

## **ENZYME INHIBITION**

### - **INHIBITORS:**

- Interfere with the action of an enzyme
- Decrease the rates of their catalysis
- Inhibitors are a great focus of many drug companies – want to develop compounds to prevent/control certain diseases due to an enzymatic activity
  1. e.g. AIDS and HIV protease inhibitors
    - HIV protease essential for processing of proteins in virus
    - Without these proteins, viable viruses cannot be released to cause further infection

### - Inhibitors can be **REVERSIBLE** or **IRREVERSIBLE**

- Irreversible Inhibitors
  - Enzyme is **COVALENTLY** modified after interaction with inhibitor
  - Derivatized enzyme is NO longer a catalyst – loses enzymatic activity
  - Original activity cannot be regenerated
  - Also called **SUICIDE INHIBITORS**
    - e.g. nerve gas, VX gas
    - Aspirin! Acetylates Ser in active site of cyclooxygenase (COX) enzyme
- Reversible Inhibitors
  - Bind to enzyme and are subsequently released
  - Leave enzyme in original condition
  - Three subclasses:
    - Competitive Inhibitors
    - Non-competitive Inhibitors
    - Uncompetitive Inhibitors
  - Can be distinguished by their kinetics of inhibition

### **How are inhibitors characterized experimentally?**

- First, perform experiment without inhibitor
  - Measure velocity at different substrate concentrations, keeping [E] constant. Choose values of [S]
  - Get [S] and V values from experiment
  - Take reciprocal of [S] and V values (“1 over”)
  - Plot on graph  $1/[S]$  (x) vs.  $1/V$  (y) – don’t use Michaelis-Menten – not reliable
- Second, do the SAME experiment in parallel using a fixed amount of inhibitor and same values for E and S
- Get V values and plot together with uninhibited reaction
- Depending on how the graph looks and using the subsequent equation of the line we can:
  - Determine type of inhibition (competitive vs. non-competitive vs. un-competitive)
  - Determine  $K_m$  and  $V_{max}$  using the NEW line equations
  - Determine  $K_i = \text{inhibition constant}$  – defined as the dissociation constant for the enzyme-inhibitor complex

## 1. COMPETITIVE INHIBITORS

- Shape and structure of inhibitor is very similar to substrate
- Inhibitor mimic substrate (or transition state) and fits into the active site
- Physically blocks substrate's access into the active site

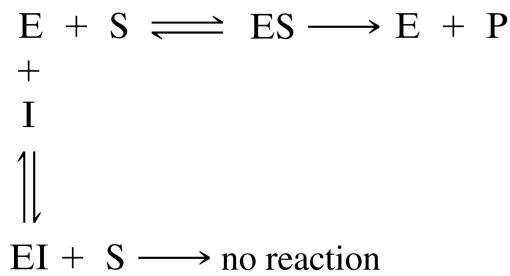
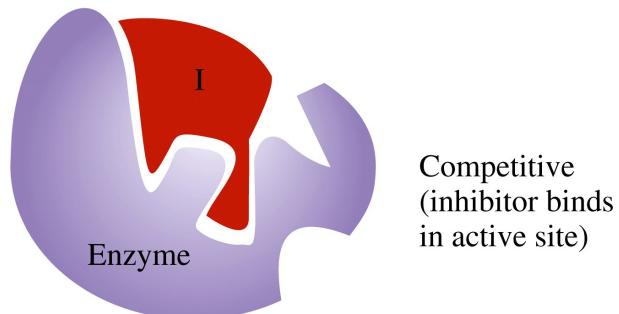
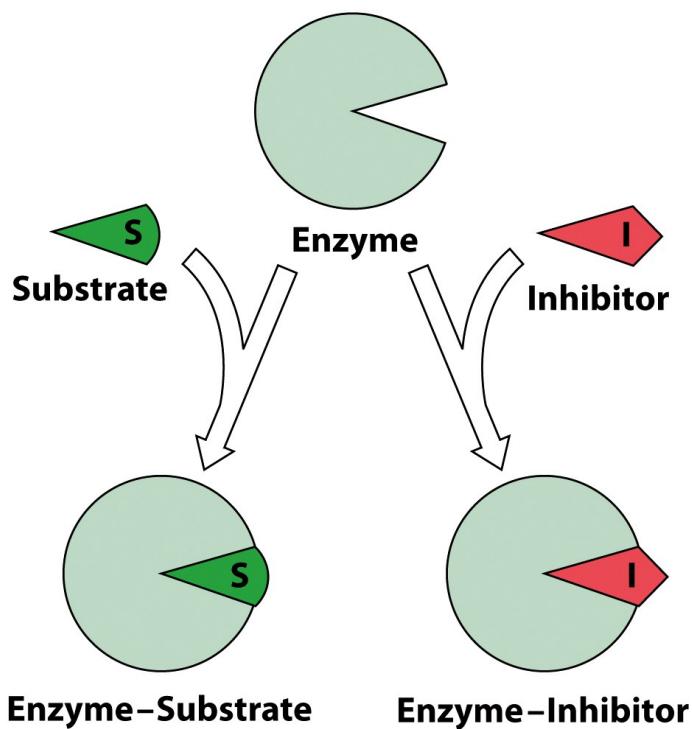


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## COMPETITIVE INHIBITION



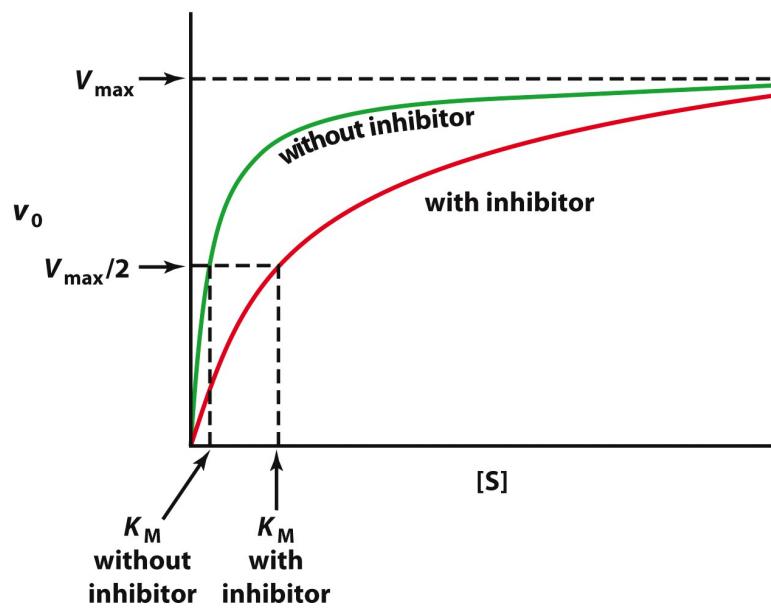
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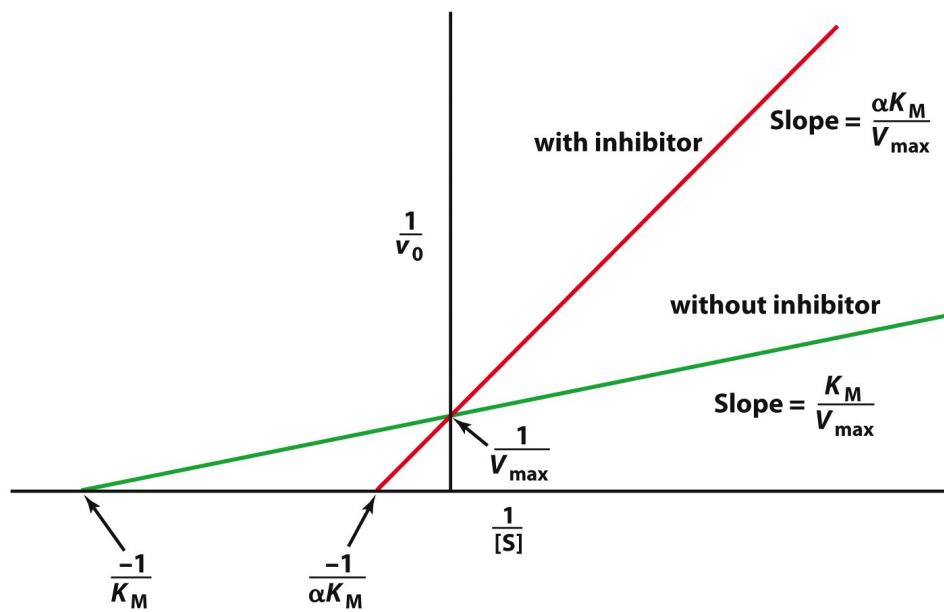
Many pharmaceutical drugs are competitive inhibitors.

Many are transition state analogs: Competitive inhibitors which mimic the transition state of an enzyme catalyzed reaction (e.g. HIV protease inhibitors such as Saquinavir and Viracept).

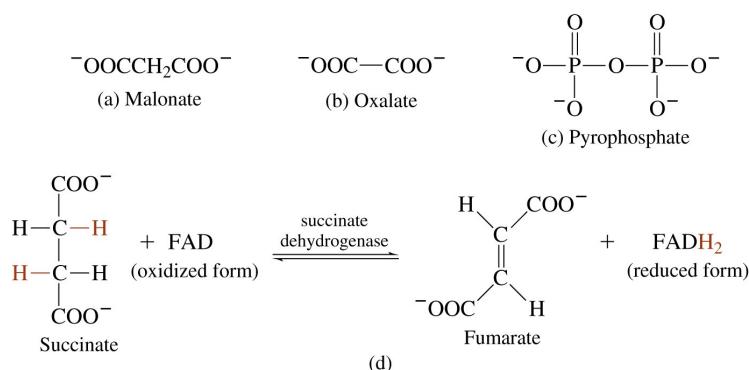
- Competitive inhibitors can be identified by the kinetics of their inhibition
- Lineweaver-Burk plot shows that in the presence of increasing concentrations of inhibitor, I,
  - **K<sub>M</sub> INCREASES**
  - **V<sub>max</sub> STAYS THE SAME**
  - Effects of competition can be overcome by increasing [S]
  - K<sub>M</sub> gets larger, more substrate needed to reach a given rate, can still, however, ultimately achieve V<sub>max</sub>
  - Regardless of the concentration of a competitive inhibitor, a sufficiently high substrate concentration will displace the inhibitor from the active site
  - The inhibitor and substrate effectively compete with each other for the site



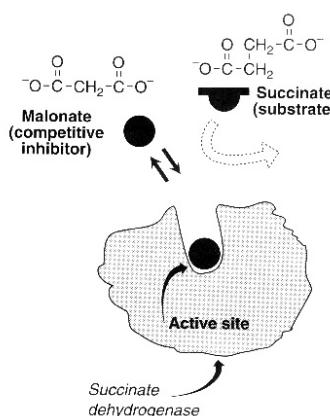
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## Competitive Inhibitors of Succinate Dehydrogenase

**Transition state analogs** are compounds that resemble the transition state of a catalyzed reaction. Usually do not undergo a chemical reaction and can act as enzyme inhibitors by blocking their active site. Like the actual transition state species, TS analogs bind much stronger to the enzyme than simple substrate or product analogs. Many drugs are transition state analogs.

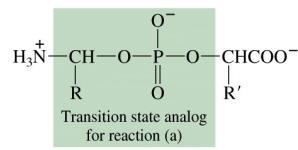
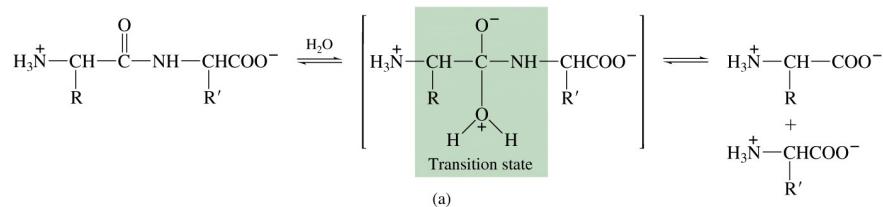
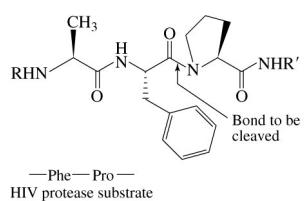
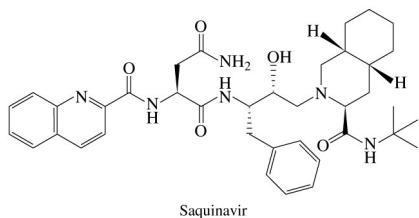


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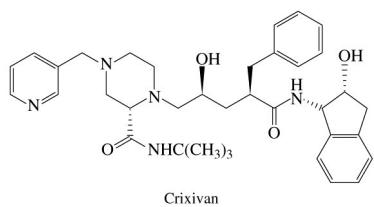
## Competitive inhibition of a peptidase by a transition state analog.



## HIV Protease Substrate



## HIV Protease Inhibitor



### HIV protease Inhibitor

# COMPETITIVE INHIBITORS DESIGNED BY TRANSITION STATE ANALOG APPROACHES

Unnumbered figure pg158 Concepts in Biochemistry, 3/e  
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## 2. NON-COMPETITIVE INHIBITORS

- Inhibitor binds to a site OTHER than the active site
  - Binding causes a change in the structure of the enzyme so that it cannot catalyze a reaction

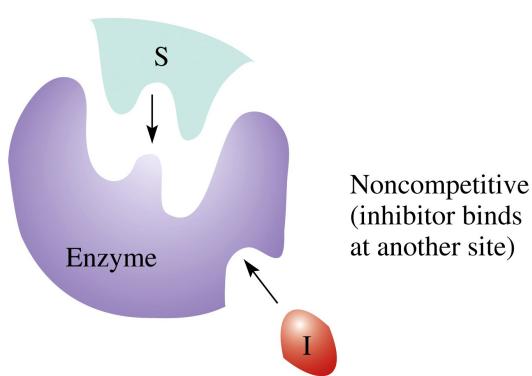
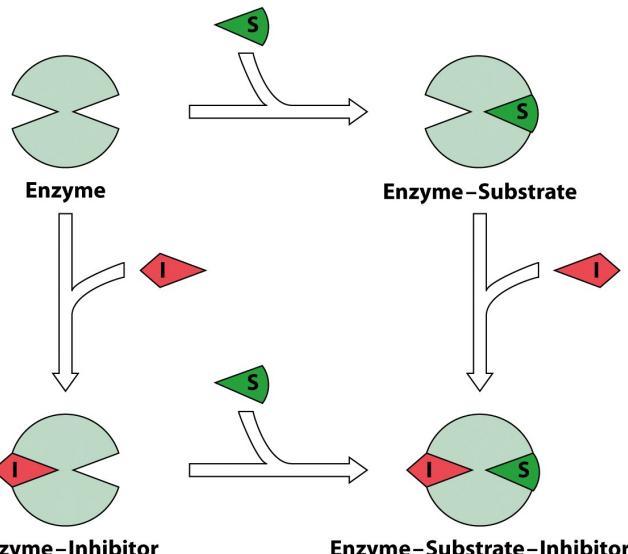
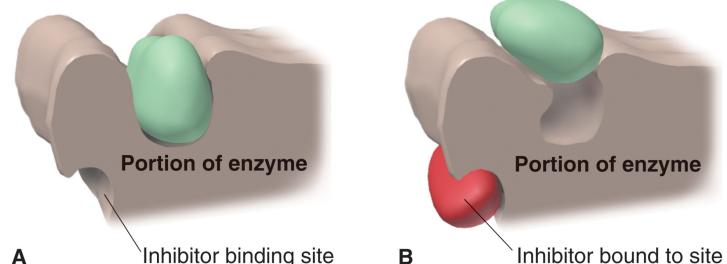


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*Active site is deformed when inhibitor is present, so substrate cannot bind and no catalysis occurs.*



Non-competitive inhibitors can also be identified by the kinetics of inhibition

- In the presence of a non-competitive inhibitor:

- **K<sub>M</sub> STAYS THE SAME**

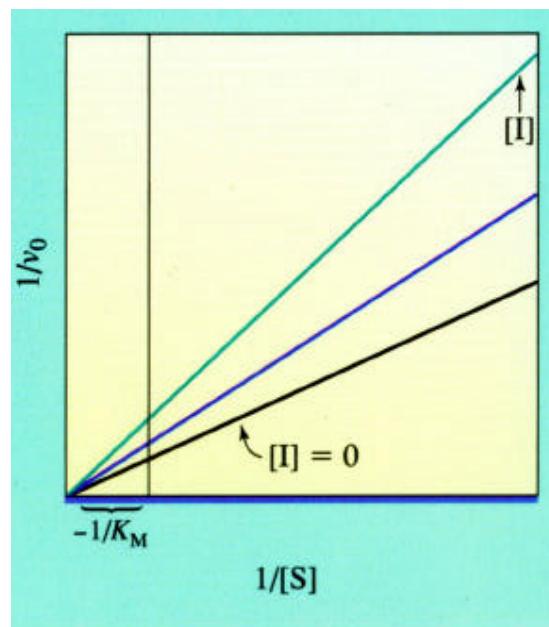
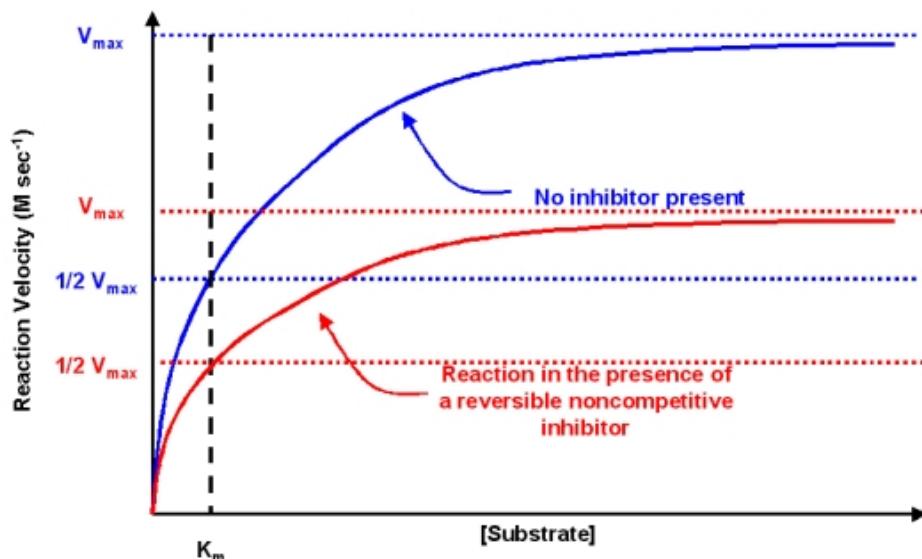
- Binding of substrate has no effect on inhibition; inhibitor can bind to ES complex

- Not competing for active site

- **V<sub>max</sub> DECREASES**

- $1/V_{max}$  increases; activity goes down

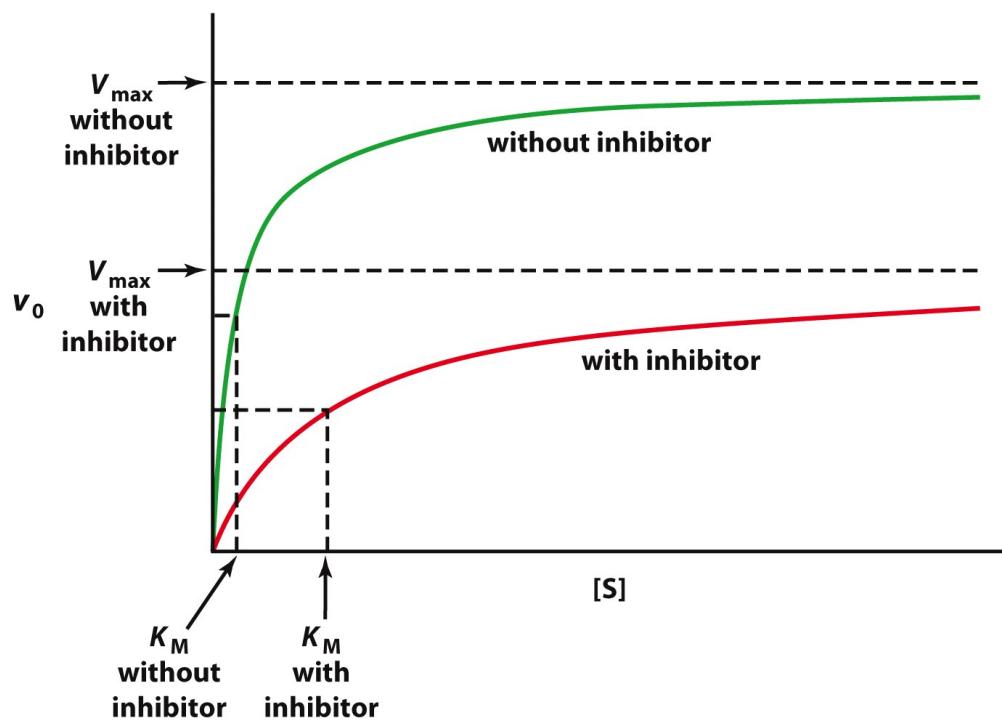
- The effects of non-competitive inhibition cannot be overcome by increasing [S]



(b) Noncompetitive inhibition

**MIXED INHIBITION:**

Inhibitor affects both substrate binding and  $V_{max}$  –  $K_M$  increases and  $V_{max}$  decreases

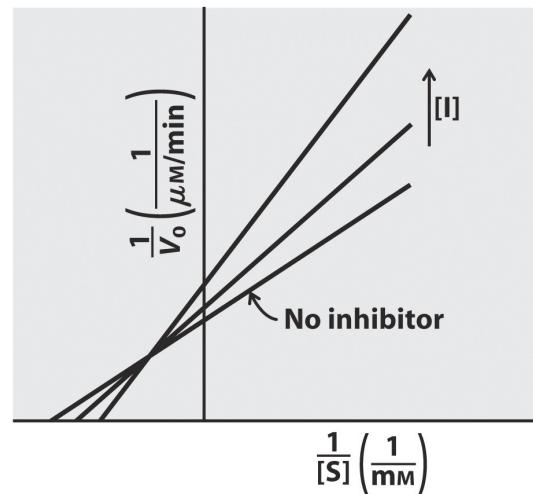
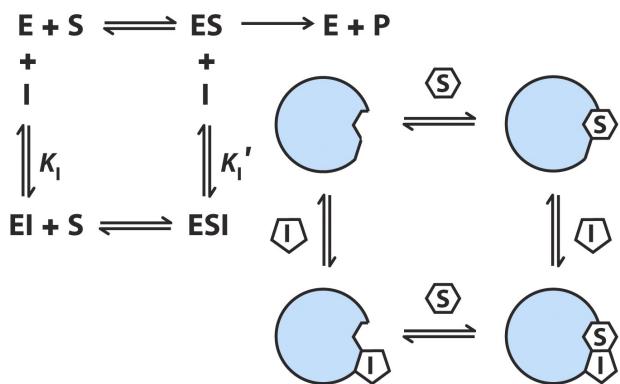


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Mixed inhibition is when the inhibitor binds to the enzyme at a location distinct from the substrate binding site. The binding of the inhibitor alters the  $K_M$  and  $V_{max}$ .

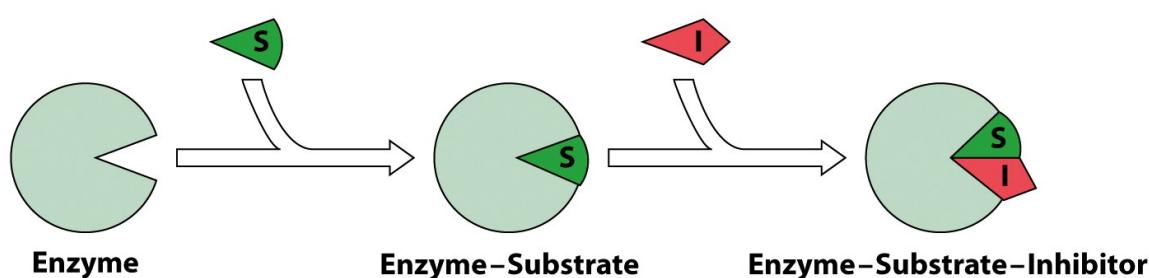
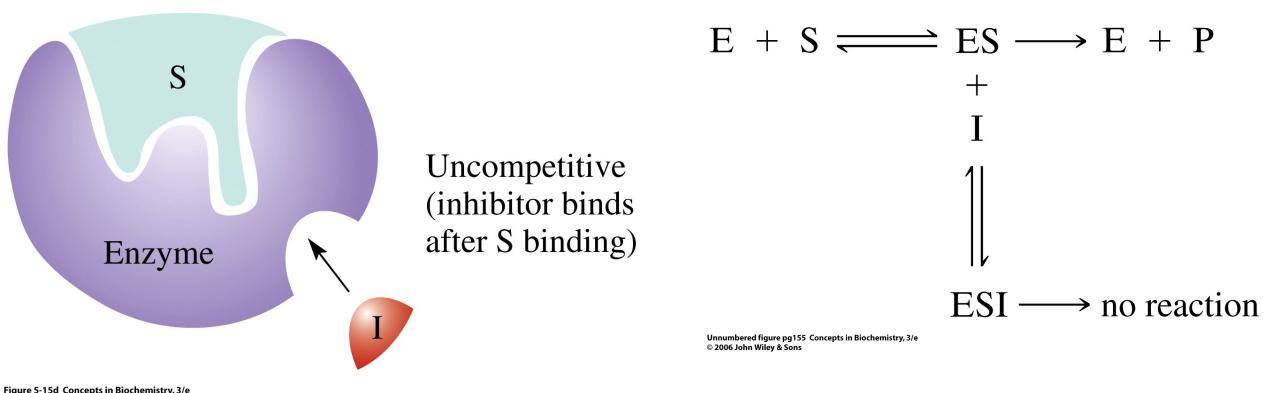
Similar to noncompetitive inhibition except that binding of the substrate or the inhibitor affect the enzyme's binding affinity for the other.

Binding affinity for the substrate is decreased when the inhibitor is present.

**(c) Mixed inhibition**

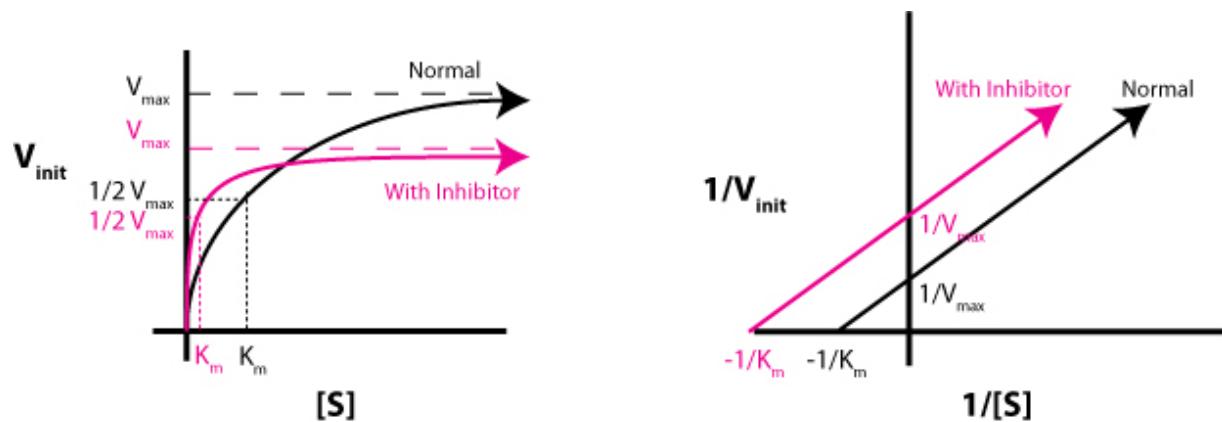
### 3. UN-COMPETITIVE INHIBITORS

- Inhibitor binds to a site other than the active site, **but only when substrate is bound (Binds to ES complex)**
  - Distorts active site; prevents reaction from occurring



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- Un-competitive inhibitors can also be identified by the kinetics of inhibition



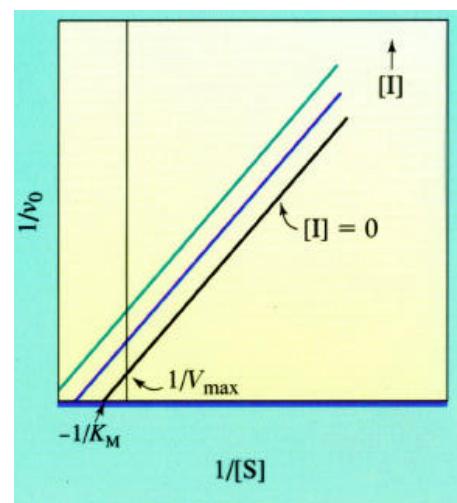
- In the presence of an **UN-COMPETITIVE inhibitor:**
- **$K_M$  DECREASES (Effectively increases the affinity for the substrate)**
- **$V_{max}$  DECREASES (Overall rate goes down)**
- The effects of un-competitive inhibition cannot be overcome by increasing [S]

**Table 5.5**  
Kinetic characteristics of reversible inhibition

Type of Inhibition	Effect of Inhibition <sup>a</sup>		
	$K_M$	$V_{max}$	$K_M/V_{max}$ (slope)
Competitive	Higher	Same	Increase
Uncompetitive	Lower	Lower	Same
Noncompetitive			
Pure	Same	Lower	Increase
Mixed	Higher	Lower	Increase

<sup>a</sup>Compared to uninhibited reaction.

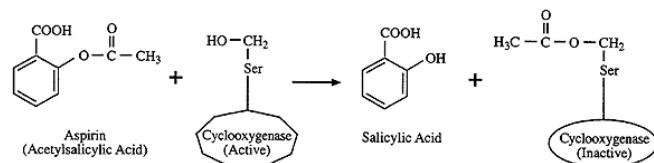
Table 5-5 Concepts in Biochemistry, 3/e  
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(c) Uncompetitive inhibition

## Irreversible inhibitors

➤ Inhibitors covalently modify the active site – permanent inhibition. Must wait for more enzyme to be made. Example: Aspirin, nerve gas



## Animations:

<http://www.wiley.com/college/fob/anim/#chap3>

## Chapter 12

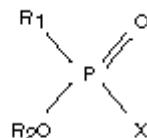
- [\*\*Fig. 12-2\*\*](#) -- The Progress Curves for a Simple Enzyme-catalyzed Reaction
- [\*\*Fig. 12-3\*\*](#) -- A Plot of Initial Velocity versus Substrate Concentration for a Simple Enzymatic Reaction
- [\*\*Fig. 12-4\*\*](#) -- A Double-reciprocal (Lineweaver-Burk) Plot
- [\*\*Fig. 12-7\*\*](#) -- A Lineweaver-Burk Plot for Competitive Inhibition
- [\*\*Fig. 12-8\*\*](#) -- A Lineweaver-Burk Plot for Uncompetitive Inhibition

## CASE STUDY: Use of Irreversible and Reversible Inhibitors

- Nerve gas and an Antidote
- **Organofluorophosphates** used as insecticides and nerve gases



**Nicholas Cage in "The Rock", ©Hollywood Pictures / Simpson-Bruckheimer Prod.**



SOURCE: AD Little (1996 ,Ch.5).

NOTE: See Table 5.1

**Figure 5.1--Chemical Structures of Nerve Agents**

**Table 5.1**  
**Nerve Agent Chemical Structure**

Agent	X	R <sub>1</sub>	R <sub>2</sub>
Tabun (GA)	CN	N(CH <sub>3</sub> ) <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>
Sarin (GB)	F	CH <sub>3</sub>	CH(CH <sub>3</sub> ) <sub>2</sub>
Soman (GD)	F	CH <sub>3</sub>	CH(CH <sub>3</sub> )C(CH <sub>3</sub> ) <sub>3</sub>
Cyclosarin (GF)	F	CH <sub>3</sub>	Cyclohexyl
VX	SCH <sub>2</sub> CH <sub>2</sub> N[CH(CH <sub>3</sub> ) <sub>2</sub> ] <sub>2</sub>	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>

SOURCE: SIPRI (1973).

X = leaving group

VX gas may be absorbed ocularly (through the eyes), percutaneously (through skin), and by inhalation

- These compounds are **irreversible inhibitors** of acetylcholinesterase, an enzyme involved in proper nerve impulse transmission
- Upon absorption, the phosphorous atom of VX covalently binds to a serine hydroxyl group in the active site of acetylcholinesterase
- Enzyme no longer functional (inactive)

**Irreversible covalent inhibition of ACE by DIFP, an organofluorophosphate**

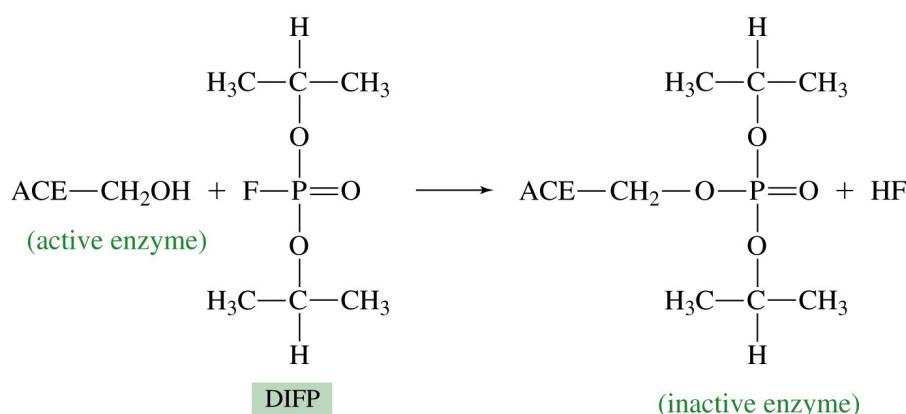
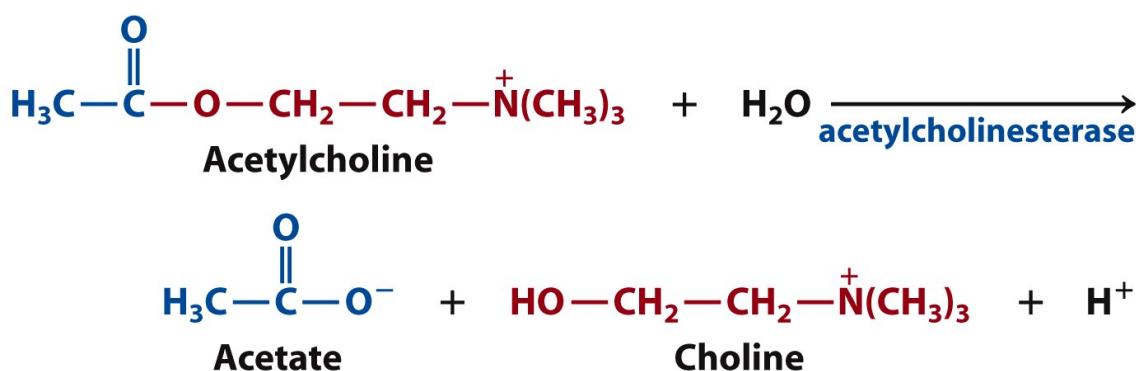


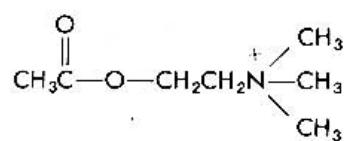
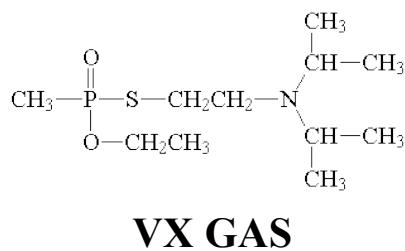
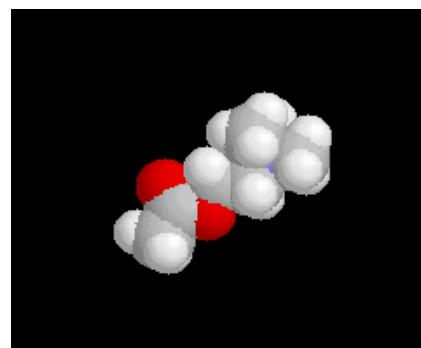
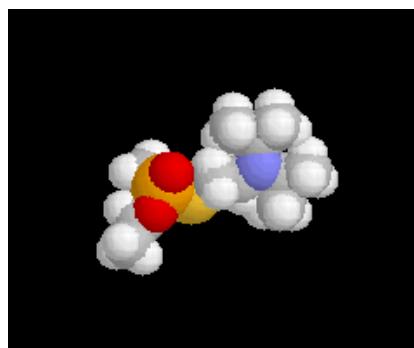
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**Reaction of diisopropylfluorophosphate (another organophosphate) with the hydroxyl group of a serine residue. This reaction is analogous to the phosphorylation that occurs with any nerve agent.**



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Below are representations of acetylcholine and VX Gas molecules. If you compare the structures you'll see how similar the structures appear.  
Notice the Phosphorus atom on the VX molecule (coloured yellow).

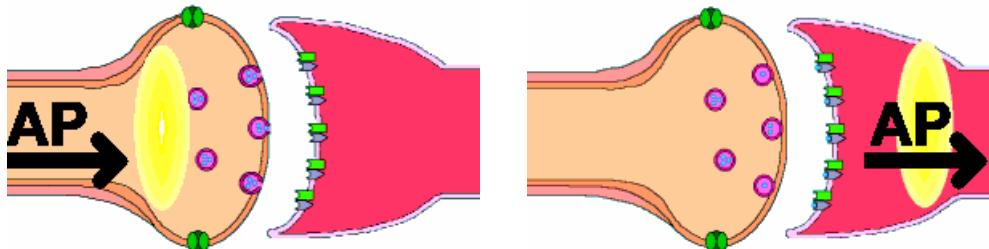


**Acetylcholine**

## How does inactivation of ACE (acetylcholinesterase) result in death?

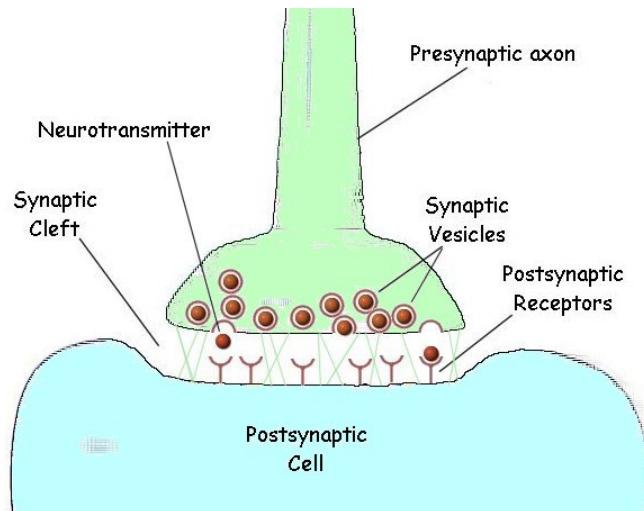
Let's talk a little about nerve conduction:

- Process starts in a presynaptic neuron
- An electrical nerve impulse (Action Potential) travels to the end of the **pre-synaptic neuron** which triggers acetylcholine-containing vesicles to fuse with the membrane and release Acetylcholine into the **synaptic cleft**.



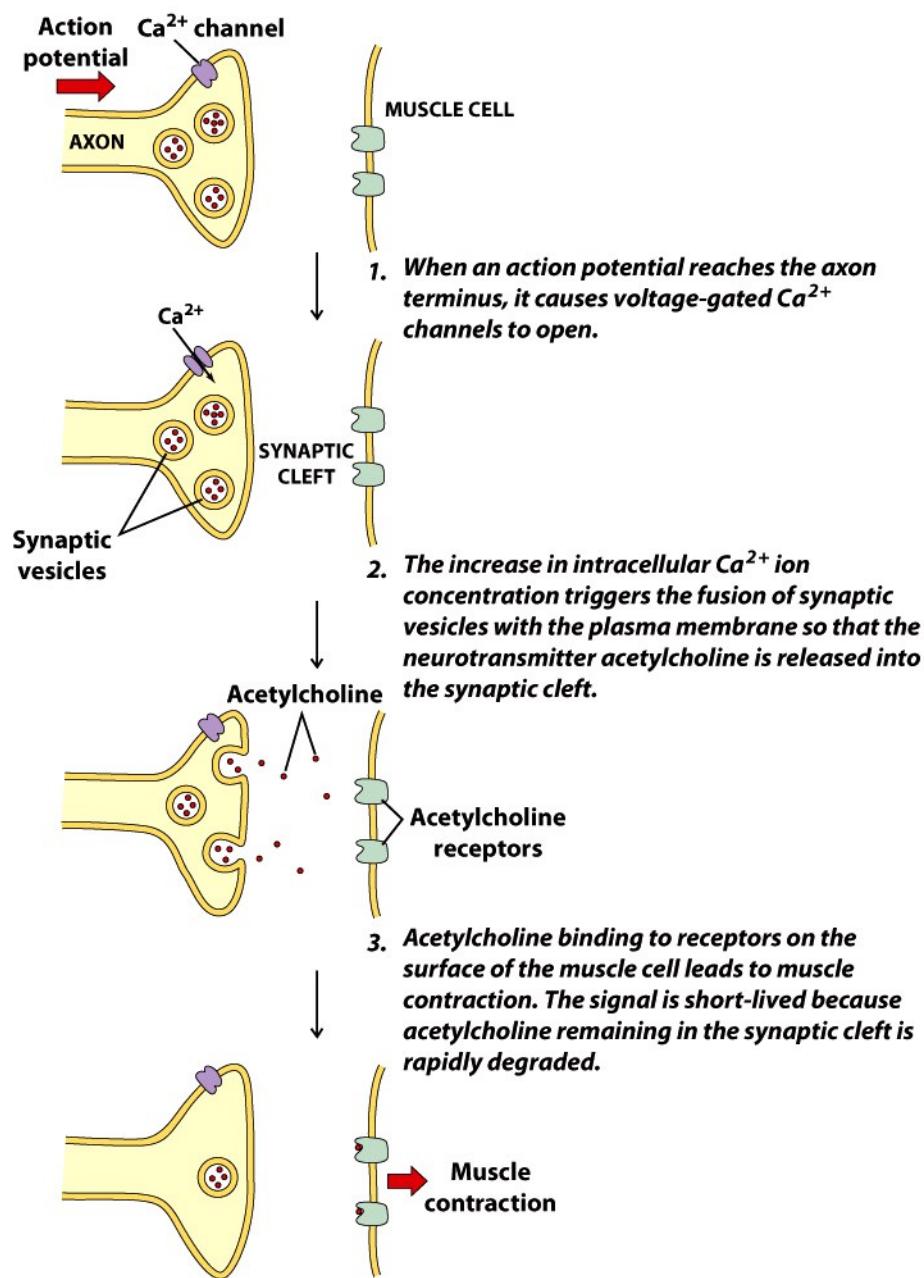
Acetylcholine moves through the synaptic cleft and diffuses to **acetylcholine receptors** on the **post-synaptic neuron**

- Ends in a post-synaptic cell
- The post-synaptic cell is stimulated by binding of neurotransmitter to the receptor. These acetylcholine/receptor interactions initiate a transmission in the post-synaptic cell:
  - If a muscle cell: causes muscle to contract
  - If another neuron: could cause further stimulation
- **Acetylcholinesterase (ACE) found in the synaptic cleft**
  - Rapidly breaks down acetylcholine to acetic acid and choline so it cannot continually stimulate
  - Acetic acid and choline diffuse back to pre-synaptic neuron and regenerates the pre-synaptic neuron for further impulses
  - When nerve gas is present and inhibits ACE, acetylcholine is NOT degraded – get prolonged nerve firing and continued stimulation
  - This ceaseless production of nervous stimulation causes tremors, convulsions, paralysis and eventual death! Receptors in brain, gut, eyes, heart and bladder.



- **Neurotransmitter = acetylcholine**
- **Receptor = acetylcholine receptors**

## CHEMICAL SYNAPSE



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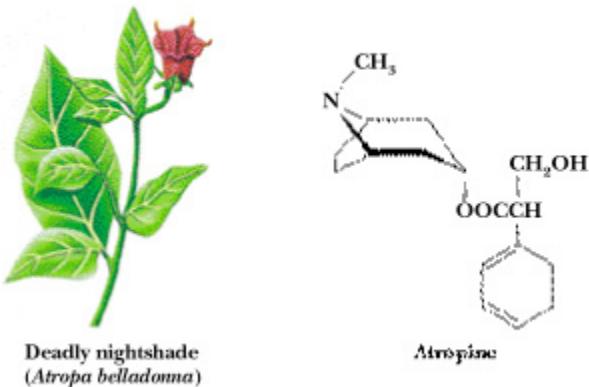
This constant nerve stimulation causes the characteristic symptoms of VX Gas poisoning.

These include:

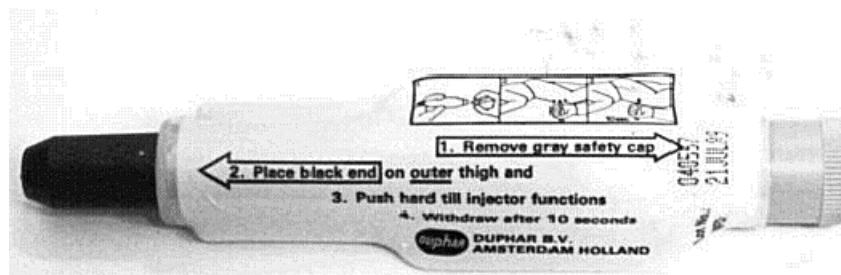
- Ataxia (lack of muscle control)
- Slurred speech
- Coma
- Areflexia (loss of reflexes)
- Generalized convulsions
- Cessation of breathing i.e. death

## ATROPINE IS AN ANTIDOTE!

- Atropine binds to the **acetylcholine receptors** and acts as a **competitive inhibitor** in muscles



- This prevents acetylcholine from binding to the receptors and sending impulses to the muscles. VX stimulates the muscles, so as atropine ceases the constant stimulation it acts as an antidote. With no acetylcholine reaching or binding to the receptors it is unimportant that the acetylcholinesterase is being destroyed by the VX nerve gas.
- i.e. atropine attenuates the constant signal. More ACE is made to eventually regain control of the system
- Oximes can also be co-administered – regenerate the enzyme



**Atropine** canister – autoinjector

This is an artist's impression of an atropine autoinjector used by the Dutch Armed Forces. Each marine is supplied with three autoinjectors. If VX exposure is indicated, a fellow soldier uses one auto-injector in the victim's thigh every six minutes.

Atropine is injected intramuscularly (into the thigh) in 2 mg doses every 3-8 minutes. If faster action is required, a direct injection into the heart will prevent death, effectively neutralizing the effect of VX.

## COENZYMES:

- Some enzymes require an additional component for activity
- These components are called **COENZYMES**
  - Used at the active site of the enzyme
  - Not covalently bound to the enzyme
  - Can be small organic molecules or metal ions
  - Many are structurally related to vitamins
  - They are regenerated for further reactions
- **PROSTHETIC GROUPS** are COENZYMES that ARE covalently bound to an enzyme and therefore are always present

Coenzymes necessary for proper metabolism		
Coenzyme	Vitamin Precursor	Reaction Type
1. Nicotinamide adenine dinucleotide (NAD <sup>+</sup> ) Reaction: $\text{NAD}^+ + \text{RCH}_2\text{OH} \rightleftharpoons \text{NADH} + \text{H}^+ + \text{RC}-\text{H}$	Niacin	Oxidation-reduction
2. Flavin-adenine dinucleotide (FAD) Reaction: $\text{HOOCCH}_2\text{CH}_2\text{COOH} + \text{FAD} \rightleftharpoons \begin{array}{c} \text{COOH} \\   \\ \text{C}=\text{C} \\   \\ \text{H} \end{array} + \text{FADH}_2$	Riboflavin	Oxidation-reduction
3. Biotin Reaction: $\text{H}_3\text{CCSCoA} + \text{ATP} + \text{HCO}_3^- \rightleftharpoons \text{CH}_2\text{CSCoA} + \text{ADP} + \text{P}_i$	Biotin	CO <sub>2</sub> fixation
4. Thiamine pyrophosphate Reaction: $\text{CH}_2\text{C}(=\text{O})\text{COO}^- \rightleftharpoons \text{CH}_2\text{C}-\text{H} + \text{CO}_2$	Thiamine (B <sub>1</sub> )	Decarboxylation
5. Coenzyme A Reaction: $\text{RCH}_2\text{COOH} + \text{ATP} + \text{CoASH} \rightleftharpoons \text{RCH}_2\text{CSCoA} + \text{ADP} + \text{P}_i$	Pantothenic acid	Acyl transfer
6. Pyridoxal phosphate Reaction: $\begin{array}{c} \text{COO}^- \\   \\ \text{H}_3\text{N}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{COO}^- \end{array} \rightleftharpoons \begin{array}{c} \text{COO}^- \\   \\ \text{O}=\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^- \end{array} + \begin{array}{c} \text{H}_3\text{N}-\text{C}-\text{H} \\   \\ \text{CH}_2-\text{CH}_2-\text{COO}^- \end{array}$	Pyridoxine (B <sub>6</sub> )	Transfer of amino group
7. Tetrahydrofolate acid Reaction: $\text{Serine} \rightleftharpoons \text{glycine} + \text{H}_2\text{O}$	Folic acid	Transfer of one-carbon unit
8. Lipoic acid Reaction: $\begin{array}{c} \text{CH}_3 \\   \\ \text{C}=\text{O} \\   \\ \text{S}-\text{S}-\text{H} \end{array} + \text{CoASH} \rightleftharpoons \begin{array}{c} \text{CoAS}-\text{C}-\text{CH}_3 \\   \\ \text{SH}-\text{S}-\text{H} \end{array} + \text{to protein}$	Lipoic acid	Acyl transfer

**Table 8.5**  
Common fat-soluble vitamins and their biological functions

Vitamin	Common Name	Chemical Characteristics	Biological Function
A	Retinol	A terpene with 20 carbons	Absorption of light in vision
D	Several forms; one is D <sub>3</sub> (cholecalciferol)	Formed from cholesterol by ultraviolet radiation	Regulation of calcium and phosphorus metabolism
E	α-Tocopherol	Aromatic ring with long hydrocarbon chain	Antioxidant; prevents oxidation damage to cellular membranes
K	Vitamin K	Bicyclic ring system with long hydrocarbon chain	Regulates blood clotting; bone formation

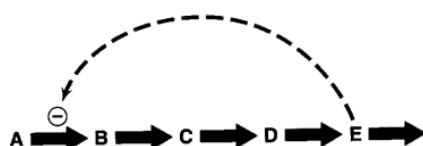
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## REGULATION OF ENZYME ACTIVITY:

- Regulation means to make an enzyme more or less active
- In general, regulation is necessary to control the rates of reactions and to properly synchronize all of the metabolic reactions in the cell. Keep it running like a finely tuned machine.

How can you change enzyme behavior in a cell?

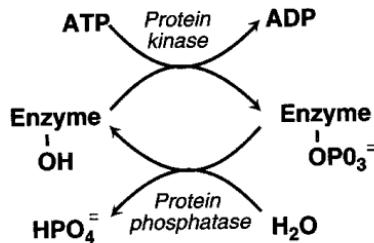
## Feedback inhibition of metabolic pathways



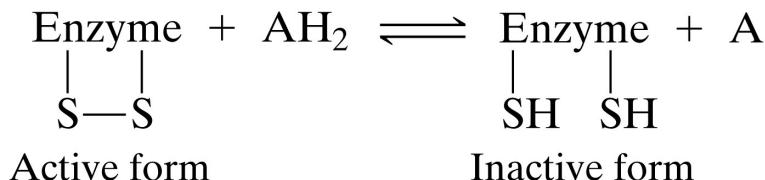
High concentrations of a downstream product of a pathway signals an upstream enzyme to shut down.

- **Induction/repression**
  - Change the rate of enzyme synthesis and/or degradation of the enzyme
  - Change cellular distribution of the enzyme
- Modify the **intrinsic properties** of the enzyme
  - **Non-covalent interactions**
    1. Bind regulatory molecules reversibly (e.g. proteins, lipids, small molecules)
  - **Reversible Covalent Modifications**
    1. **Phosphorylation** of serine, threonine or tyrosine
    2. **Methylation** of glutamate residues
      - Used in bacteria as food sensor

## Regulation by covalent modification of an enzyme



### 3. Creation or reduction of disulfide bonds



Unnumbered figure pg177b Concepts in Biochemistry, 3/e  
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- **Irreversible Covalent Modifications**

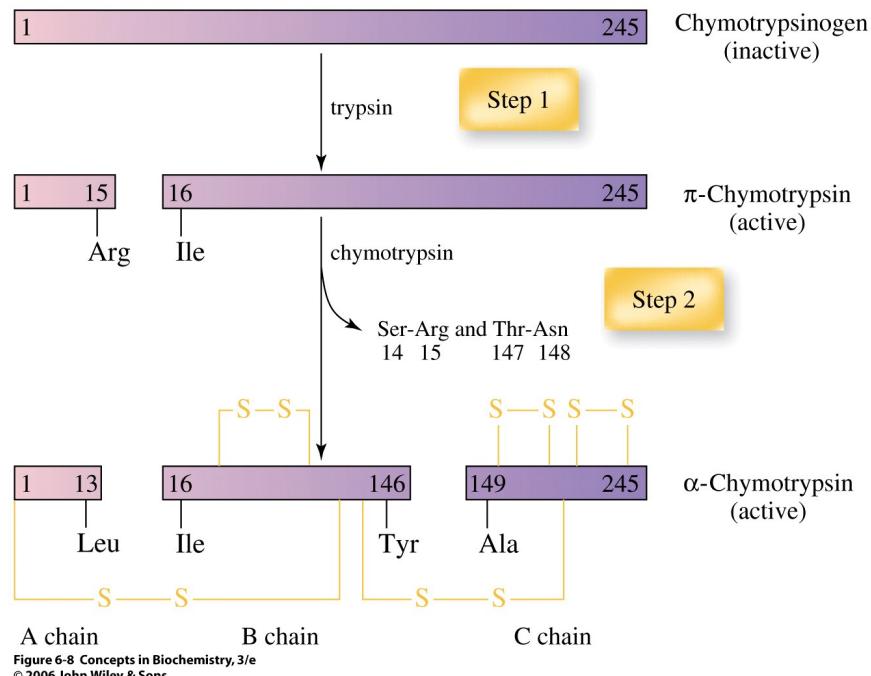
1. **Isoprenylation, acylation, palmitoylation** – addition of fatty acids and fatty acid derivatives
2. **Glycosylation** – addition of sugars to Asparagine

3. **Proteolytic cleavage**

- **ZYMOGENS** – inactive precursor to an enzyme; activated by cleavage of a specific peptide bond
- Why would this be useful? Let's look at examples:
  - Proteolytic enzymes **TRYPSIN** and **CHYMOTRYPSIN**
  - Initially synthesized as **trypsinogen** and **chymotrypsinogen** which are both inactive
  - Formed in the pancreas where they would do damage if active
  - In the small intestine, where their digestive properties are needed, they are **ACTIVATED** by cleavage of specific peptide bonds.

## ZYMOGENS ARE INACTIVE UNTIL REACH PROPER ENVIRONMENT!!

### CHYMOTRYPSINOGEN: INACTIVE PRECURSOR OF CHYMOTRYPSIN



**Table 6.3**  
**Digestive enzymes (peptidases) that exist as zymogens**

Active Enzyme	Zymogen Form	Site of Zymogen Synthesis
π- or α-Chymotrypsin	Chymotrypsinogen	Pancreas
Pepsin	Pepsinogen	Stomach
Trypsin	Trypsinogen	Pancreas
Carboxypeptidase	Procarboxypeptidase	Pancreas
Elastase	Proelastase	Pancreas

Table 6-3 Concepts in Biochemistry, 3/e  
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