Enzyme Catalysis

10/08/2009

Regulation of Enzymatic Activity

There are two general ways to control enzymatic activity.

1. **Control the amount or availability of the enzyme.**
2. **Control or regulate the enzymes catalytic activity.**

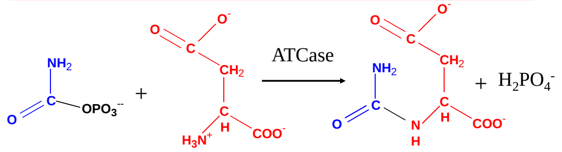
Each topic can be subdivided into many different categories.

Enzyme amounts in a cell depend upon the rate in which it is synthesized and the rate it is degraded. Synthesis rates can be transcriptionally or translationally controlled. Degradation rates of proteins are also controlled.

However, We will be focusing on the regulation of enzymatic activity.

Aspartate Transcarbamoylase:

the first step in pyrimidine biosynthesis.

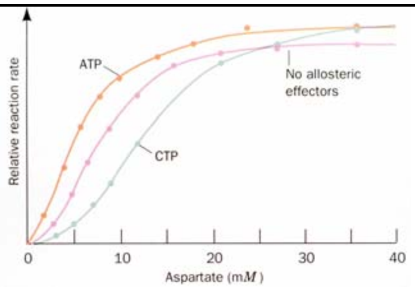


Carbamoyl phosphate

Aspartate

N-Carbamoyl aspartate

This enzyme is controlled by Allosteric regulation and Feedback inhibition

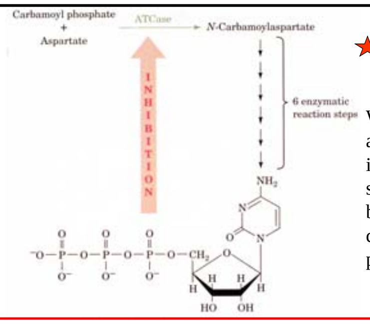


Notice the S shaped curve (pink) cooperative binding of aspartate

Positively homotropic cooperative binding

Hetertropically inhibited by CTP

Hetertropically activated by ATP



Feedback inhibition

Where the product of a metabolic pathway inhibits is own synthesis at the beginning or first committed step in the pathway

CTP is the product of this pathway and it is also a precursor for the synthesis of DNA and RNA (nucleic acids). The rapid synthesis of DNA and/or RNA depletes the CTP pool in the cell, causing CTP to be released from ATCase and increasing its activity. When the activity of ATCase is greater than the need for CTP, CTP concentrations rise rapidly and rebinds to the enzyme to inhibit the activity. ATP activates ATCase. Purines and Pyrimidines are needed in equal amounts. When ATP concentrations are greater than CTP, ATP binds to ATCase activating the enzyme until the levels of ATP and CTP are about the same.

Enzymatic catalysis and mechanisms

- **A. Acid - Base catalysis**
- **B. Covalent catalysis**
- **C. Metal ion aided catalysis**
- **D. Electrostatic interactions**
- **E. Orientation and Proximity effects**
- **F. Transition state binding**

General Acid Base

Rate increase by partial proton abstraction by a Bronsted base

or

Rate increase by partial proton donation by a Bronsted Acid

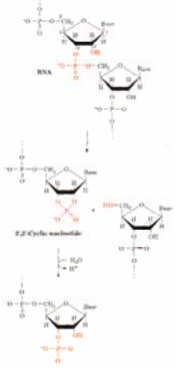
Many biochemical reactions require acid base catalysis

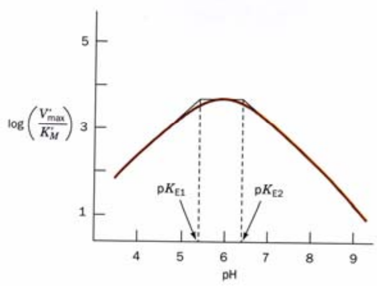
- Hydrolysis of peptides
- Reactions with Phosphate groups
- Tautomerizations
- Additions to carboxyl groups

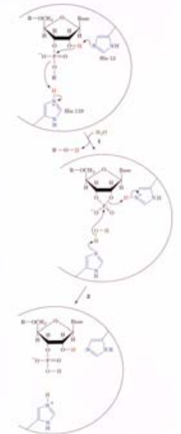
Asp, Glu, Cys, Tyr, His, and Lys have pK's near physiological pH and can assist in general acid-base catalysis.

Enzymes arrange several catalytic groups about the substrate to make a concerted catalysis a common mechanism.

RNase uses a acid base mechanism







Two histidine residues catalyze the reaction. Residue His 12 is deprotonated and acts as a general base by abstracting a proton from the 2' OH.

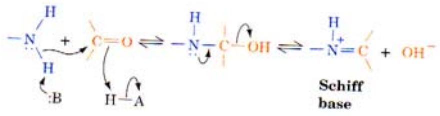
His 119 is protonated and acts as a general acid catalysis by donating a proton to the phosphate group.

The second step of the catalysis His 12 reprotonates the 2'OH and His 119 reacts with water to abstract a proton and the resulting OH⁻ is added to the phosphate.

This mechanism results in the hydrolysis of the RNA phosphate linkage.

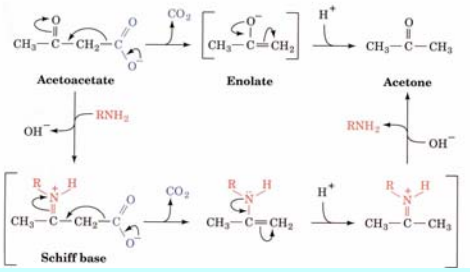
Covalent catalysis

Covalent catalysis involves the formation of a transient covalent bond between the catalyst and the substrate



Nucleophiles donate electrons - Lewis bases.

Electrophiles accept electrons - Lewis acids.



Catalysis has both an nucleophilic and an electrophilic stage

1 Nucleophilic reaction forms the covalent bond

2 Withdrawal of electrons by the now electrophilic catalyst

3 Elimination of the catalyst (almost the reverse of step 1)

Metal ion catalysts

One-third of all known enzymes needs metal ions to work!!

1. Metalloenzymes: contain tightly bound metal ions: I.e. Fe^{++}, Fe^{+++}, Cu^{++}, Zn^{++}, Mn^{++}, or Co^{++}.

2. Metal-activated enzymes- loosely bind ions Na^{+}, K^{+}, Mg^{++}, or Ca^{++}.

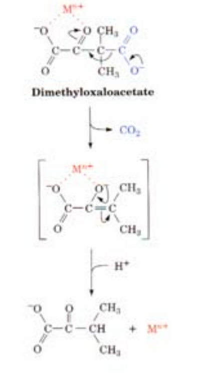
They participate in one of three ways:

a. They bind substrates to orient then for catalysis

b. Through redox reactions gain or loss of electrons.

c. electrostatic stabilization or negative charge shielding

Charge stabilization by metal ions



Metal ions are effective catalysts because unlike protons the can be present at higher concentrations at neutral pH and have charges greater than 1.

Metal ions can ionize water at higher concentrations

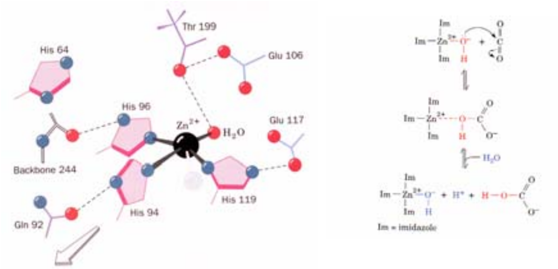
The charge on a metal ion makes a bound water more acidic than free H_2O and is a source of HO^- ions even below pH 7.0

$$(NH_3)_5Co^{3+}(H_2O) \Leftrightarrow (NH_3)_5Co^{3+}(OH^-) + H^+$$

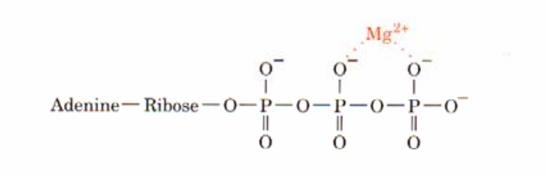
The resultant metal bound OH- is a potent nucleophile

Carbonic Anhydrase

$$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+$$

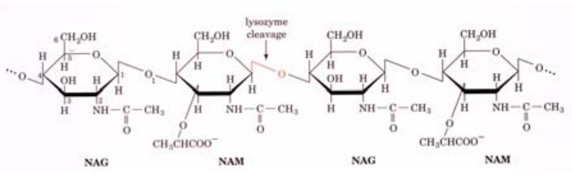


Charge shielding



Mechanism of lysozyme

Lysozyme digests bacterial cell walls by breaking β(1- 4) glycosidic bonds between (N- acetylmuramic acid (NAM) and N-acetylglucosamine (NAG)



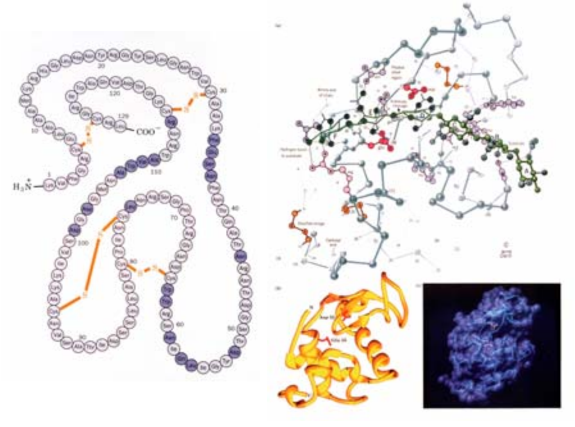
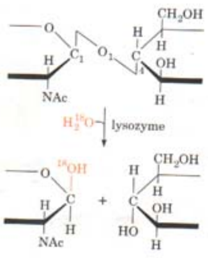


TABLE 14-2. RATES OF HEW LYSOZYME-CATALYZED HYDROLYSIS OF SELECTED OLIGOSACCHARIDE SUBSTRATE ANALOGS

Compound	$k_{cat}(s^{-1})$
$(NAG)_2$	2.5×10^{-8}
$(NAG)_3$	8.3×10^{-6}
$(NAG)_4$	6.6×10^{-5}
$(NAG)_5$	0.033
$(NAG)_6$	0.25
$(NAG-NAM)_3$	0.5

Source: Imoto, T., Johnson, L.N., North, A.C.T., Phillips, D.C., and Rupley, J.A., *in* Boyer, P.D. (Ed.), *The Enzymes* (3rd ed.), Vol. 7, *p.* 842, Academic Press (1972).



···–NAG–NAM–NAG–NAM–NAG–NAM–···→(reducing end)
A B C D E F

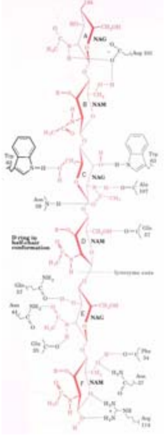
Lysozyme Phillips mechanism

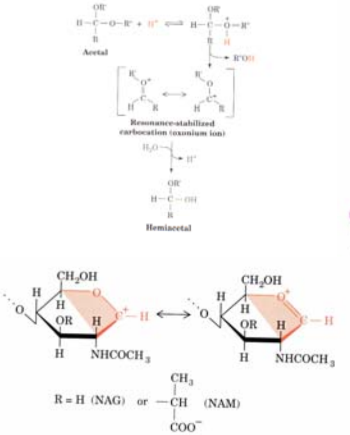
1. Binds a hexasaccharide unit on a bacterial cell wall, distorting sugar D to a half-chair configuration.

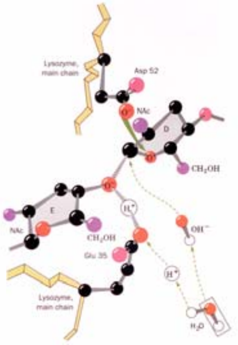
2. Glu 35 transfers its proton to the O1 of the D ring (general acid catalysis) C1-O1 bond is cleaved generating an oxonium ion at C1.

3. Asp 52 stabilizes the oxonium ion through charge-charge interactions. Reaction via a S_N2 mechanism with transient formation of a C--O bond to the enzyme.

4. E ring group is released from the enzyme yielding a glycosyl-enzyme intermediate which adds water to reverse the chemistry and reprotonate Glu 35.







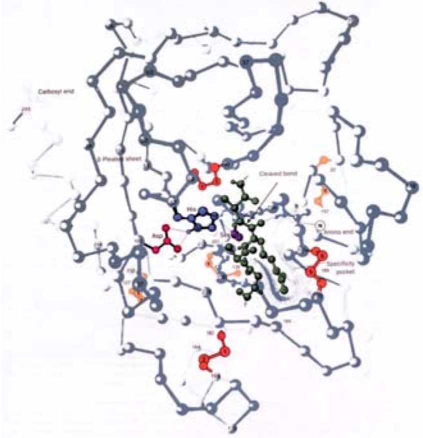
Serine proteases

- Diverse and widespread proteolytic enzymes
- Involved in digestion, development, clotting, inflammation…
- Common catalytic mechanism

TABLE 14-4. A SELECTION OF SERINE PROTEASES

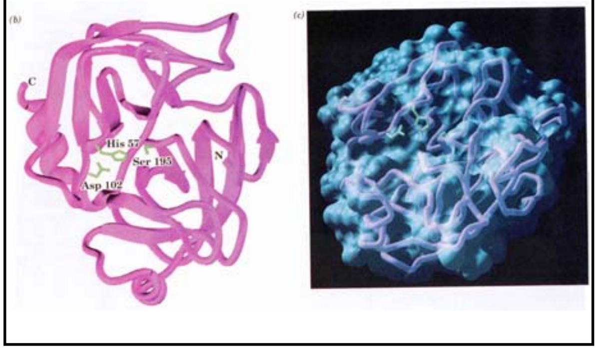
Enzyme	Source	Function
Trypsin	Pancreas	Digestion of proteins
Chymotrypsin	Pancreas	Digestion of proteins
Elastase	Pancreas	Digestion of proteins
Thrombin	Vertebrate serum	Blood clotting
Plasmin	Vertebrate serum	Dissolution of blood clots
Kallikrein	Blood and tissues	Control of blood flow
Complement C1	Serum	Cell lysis in the immune response
Acrosomal protease	Sperm acrosome	Penetration of ovum
Lysosomal protease	Animal cells	Cell protein turnover
Cocoonase	Moth larvae	Dissolution of cocoon after metamorphosis
α-Lytic protease	*Bacillus sorangium*	Possibly digestion
Proteases A and B	*Streptomyces griseus*	Possibly digestion
Subtilisin	*Bacillus subtilus*	Possibly digestion

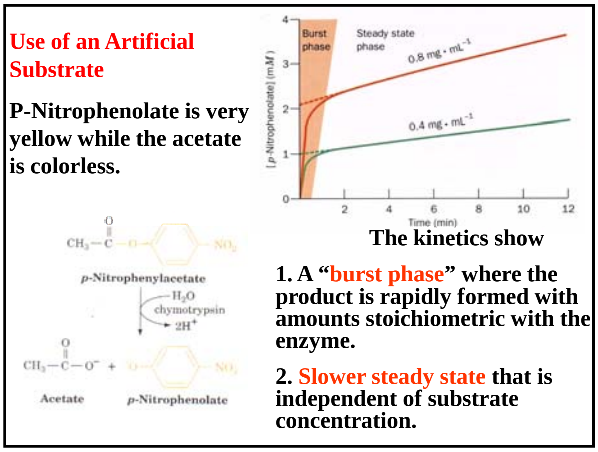
Source: Stroud, R.M., *Sci. Am.* 231(1): 86 (1974).



Bovine Trypsin

Bovine trypsin





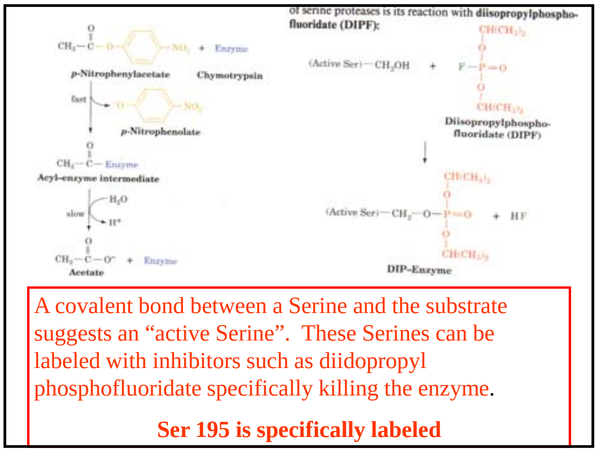
Use of an Artificial Substrate

P-Nitrophenolate is very yellow while the acetate is colorless.

The kinetics show

1. A “burst phase” where the product is rapidly formed with amounts stoichiometric with the enzyme.

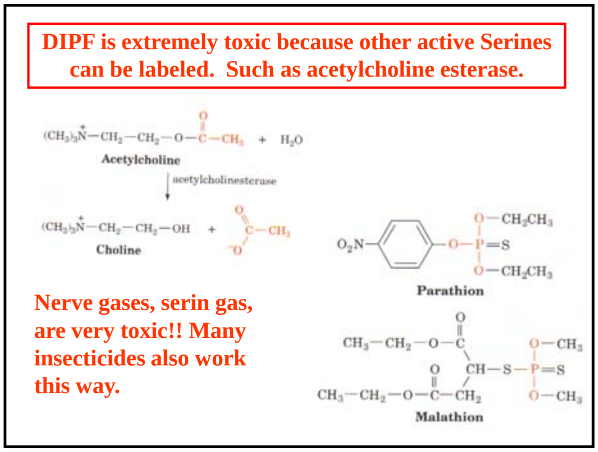
2. Slower steady state that is independent of substrate concentration.



of serine proteases is its reaction with **diisopropylphosphofluoridate (DIPF):**

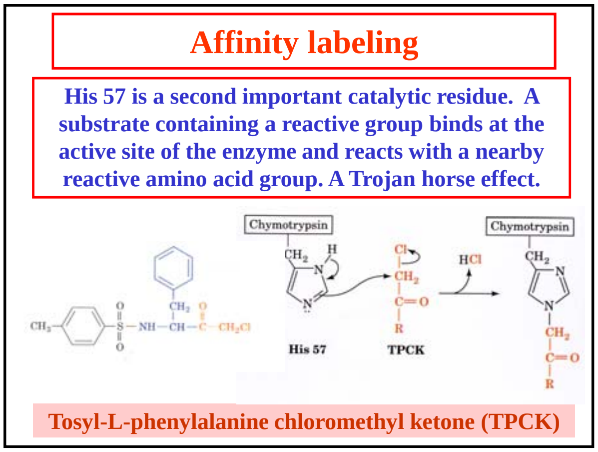
A covalent bond between a Serine and the substrate suggests an “active Serine”. These Serines can be labeled with inhibitors such as diidopropyl phosphofluoridate specifically killing the enzyme.

Ser 195 is specifically labeled



DIPF is extremely toxic because other active Serines can be labeled. Such as acetylcholine esterase.

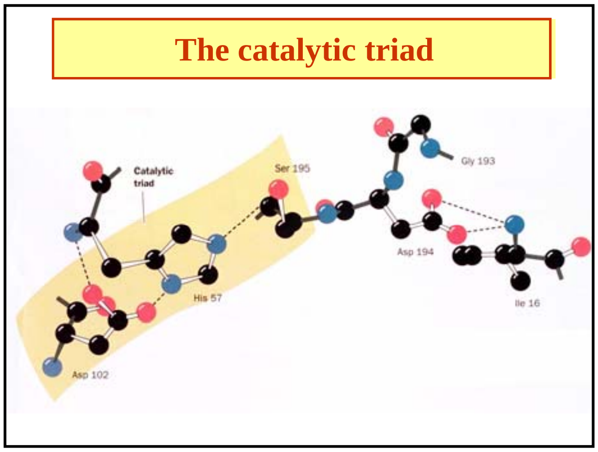
Nerve gases, serin gas, are very toxic!! Many insecticides also work this way.



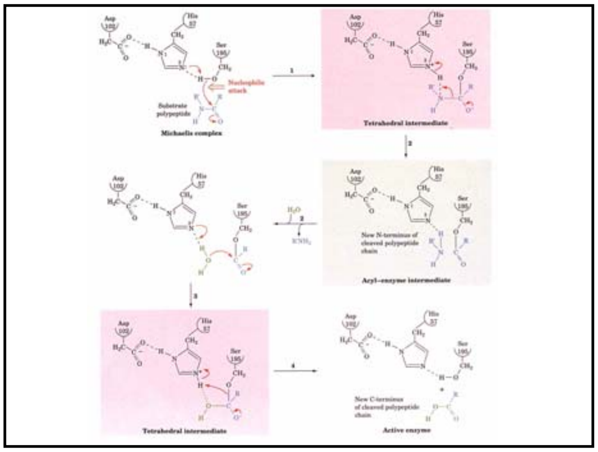
Affinity labeling

His 57 is a second important catalytic residue. A substrate containing a reactive group binds at the active site of the enzyme and reacts with a nearby reactive amino acid group. A Trojan horse effect.

Tosyl-L-phenylalanine chloromethyl ketone (TPCK)



The catalytic triad

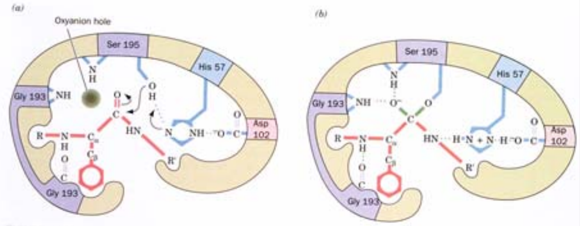


Catalytic mechanism

1. After the substrate binds Ser 195 nucleophilically attacks the scissile peptide bond to form a transition state complex called the tetrahedral intermediate (covalent catalysis) the imidazole His 52 takes up the proton Asp 102 is hydrogen bonded to His 57. Without Asp 102 the rate of catalysis is only 0.05% of wild-type.

2. Tetrahedral intermediate decomposes to the acyl-enzyme intermediate. His 57 acts as an acid donating a proton.

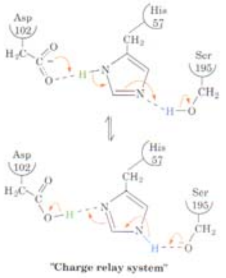
3. The enzyme is deacylated by the reverse of step 1 with water the attacking nucleophile and Ser 195 as the leaving group.



1. Conformational distortion forms the tetrahedral intermediate and causes the carboxyl to move close to the oxyanion hole

2. Now it forms two hydrogen bonds with the enzyme that cannot form when the carbonyl is in its normal conformation.

3. Distortion caused by the enzyme binding allows the hydrogen bonds to be maximal.



Triad charge transfer complex stabilization

Lecture 15
Tuesday 10/13/09
Enzyme Kinetics