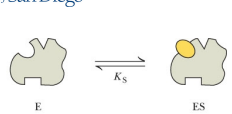
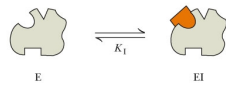
Enzyme Inhibitors

Molecules which bind and act to alter the reaction kinetics of an enzyme. By binding to enzyme reducing the affinity, velocity, catalytic turnover or all of the above. - examples include poisons and drugs

- Some inhibitors **reversibly** bind directly to active site competing with normal substrate (competitive inhibitor)
- Some inhibitors **reversibly** bind to the protein away from active site – altering structure of protein so the enzyme does not function (either won't bind or won't react substrate). Non and Un-competitive and allosteric…
- Other inhibitors bind covalently (**irreversibly**) to the active site, leaving the enzyme "dead". Uncompetitive – sometimes called suicide Inhibitor

Competitive Inhibitors Compete With Substrate for the Same Site on the Enzyme





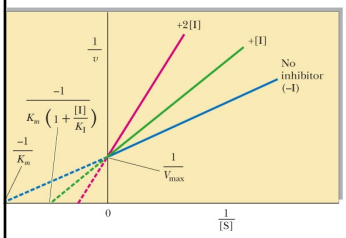
- Inhibitor is similar to substrate and both bind to or near active site. compete' for binding
- Inhibitor is unreactive - EI state
- Lineweaver Burke intersect at the Y axis

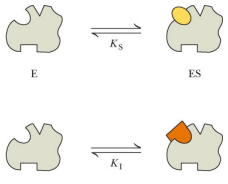
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$+ \quad I \quad \Big\| K_I$$

$$EI + S \longrightarrow \text{NO REACTION}$$

Competitive Inhibitors Compete With Substrate for the Same Site on the Enzyme

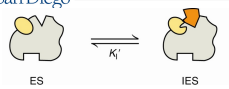




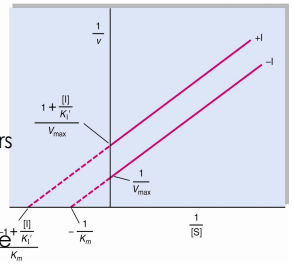
Lineweaver-Burk plot of competitive inhibition, showing lines for no I, [I], and 2[I].

Km is reduced but Vmax is unchanged

Uncompetitive Inhibition, where I combines only with ES, but not with Free E



- binds covalently in the transition state
- **Some are** suicide inhibitors
- binds to the ES complex
- lowers affinity and velocity
- lineweaver Burke plots are parallel



Note that both intercepts change but the slope (K_m/V_{max}) remains constant in the presence of I.

Uncompetitive Inhibition, where I combines only ES, but not with Free E

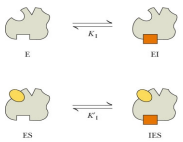
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$+ \quad I \quad \Big\| K_I'$$

$$ESI \longrightarrow \text{NO REACTION}$$

Note that both intercepts change but the slope (K_m/V_{max}) remains constant in the presence of I.

Pure Noncompetitive Inhibition – where S and I bind to different sites on the enzyme



- inhibitor binds distal to active site
- effects enzyme rate not affinity
- binds E in E S or E
- Reversible
- Lineweaver Burke intersect at the Y axis

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

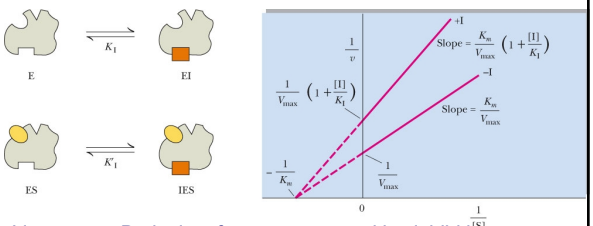
$$+ \quad I \quad \Big\| K_I \qquad + \quad I \quad \Big\| K_I'$$

$$EI \qquad\qquad ESI \longrightarrow \text{NO REACTION}$$

- Both E and ES bind to I
- Noncompet I bind to enzyme site that participates in both substrate binding AND catalysis

Also Known as Mixed

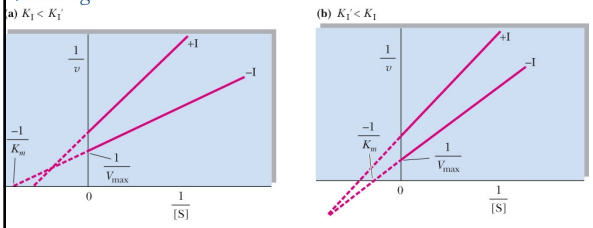
Pure Noncompetitive Inhibition – where S and I bind to different sites on the enzyme



Lineweaver-Burk plot of pure noncompetitive inhibition.

Note that I does not alter K_m but that it decreases V_{max}.

Mixed OR Noncompetitive Inhibition: binding of I by E influences binding of S by E

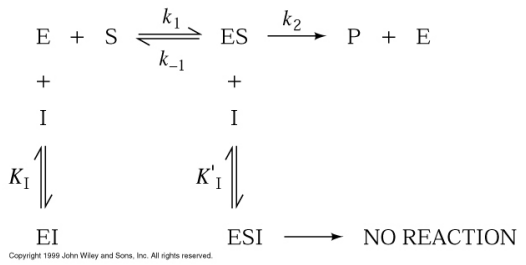


Inhibitor binds to enzyme site that involves both S binding and catalysis

Lineweaver-Burk plot of mixed noncompetitive inhibition.

Note that both intercepts and the slope change in the presence of I.

Mixed Inhibition



Kinetic Behavior of Bimolecular Reactions?

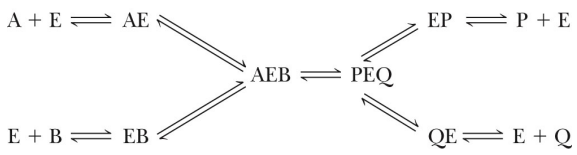
Enzymes often catalyze reactions involving two (or more) substrates

Bisubstrate reactions may be **sequential** or **single-displacement** reactions or **double-displacement** (**ping-pong**) reactions

Single-displacement reactions can be of two distinct classes:

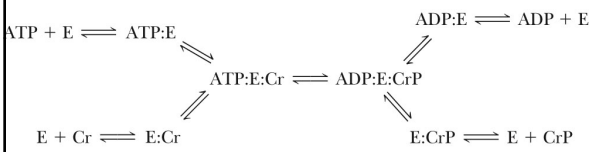
1. **Random**, where either substrate may bind first, followed by the other substrate
2. **Ordered**, where a **leading substrate** binds first, followed by the other substrate

Conversion of AEB to PEQ is the Rate-Limiting Step in Random, Single-Displacement Reactions



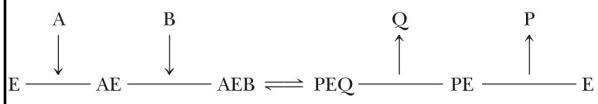
In this type of sequential reaction, all possible binary enzyme-substrate and enzyme-product complexes are formed rapidly and reversibly when enzyme is added to a reaction mixture containing A, B, P, and Q.

Creatine Kinase Acts by a Random, Single-Displacement Mechanism



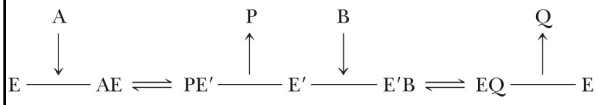
The overall direction of the reaction will be determined by the relative concentrations of ATP, ADP, Cr, and CrP and the equilibrium constant for the reaction.

In an Ordered, Single-Displacement Reaction, the Leading Substrate Must Bind First



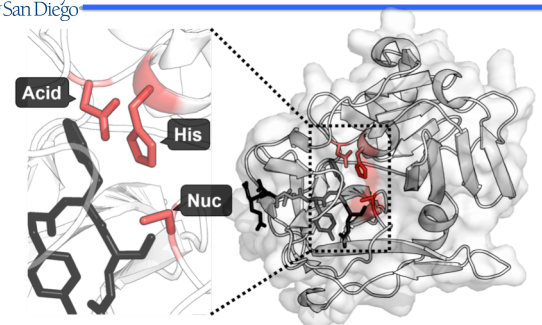
The leading substrate (A) binds first, followed by B. Reaction between A and B occurs in the ternary complex and is usually followed by an ordered release of the products, P and Q.

The Double Displacement (Ping-Pong) Reaction



Double-Displacement (Ping-Pong) reactions proceed via formation of a covalently modified enzyme intermediate. Reactions conforming to this kinetic pattern are characterized by the fact that the product of the enzyme's reaction with A (called P in the above scheme) is released prior to reaction of the enzyme with the second substrate, B.

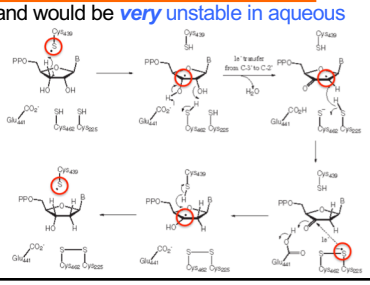
How do enzymes work?



It's the amino acids!

Stabilization of reactive intermediates

Enzyme-catalyzed reactions generate *reactive intermediates*, such as carbanions, carbocations, and radicals, that normally would require strong reaction conditions to generate in chemical reactions and would be ***very*** unstable in aqueous solution.



Its about transition state!

Enzymes stabilize the transition state and catalyze the reaction by providing appropriate functional groups of the protein or cofactor spatially and temporally appropriate manner.

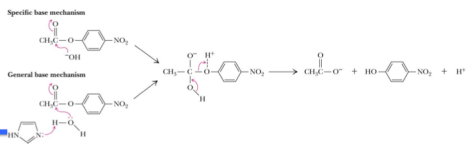
- There are five general mechanisms by which enzymes contribute to catalysis:

Acid/Base, covalent and metal ion catalysis.
Proximity and orientation (strain) effects and preferential binding to transition state complex

Acid / Base catalysis

Chemical groups are made more reactive by adding or removing a proton from substrate to reduce (stabilizing) transition state free energy.

- Result of Acid/Base catalysis is making a reactive group more reactive by increasing its intrinsic electrophilic or nucleophilic character
- This can increase the rate 10-100 fold
- Microenvironment shifts in pKa allow for several amino acids to be involved: Asp, Glu, His, Cys, Tyr, and Lys often involved



Metals can help too

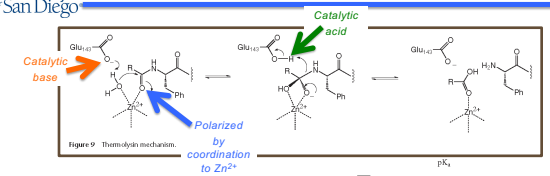


Figure 9 Thermolysin mechanism.

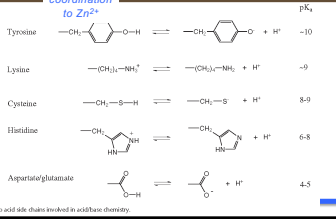
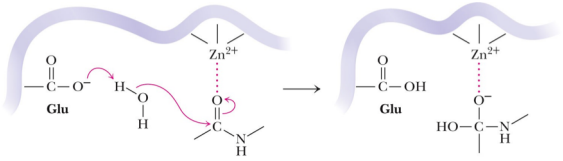


Figure 7 Amino acid side chains involved in acid/base chemistry.

Metal Ion Catalysis

metalloenzymes require metal ions for maintain the native structure/state of the enzyme

metal activated enzymes bind metals more weakly, potentially in the catalytic cycle



Initial step of thermolysin reaction

Metal Ion Catalysis

Transition metals as well as divalent cations (Mg^{+2} and Ca^{+2}) are useful for this type of catalysis. Metal ions act as a lewis acid and accept electrons. Therefore they are effective electrophiles. Another important reason for involving metals is the positive charge at any physiological pH.

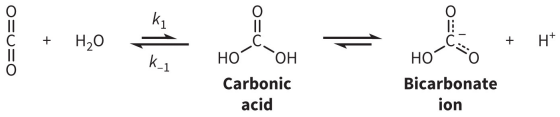
- Called Metalloenzymes
- Involved in redox reactions
- Metals such as zinc activate water - to "acidify" or polarize the water so the OH group can act as a nucleophile.
- Electrostatically stabilize or shield negative charges (ATP and Mg^{+2})
- Carbonic anhydrase is an example of this type of reaction

Anhydrase: An Example of Metal Ion Catalysis

Found in erythrocytes

Catalyzes the conversion of carbon dioxide to carbonic acid

Zn^{2+} is in the active site.

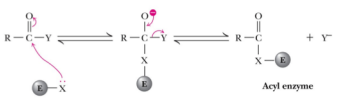


Carbonic anhydrase structure and reaction.

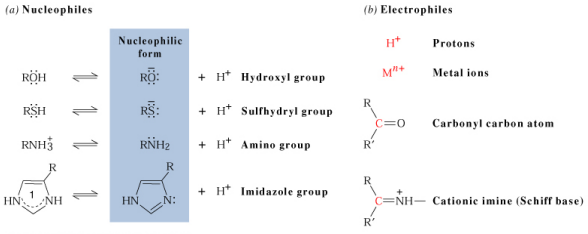
Covalent Catalysis

A covalent bond between substrate and enzyme is generated to lower transition state

Takes place with enzyme side chain nucleophiles with substrates that have electrophilic centers



Important nucleophiles of enzyme and electrophiles of substrates



Covalent Catalysis

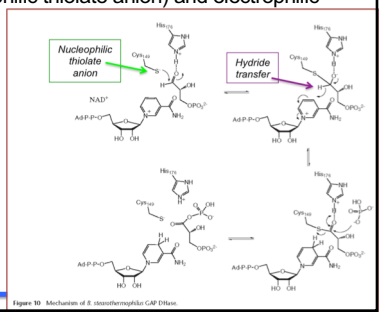
In some enzymes a nucleophilic side chain group forms an unstable covalent bond with the substrate. The enzyme-substrate complex them forms product. The pathway can require that the intermediate is more susceptible to nucleophilic attack by water than the original substrate.

Three stages of covalent catalysis:

- Formation of a bond between substrate and enzyme
- Removal of electrons to make a reactive center
- Elimination of the bond that was formed in step one

Covalent Catalysis

1. Bond formation between deprotonated Cys nucleophile (STRONG nucleophilic thiolate anion) and electrophilic substrate
2. Lewis base transfering H^+ & e- Intermediate
3. Elimination of (phosphate anion attack) bond forms new product



Lysozyme: An Example of Acid–Base Catalysis

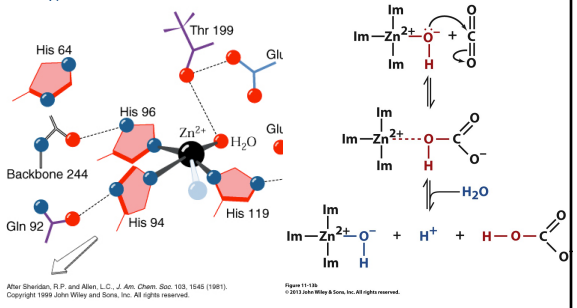
A natural antibiotic

An enzyme found in egg whites, tears, and mucus

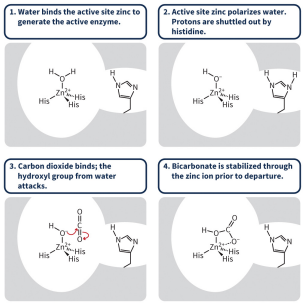
The active site contains two negatively charge residues

Glutamic acid is protonated, whereas aspartate is not

Role of Zn+2 in Carbonic Anhydrase



Mechanism of Anhydrase



Mechanism of carbonic anhydrase.

Chymotrypsin: A Covalent Catalysis

Serine protease

Cleaves dietary protein on the C-terminus of Tyr, Phe, and Trp

Secreted from the pancreas

Contains a catalytic triad containing Asp, His, and Ser

Inactive form = chymotrypsinogen