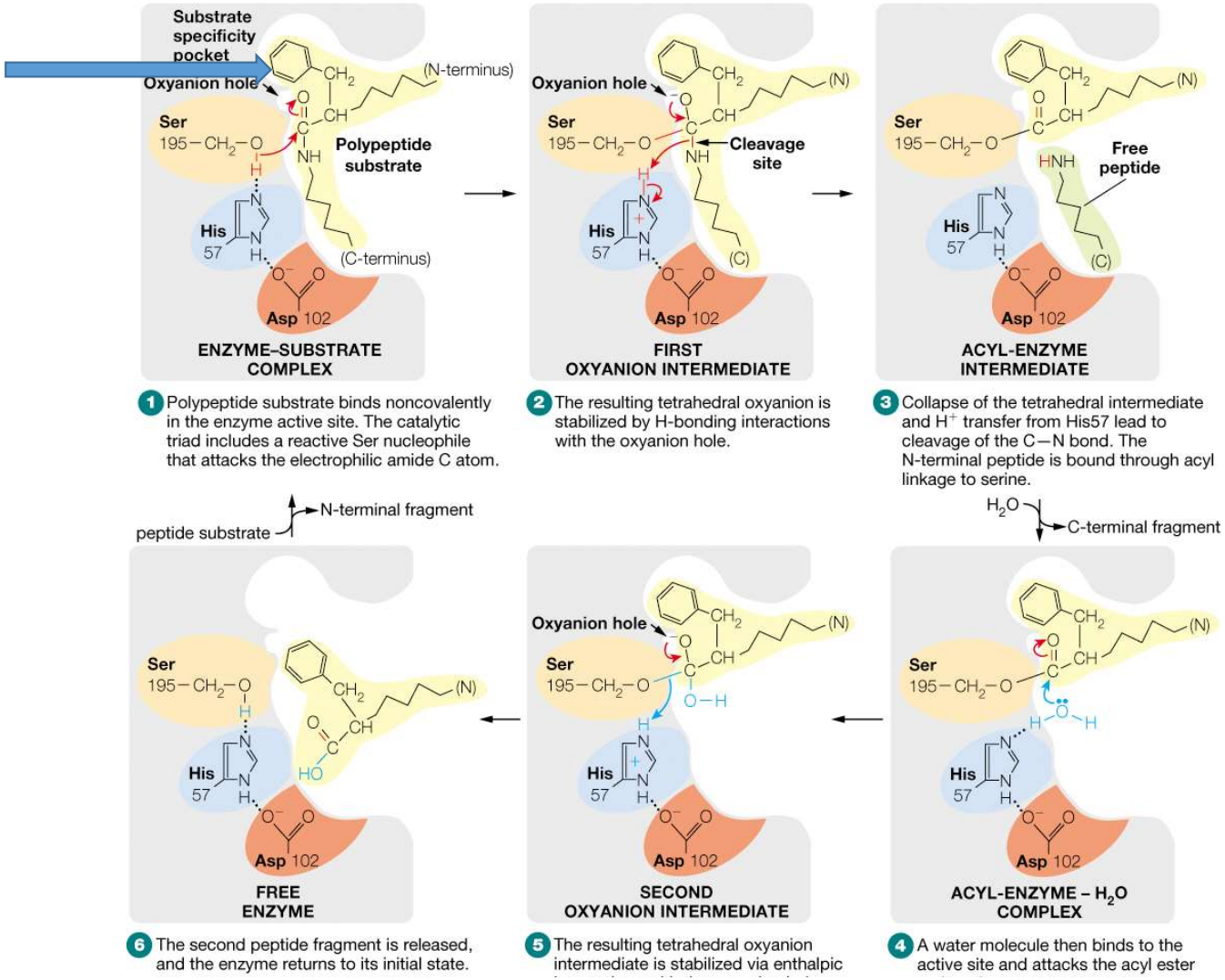


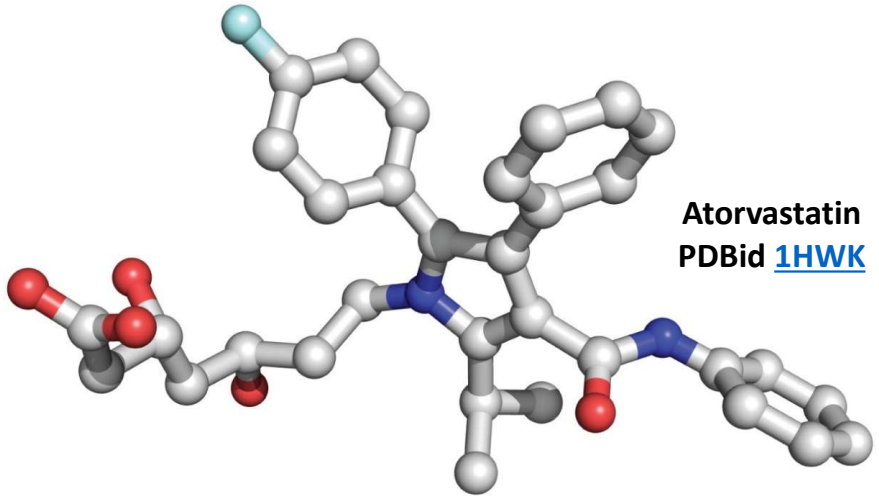
Figure 8.15 Catalysis of peptide bond hydrolysis by chymotrypsin

The enzyme active site - Summary

- The active site of an enzyme is a completely different environment to the surrounding solution
  - Can be more acidic or basic than the solution
  - Usually excludes solvent molecules and provides a hydrophobic environment
  - Restricts the orientation in which the substrate binds: i.e. stereospecificity
  - Possibility for electrophilic or nucleophilic functional groups
    - Reaction with the enzyme is possible but the enzyme is regenerated.
  - Critical for inhibitor design in biotech and drug design.

Enzyme inhibition: Atorvastatin (Lipitor®)

- One of the most commonly prescribed drugs for lowering cholesterol
- Binds to and **inhibits** the activity of the **enzyme** HMG-CoA reductase



Atorvastatin
PDBid 1HWK

Drugs, Toxins, and Enzymatic Activity

- Many prescription drugs are enzyme inhibitors
  - The development of some drugs has been advanced by kinetic studies, as well as structural studies of the target enzymes
- Also, the effects of some natural and synthetic toxins are the result of enzyme inhibition or inactivation
- There are two classes of enzyme inhibitors:
  - reversible inhibitors (noncovalently bound)
  - irreversible inhibitors (covalently bound)

Enzyme inhibition

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

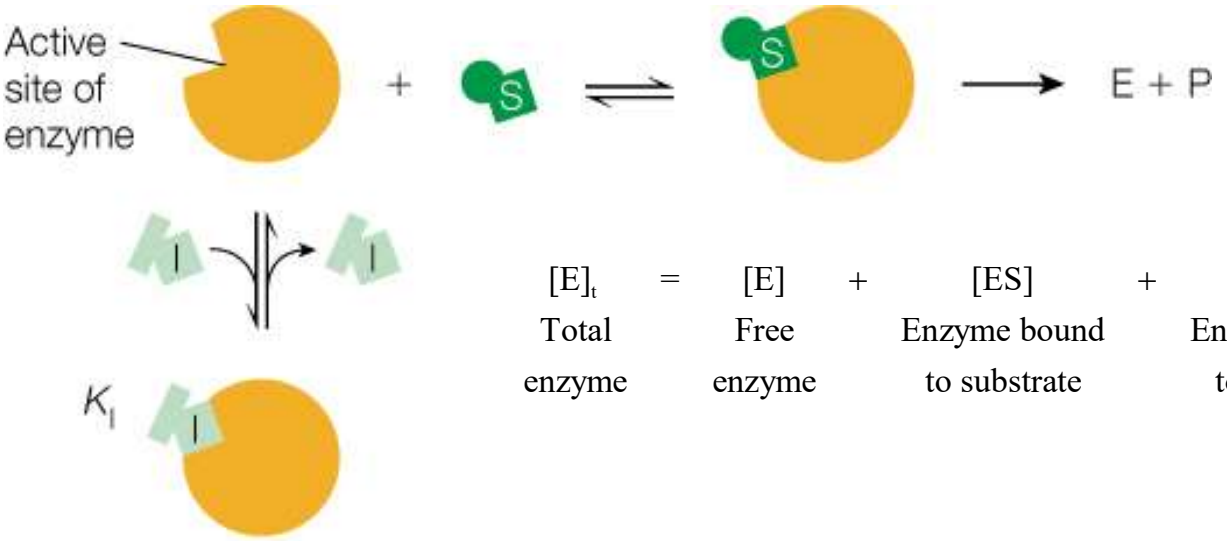
- An enzyme (E) binds the substrate (S) reversibly forming the ES complex, which then forms product (P), with recovery of the enzyme.
- An inhibitor (I) disrupts the above reaction by a number of mechanisms, involving the inhibitor binding in some way (reversibly or irreversibly) to the enzyme (E)
  - The inhibitor can bind strongly to the **active site** and prevent the substrate (S) from binding, **or**
  - It can bind to **some other site** on the enzyme and affect the reaction indirectly.

$$E + I \rightleftharpoons EI \longrightarrow \text{no products}$$

Reversible inhibition

- While in a reversible inhibition the inhibitor interacts with an enzyme noncovalently, in an irreversible reaction the inhibitor is bound covalently
- There are at least three different mode of reversible inhibition:
  1) competitive inhibition
  2) uncompetitive inhibition
  3) mixed or noncompetitive inhibition

Competitive inhibition



$$[E]_t = [E] + [ES] + [EI]$$

$[E]_t$		[E]		[ES]		[EI]
Total enzyme	=	Free enzyme	+	Enzyme bound to substrate	+	Enzyme bound to inhibitor

- Both substrate (S) and inhibitor (I) can fit the enzyme's active site.
- The substrate can be processed, but not the inhibitor

Competitive Inhibition

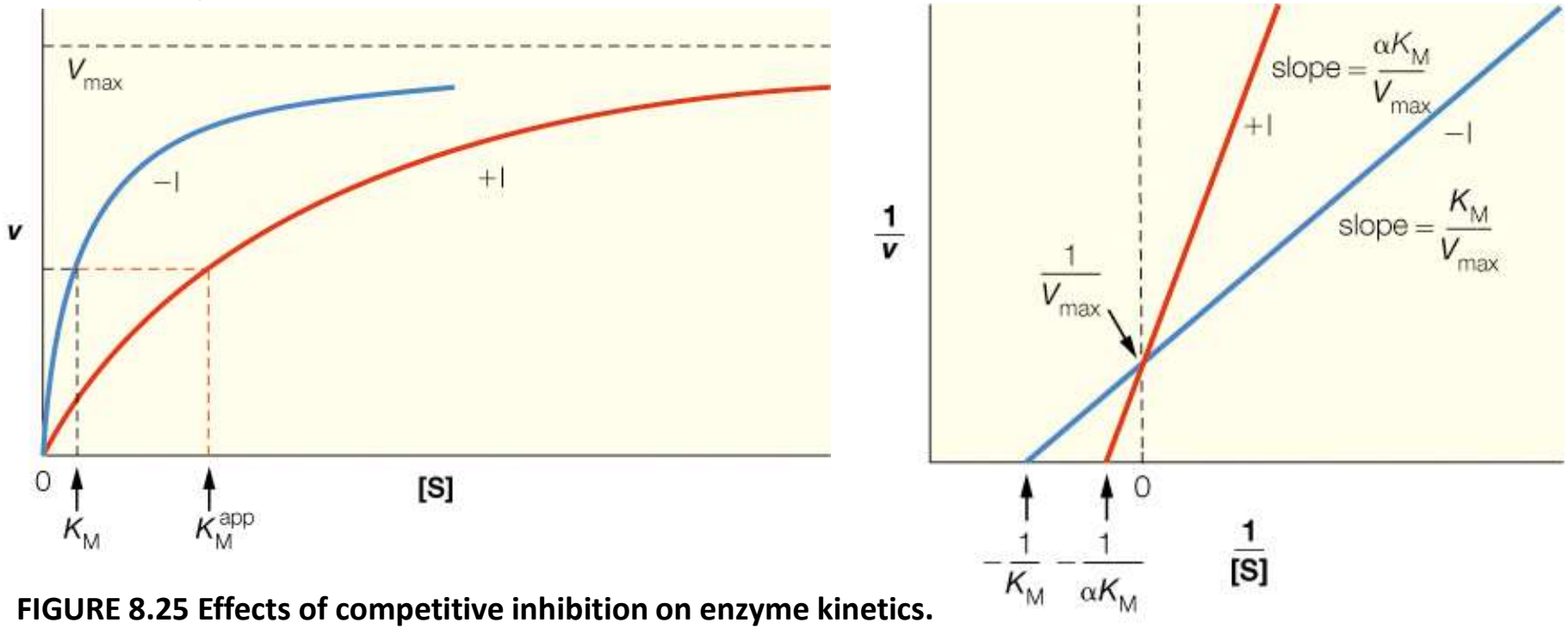


FIGURE 8.25 Effects of competitive inhibition on enzyme kinetics.

- **Inhibitor** binds to the **active site** and reduces amount of free enzyme available for catalysis by ***competing with substrate***
  - $\underline{K_m}$ changes but $\boldsymbol{V_{max}}$ **remains constant**

The apparent K_M is increased, while the V_{max} is unchanged

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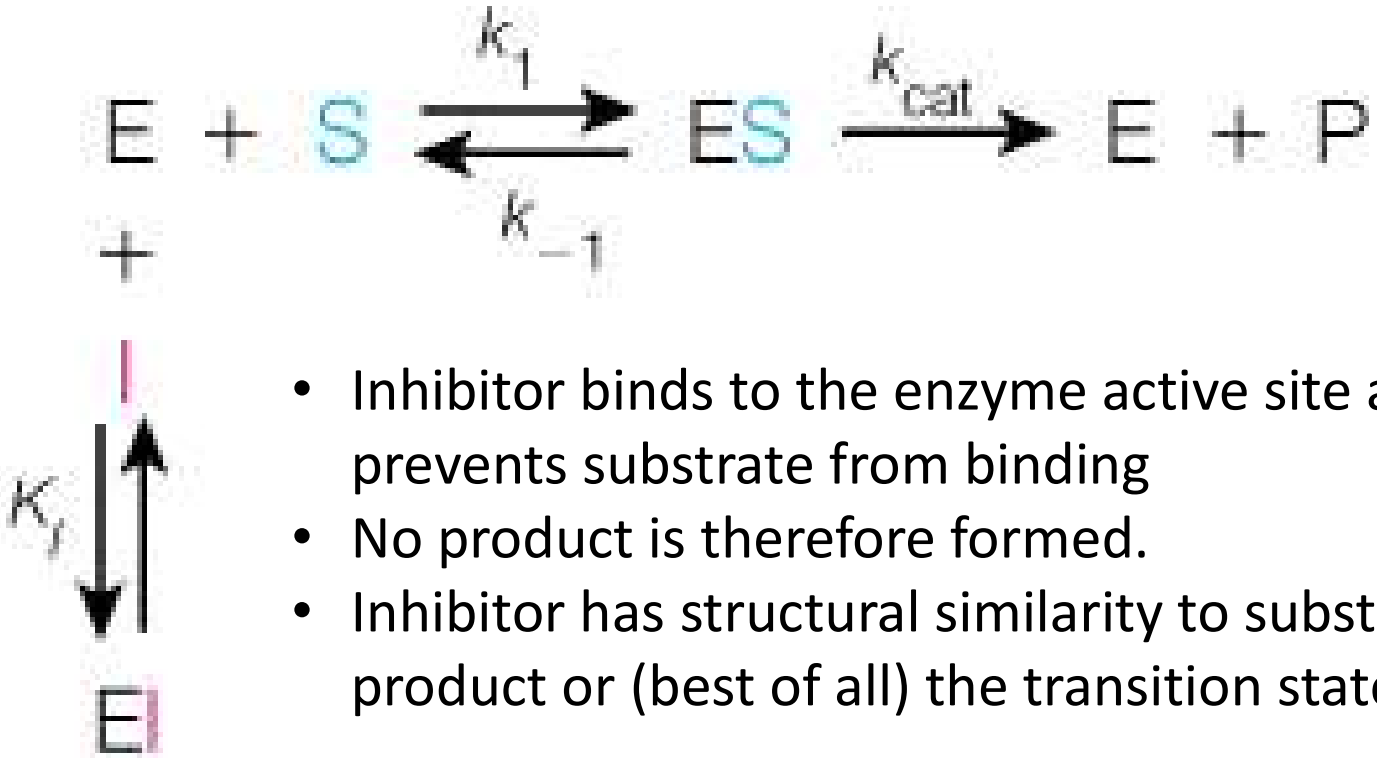
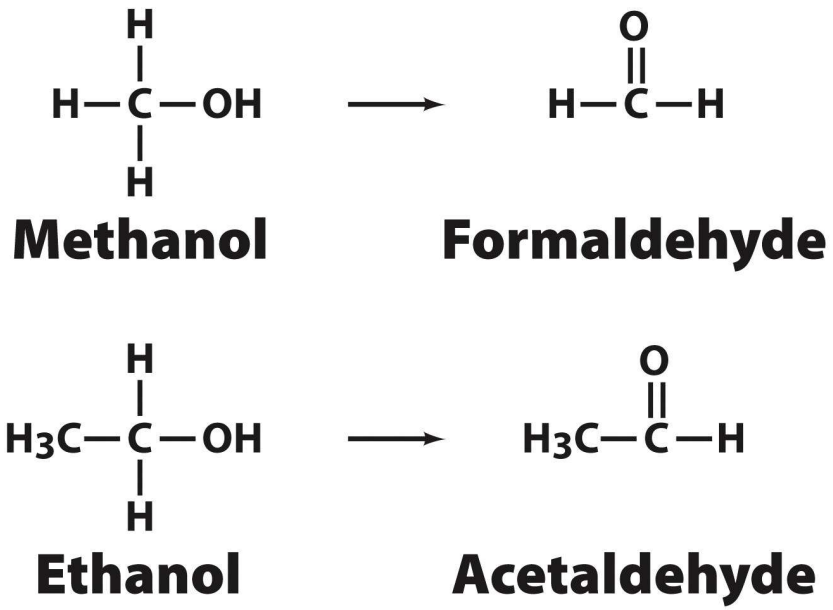
Competitive Enzyme Inhibition



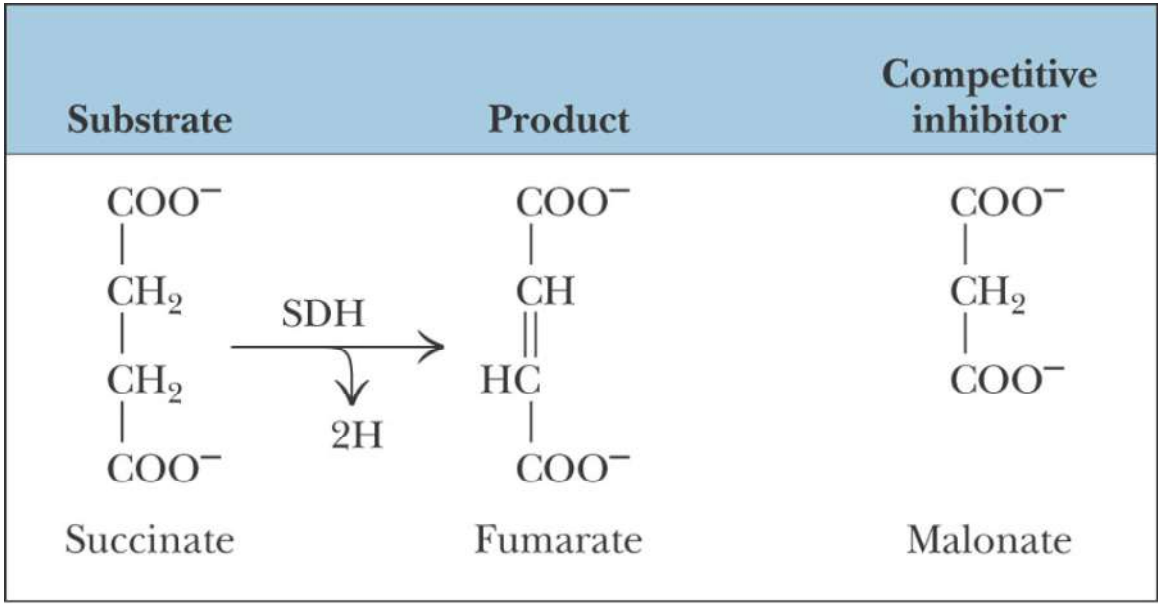
- Inhibitor binds to the enzyme active site and prevents substrate from binding
- No product is therefore formed.
- Inhibitor has structural similarity to substrate, product or (best of all) the transition state.

Competitive Inhibition: Ethanol Treatment of Methanol Poisoning



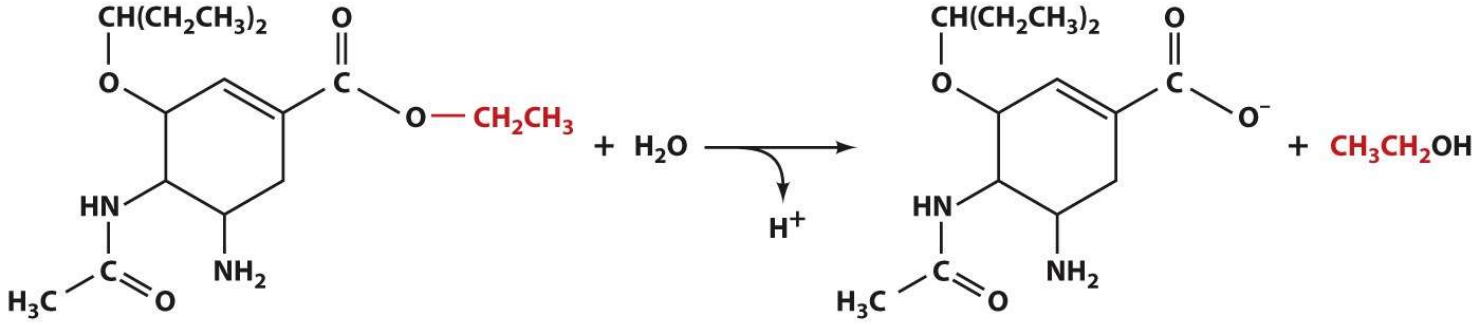
- Alcohol dehydrogenase (ADH) is the enzyme.
- Ethanol competes for the active site with methanol.
- Methanol is harmlessly excreted *via* urine, instead of getting converted to toxic formaldehyde.

Malonate competitively inhibits succinate dehydrogenase (citric acid cycle)



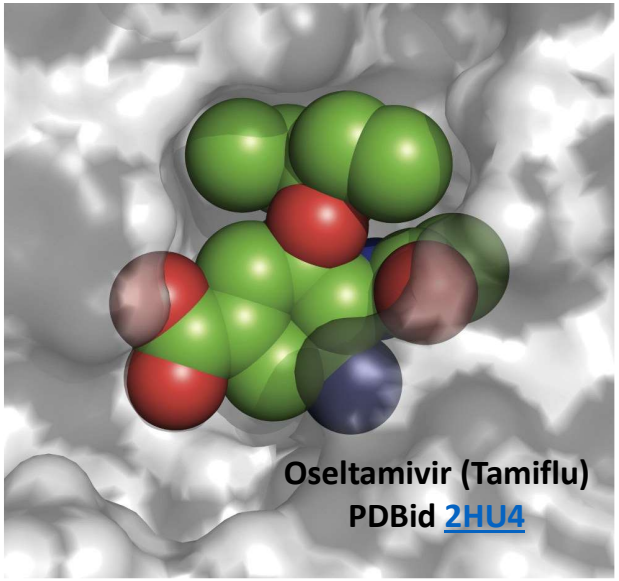
Note: structural similarity between S and I

Drugs are designed to fit the target enzyme's active site by competitive inhibition



Oseltamivir (Tamiflu) **Oseltamivir carboxylate**

- Oseltamivir (Tamiflu®)-Avian Flu Neuraminidase Complex
- Neuraminidase catalyses the hydrolysis of neuraminic acid (sialic acid) to help viral particles escape from the host cell surface



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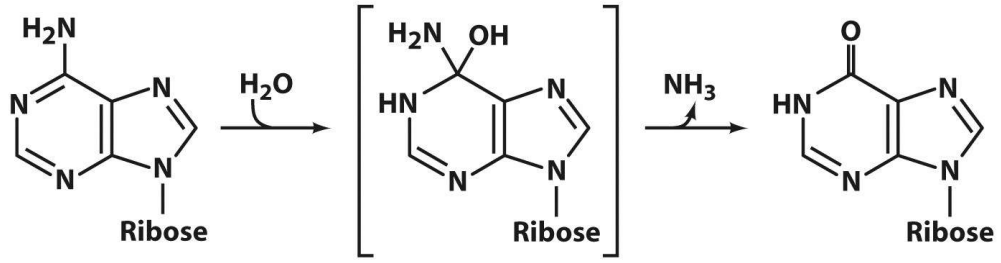
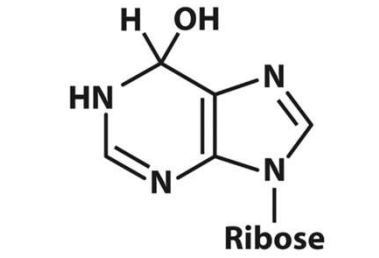
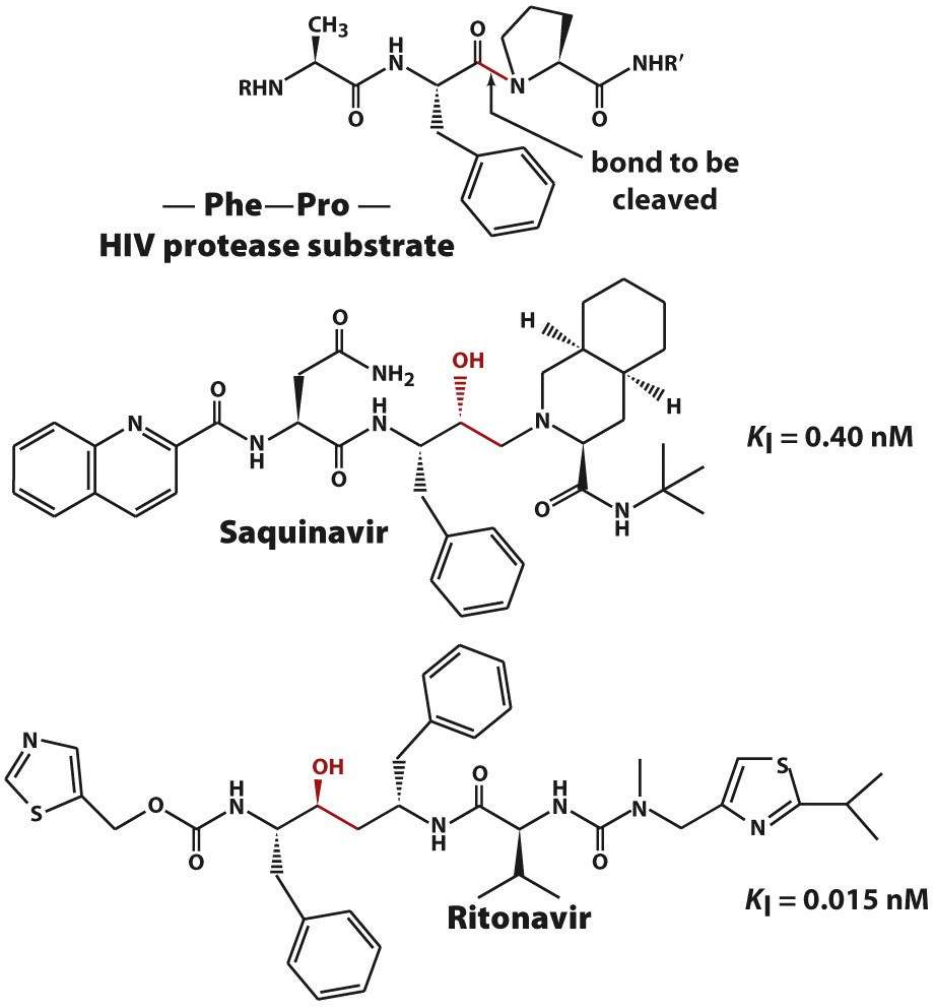
Adenosine Deaminase: Transition State Analog Inhibitor for Leukemia Treatment





HIV Enzyme Inhibitors



Box 12-3 part 3

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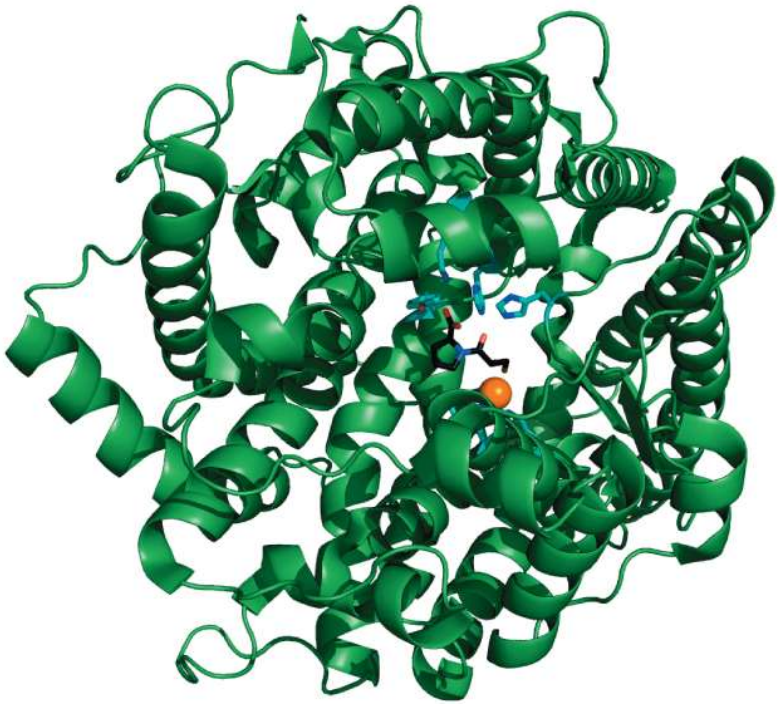
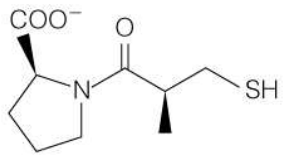
ACE inhibitor





Captopril

K_I = 1.7 nM for captopril
K_M = 52 μM for angiotensin I

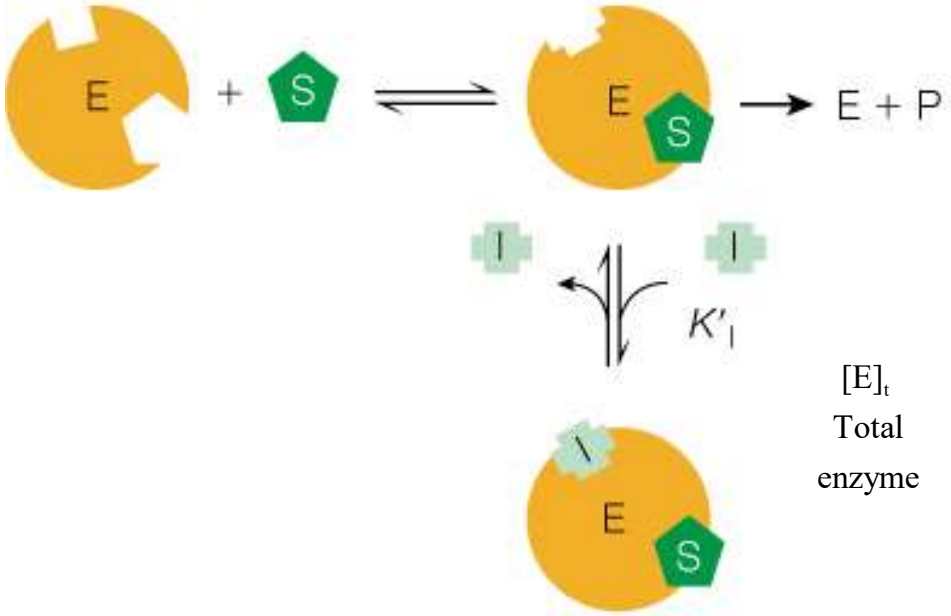
(a) A cartoon rendering of human ACE is shown in green with captopril (black) in sticks. The active site Zn^{2+} ion is shown as an orange sphere, and the side chains that bind captopril are shown as cyan sticks.

- Angiotensin I is a peptide hormone that causes vasoconstriction and an increase in blood pressure.

  Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
- It is cut after Pro by the angiotensin converting enzyme, ACE to form angiotensin II.
- ACE inhibitors prevent congestive heart failure.

Figure 8.26a, Captopril is a competitive inhibitor of angiotensin-converting enzyme.

Uncompetitive inhibition

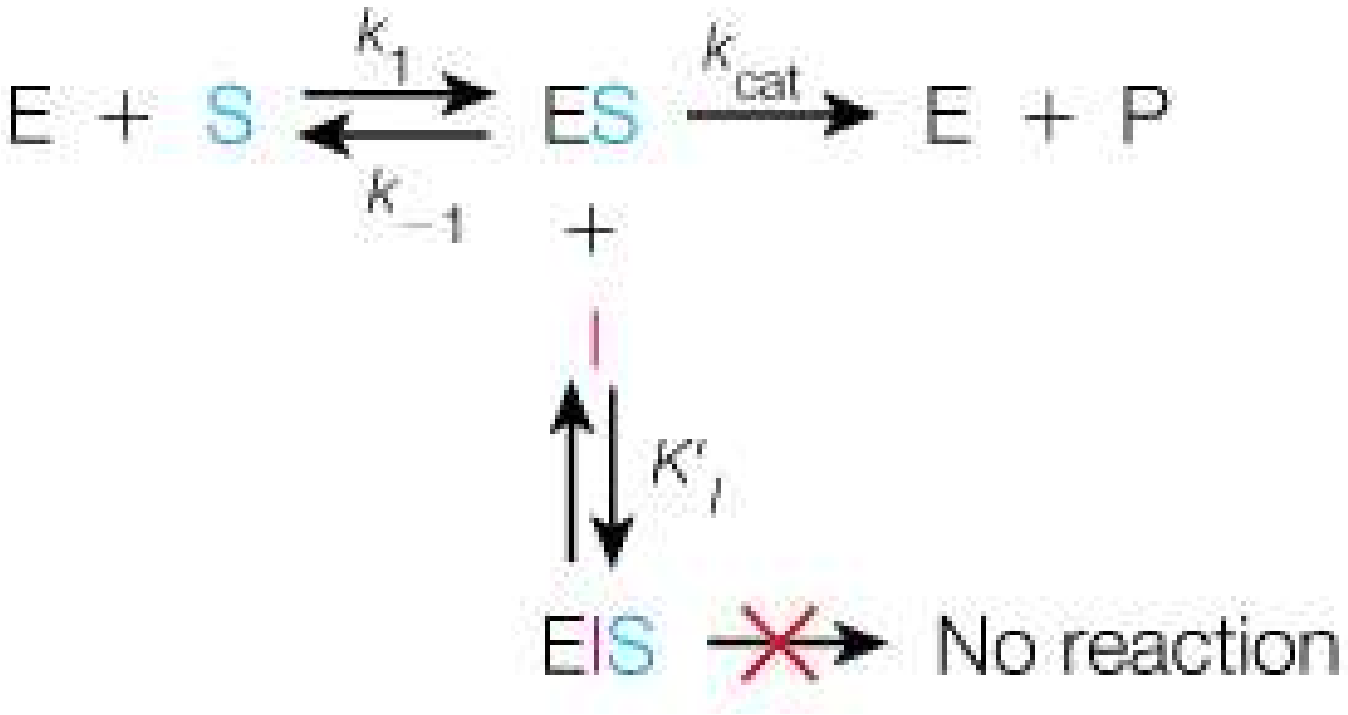


$$[E]_t = [E] + [ES] + [EI]$$

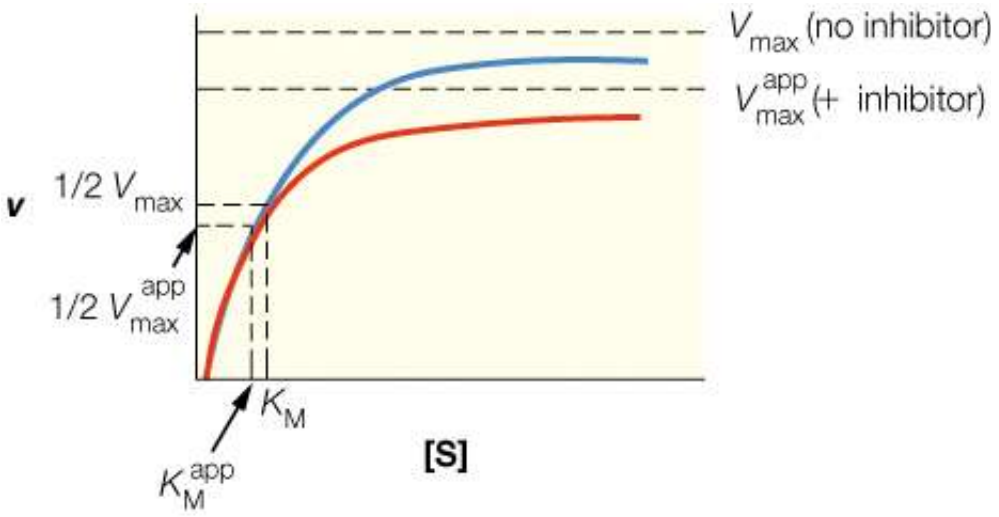
$[E]_t$	=	[E]	+	[ES]	+	[EI]
Total enzyme		Free enzyme		Enzyme bound to substrate		Enzyme bound to inhibitor

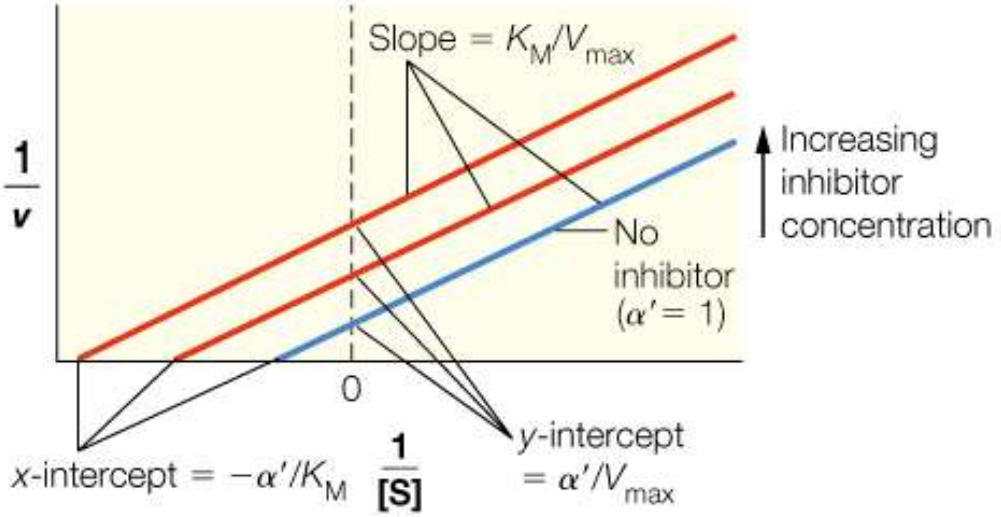
- The inhibitor binds at a site on the enzyme different from that of the substrate, thereby diminishing the enzyme's catalytic activity
- This is a **regulatory site** where a bound **effector** acts by an **allosteric** mechanism.
- Inhibitor **I** binds to enzyme <u>after ES has formed</u>
  - **I prevents product formation**
  - <u>Dead-end complex</u>

Uncompetitive Enzyme Inhibition



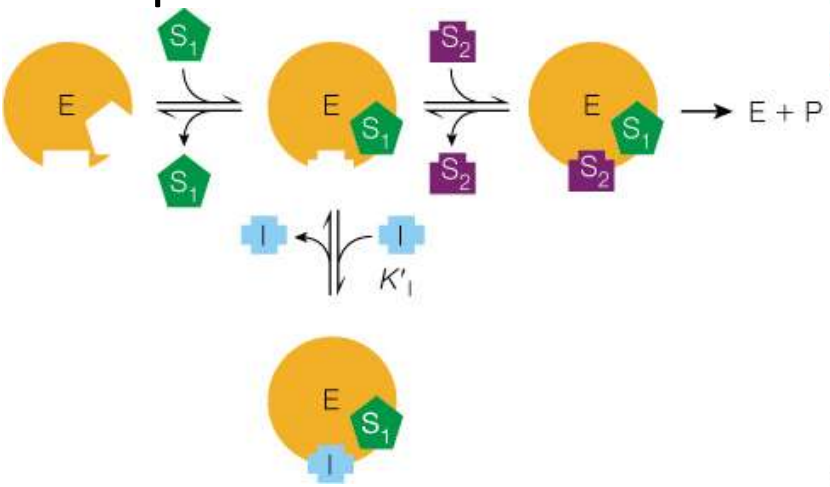
Uncompetitive Inhibition

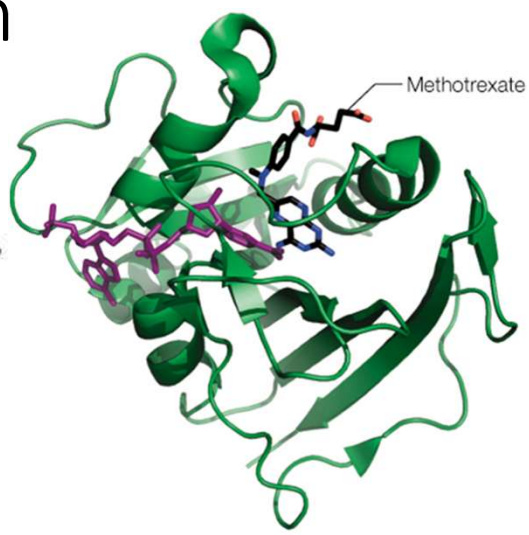




- Both <u>V_{max} and K_m change</u> in this case.
- **Slope (K_m /V_{max}) is the same**
- Both, the apparent K_M and apparent V_{max}, are decreased

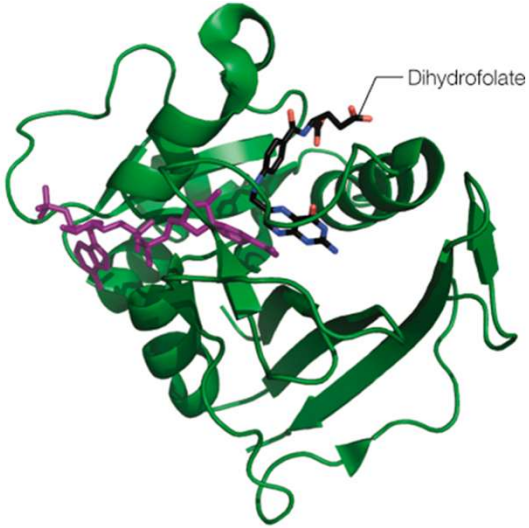
Uncompetitive Inhibition Example





Methotrexate $K_I' < 1$ nM

(a) Methotrexate (black sticks) binds tightly to the complex of dihydrofolate reductase (green cartoon) and NADPH (magenta sticks). NADPH is a phosphorylated derivative of NADH. PDB ID: 1u72.

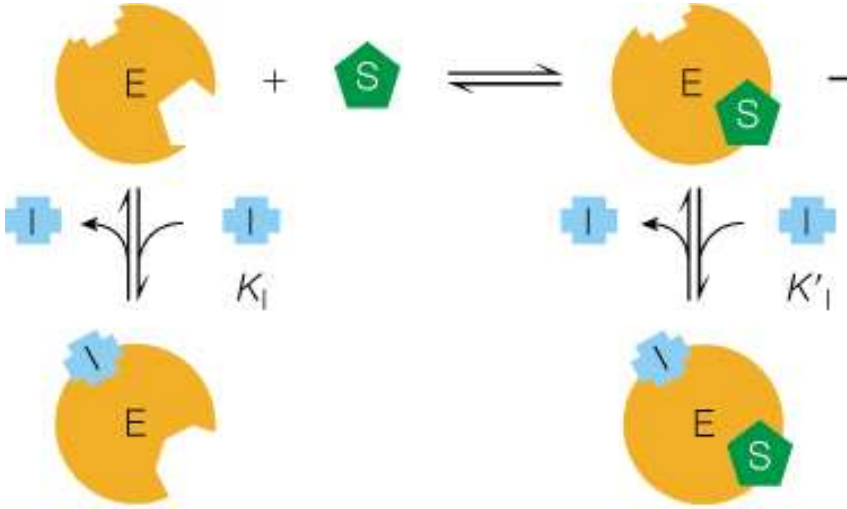


NADPH $K_M = 4.0\ \mu M$
DHF $K_M = 2.7\ \mu M$

(b) Dihydrofolate reductase bound to both its normal substrates NADPH (magenta sticks) and dihydrofolate (black sticks). PDB ID: 2w3m.

- Bisubstrate reaction with ordered substrate binding involving an inhibitor that is uncompetitive with respect to substrate S1 and competitive with respect to substrate S2
- Inhibition of dihydrofolate reductase by the anticancer drug methotrexate; uncompetitive for NADPH (S1, cofactor) binding and competitive for dihydrofolate (S2) binding.

Mixed or noncompetitive inhibition

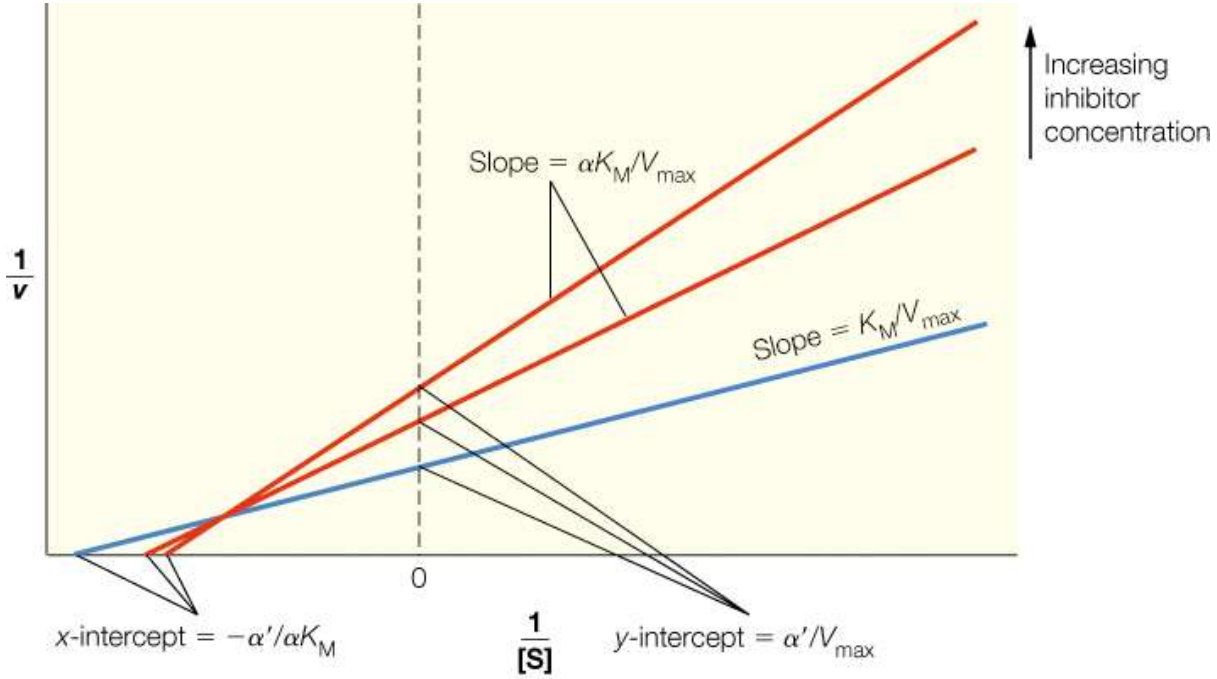


$$[E]_t = [E] + [ES] + [EI]$$

$[E]_t$	=	[E]	+	[ES]	+	[EI]
Total enzyme		Free enzyme		Enzyme bound to substrate		Enzyme bound to inhibitor

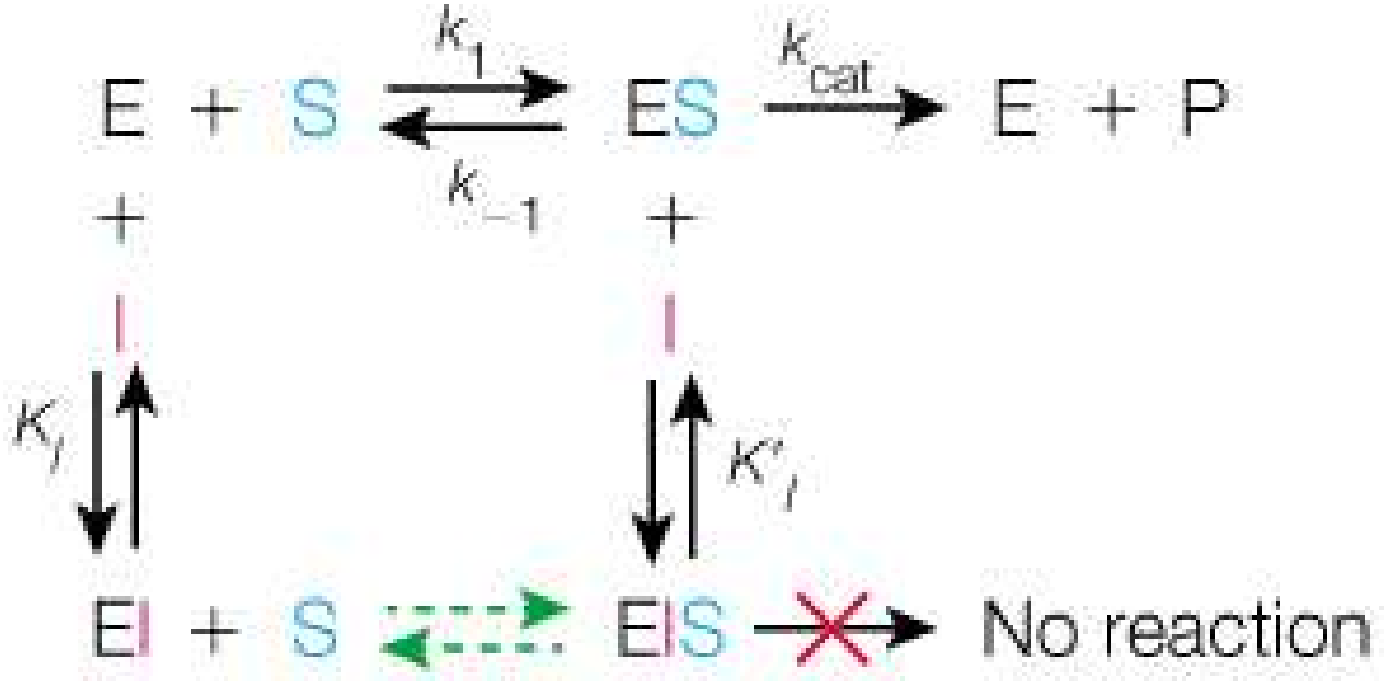
- The inhibitor binds at a site on the enzyme other than the active site, thereby diminishing the enzyme's catalytic activity
- Inhibitor binding occurs at any stage during catalysis
  - I does not compete with S for the active site
  - Both E and ES can bind I

Mixed or Noncompetitive Inhibition



The apparent K_M is almost unchanged, while the apparent V_{max} is decreased

Mixed (Noncompetitive) Enzyme Inhibition



Enzyme Inhibitor Effects

Type of Inhibition	Effect of Inhibitor
None	None
Competitive	Increases K_M^{app}
Uncompetitive	Decreases K_M^{app} and V_{max}^{app}
Mixed (noncompetitive)	Decreases V_{max}^{app}; may increase or decrease K_M^{app}

Irreversible Inhibition

Covalent bonding to an enzyme's active site

- Example: diisopropyl fluorophosphate (DFP) binds to the active site serine of acetylcholinesterase, an enzyme involved in nerve conduction.
- Inhibition of this enzyme causes rapid paralysis

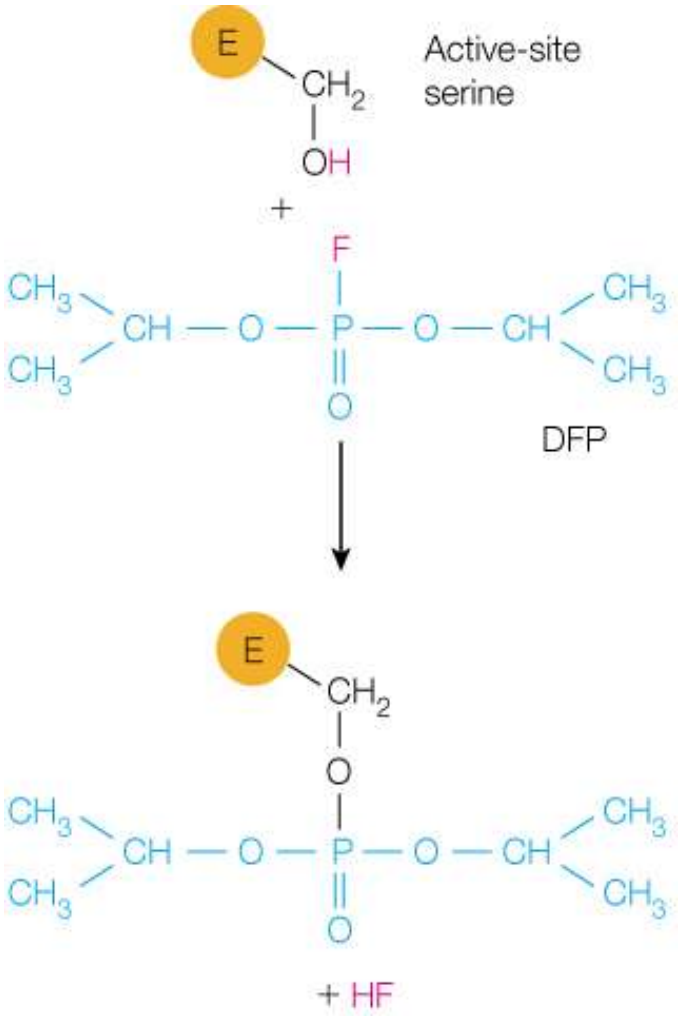


FIGURE 8.32 Irreversible inhibition by adduct formation.

Enzyme Inhibition Summary

- Enzyme inhibitors interact reversibly or irreversibly with an enzyme to alter its K_M and/or V_{max} values.
- A competitive inhibitor binds to the enzyme's active site and increases the apparent K_M for the reaction.
- An uncompetitive enzyme inhibitor affects catalytic activity such that both the apparent K_M and the apparent V_{max} decrease (but ratio remains the same).
- A mixed enzyme inhibitor alters both catalytic activity and substrate binding such that the apparent V_{max} decreases and the apparent K_M may increase or decrease slightly.
- Irreversible inhibition is by covalent bond formation.

Enzyme activity can be controlled

- By enzyme availability: how much is present or produced? Controlled by the cell and can change dramatically.
  - "Gene level control" can also lead to the generation of multiple isomeric forms called isozymes
- By allosteric mechanisms:
  - Structural changes affecting substrate binding
- By covalent modification
  - Usually phosphorylation (by kinases) and dephosphorylation (by phosphatases) of specific residues: Ser, Thr or Tyr.
  - Can activate or deactivate enzymes: **on or off switches**

Control by Multiple Forms of Enzymes

- Isoenzymes or isozymes are enzymes that are encoded by different genes.
- They catalyze the same reaction but display different regulatory properties.
- They may be expressed in a tissue-specific or developmentally specific pattern.
- The appearance of certain isozymes in the blood is a sign of tissue damage.

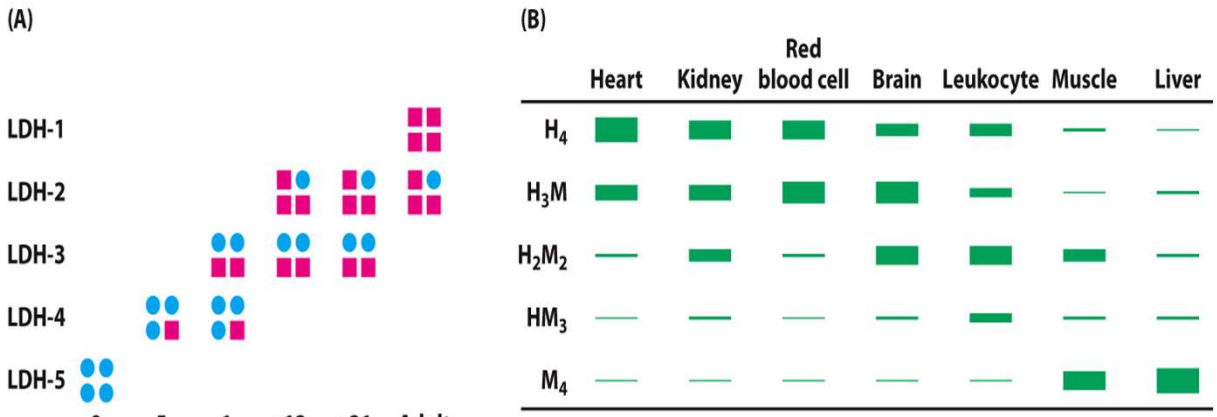


Figure 10.15
Biochemistry, Eighth Edition

Control by Multiple Forms of Enzymes

Studies of individual enzyme molecules suggest that some enzymes (isozymes) may exist in multiple conformations that are in equilibrium.

These different conformations may have different catalytic or regulatory properties – more in amino acid metabolism.

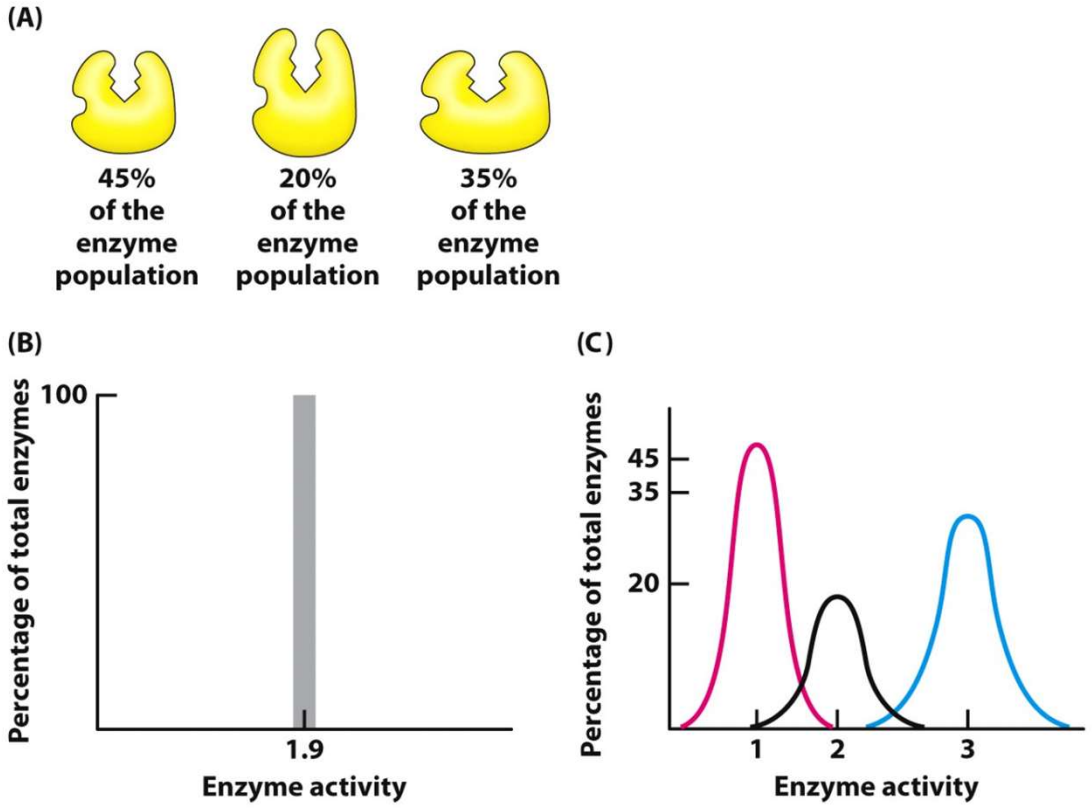
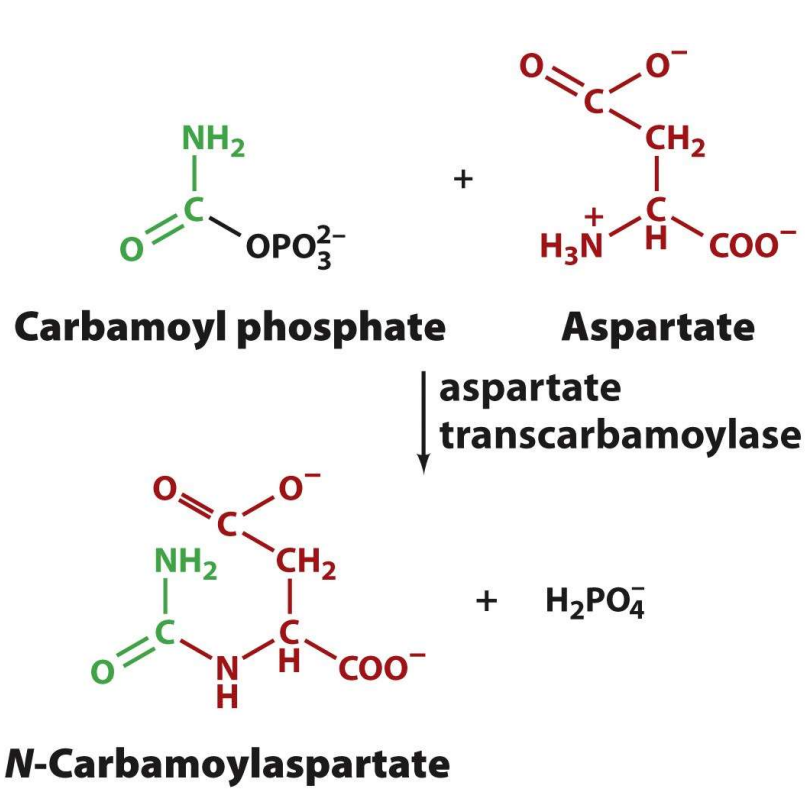
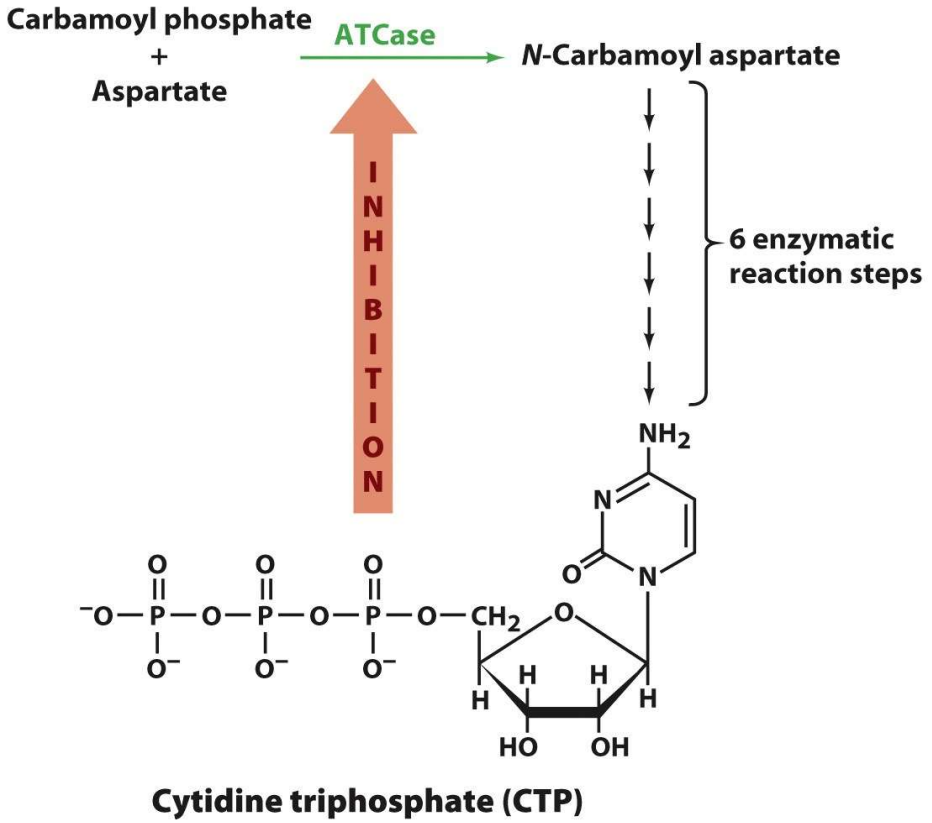


Figure 8.34
Biochemistry, Seventh Edition

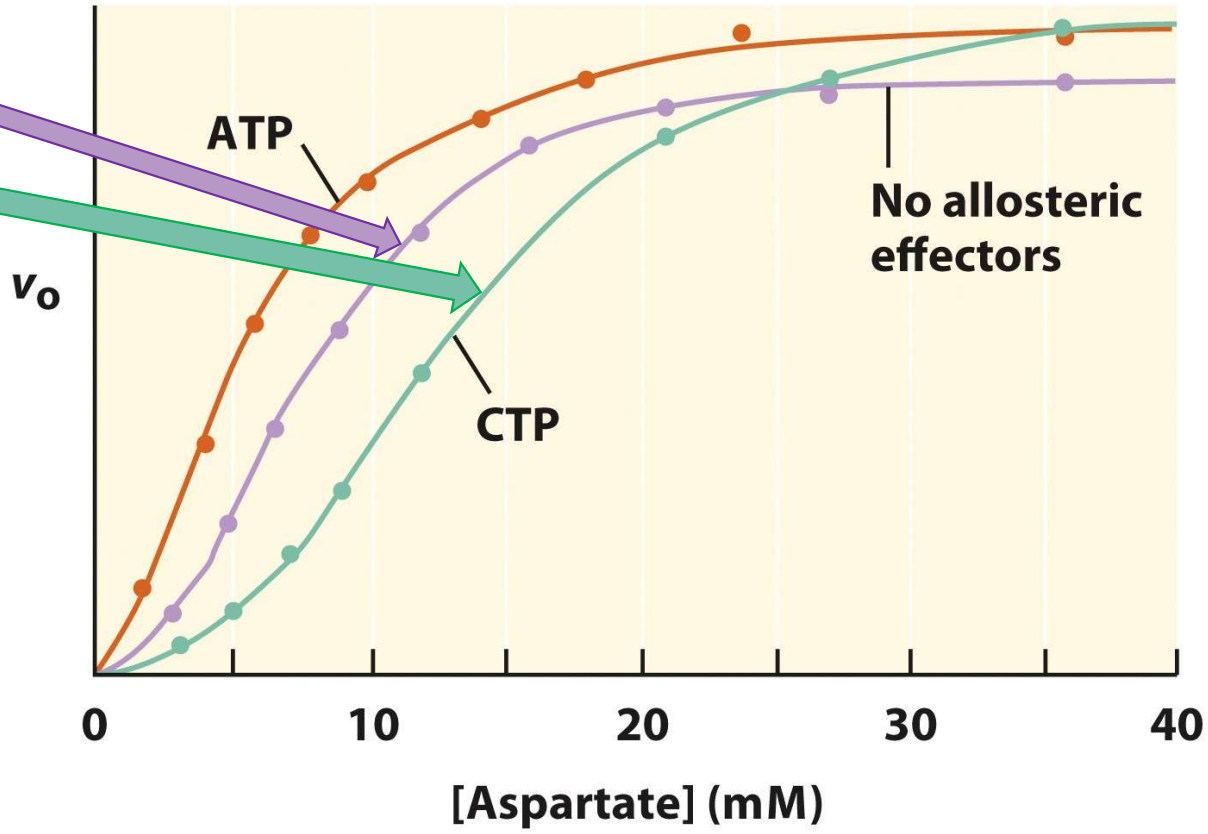
Allosteric control: Pyrimidine Biosynthesis: Aspartate transcarbamolyase (ATCase) Feedback Inhibition





Allosteric Effectors: ATCase Reaction

- Reaction velocity curve changes from hyperbolic to sigmoidal
- ATP is a positive effector, while CTP is a negative effector
- Final product in a multi-step reaction inhibits the enzyme catalysing the first step
- Conserves the cell's resources
- Effector binds to the allosteric site, not active site
- *Another example in Prac 5*



Control by Reversible Covalent Modification

TABLE 10.1 Common covalent modifications of protein activity

Modification	Donor molecule	Example of modified protein	Protein function
Phosphorylation	ATP	Glycogen phosphorylase	Glucose homeostasis; energy transduction
Acetylation	Acetyl CoA	Histones	DNA packing; transcription
Myristoylation	Myristoyl CoA	Src	Signal transduction
ADP ribosylation	NAD^+	RNA polymerase	Transcription
Farnesylation	Farnesyl pyrophosphate	Ras	Signal transduction
γ-Carboxylation	HCO_3^-	Thrombin	Blood clotting
Sulfation	3′-Phosphoadenosine-5′-phosphosulfate	Fibrinogen	Blood-clot formation
Ubiquitination	Ubiquitin	Cyclin	Control of cell cycle

Table 10.1
Biochemistry, Eighth Edition

Control by Covalent Modification: Phosphorylation

Protein kinases modify proteins by attaching a phosphate to a serine, threonine, or tyrosine residue. ATP serves as the phosphate donor.

TABLE 10.2 Examples of serine and threonine kinases and their activating signals

Signal	Enzyme
Cyclic nucleotides	Cyclic AMP-dependent protein kinase Cyclic GMP-dependent protein kinase
Ca^{2+} and calmodulin	Ca^{2+}—calmodulin protein kinase Phosphorylase kinase or glycogen synthase kinase 2
AMP	AMP-activated kinase
Diacylglycerol	Protein kinase C
Metabolic intermediates and other "local" effectors	Many target-specific enzymes, such as pyruvate dehydrogenase kinase and branched-chain ketoacid dehydrogenase kinase

Source: Information from D. Fell, *Understanding the Control of Metabolism* (Portland Press, 1997), Table 7.2.

Table 10.2
Biochemistry, Eighth Edition

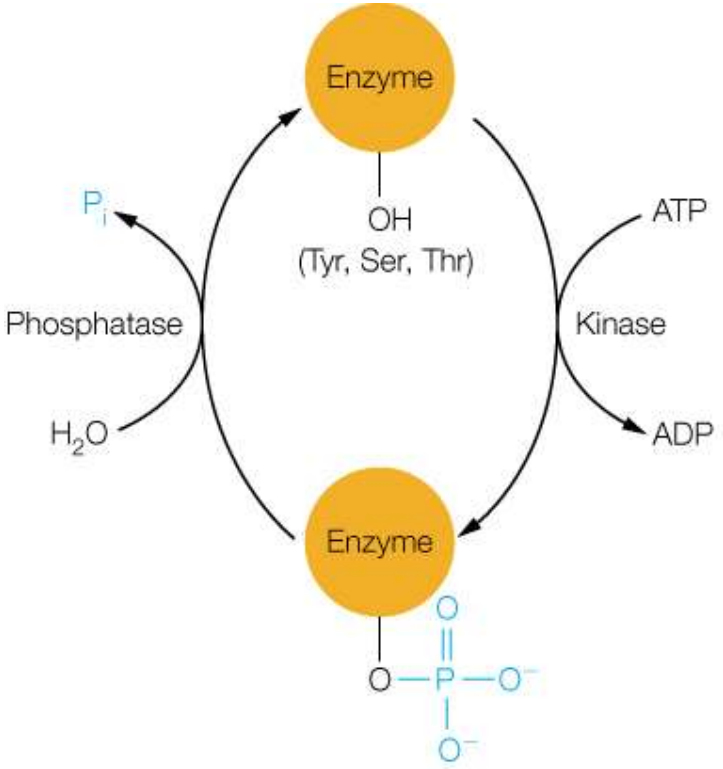
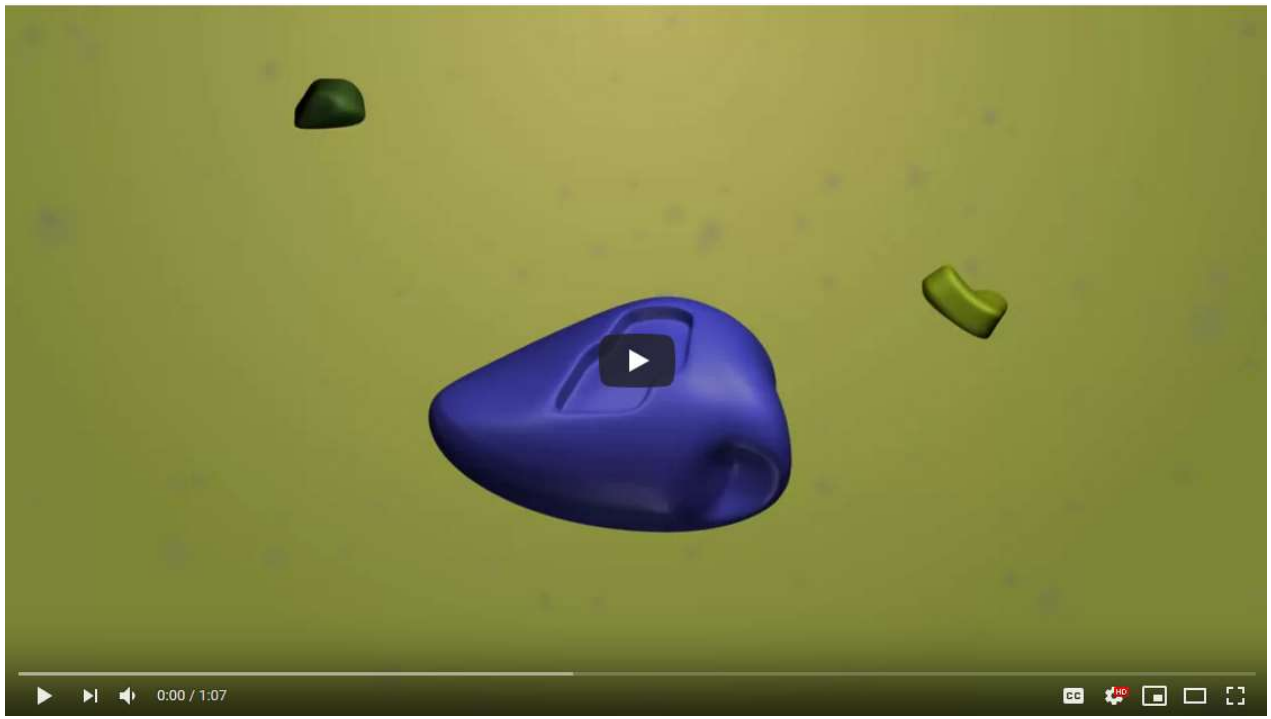


FIGURE 8.41 Reversible covalent modification by kinases/phosphatases.

Control of Enzyme Activity Summary

- Activities of enzymes can be regulated in various ways by: e.g., gene level control, allosteric mechanisms, or reversible/irreversible covalent protein modification
- Allosteric effectors bind to multisubunit enzymes such as aspartate transcarbamoylase (ATCase), thereby inducing cooperative conformational changes that alter the enzyme's catalytic activity.
- An example of reversible modification is the phosphorylation and dephosphorylation of enzymes by kinases and phosphatases, respectively
  - Phosphorylation and dephosphorylation of an enzyme such as glycogen phosphorylase can control its activity by shifting the equilibrium between more active and less active conformations.

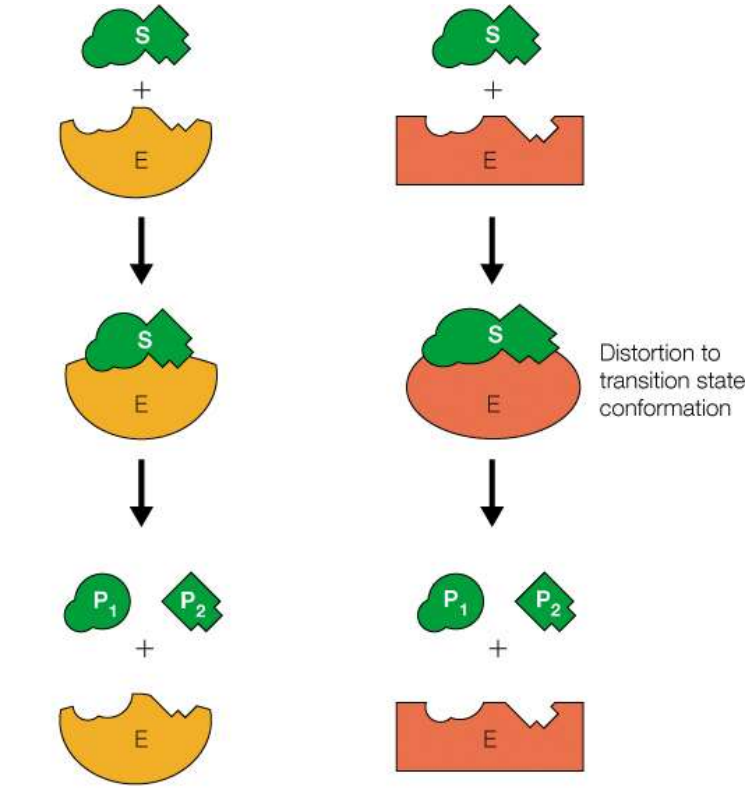
Enzyme function and inhibition



https://www.youtube.com/watch?v=PILzvT3spCQ

Enzyme mechanisms – how enzymes work

- Lock and key model (Fischer, 1894)
  - The substrate fits into the active site of the enzyme like a key fits into a lock (historic).
- **Induced fit model**
  - The active site *does not quite fit* the substrate.
  - So the active site is changed to accommodate the substrate.
    - ✓ This flexibility can help the substrate enter the transition state and the product to leave.
    - ✓ Catalytically active antibodies are made against transition state analogues.
  - Instead of the enzyme, the substrate can be fitted to the active site.



(a) Lock-and-key model In this early model, the active site of the enzyme fits the substrate as a lock does a key.

(b) Induced fit model In this elaboration of the lock-and-key model, both enzyme and substrate are distorted on binding. The substrate is forced into a conformation approximating the transition state; the enzyme keeps the substrate under strain.

FIGURE 8.8 Two models for enzyme–substrate interaction.

Induced fit model from experiment

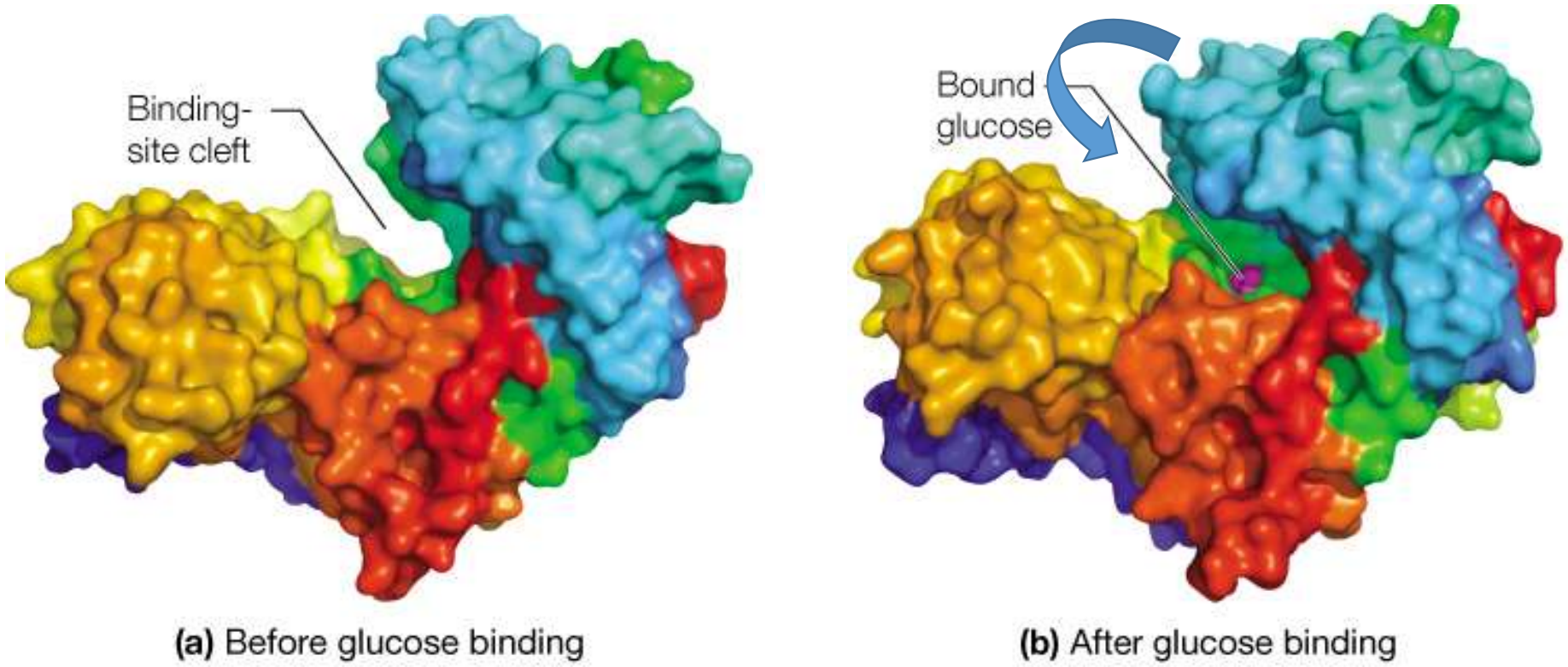


FIGURE 8.9 The induced conformational change in hexokinase.