

The Behavior of Proteins: Enzymes, Mechanisms, and Control

Chapter Seven

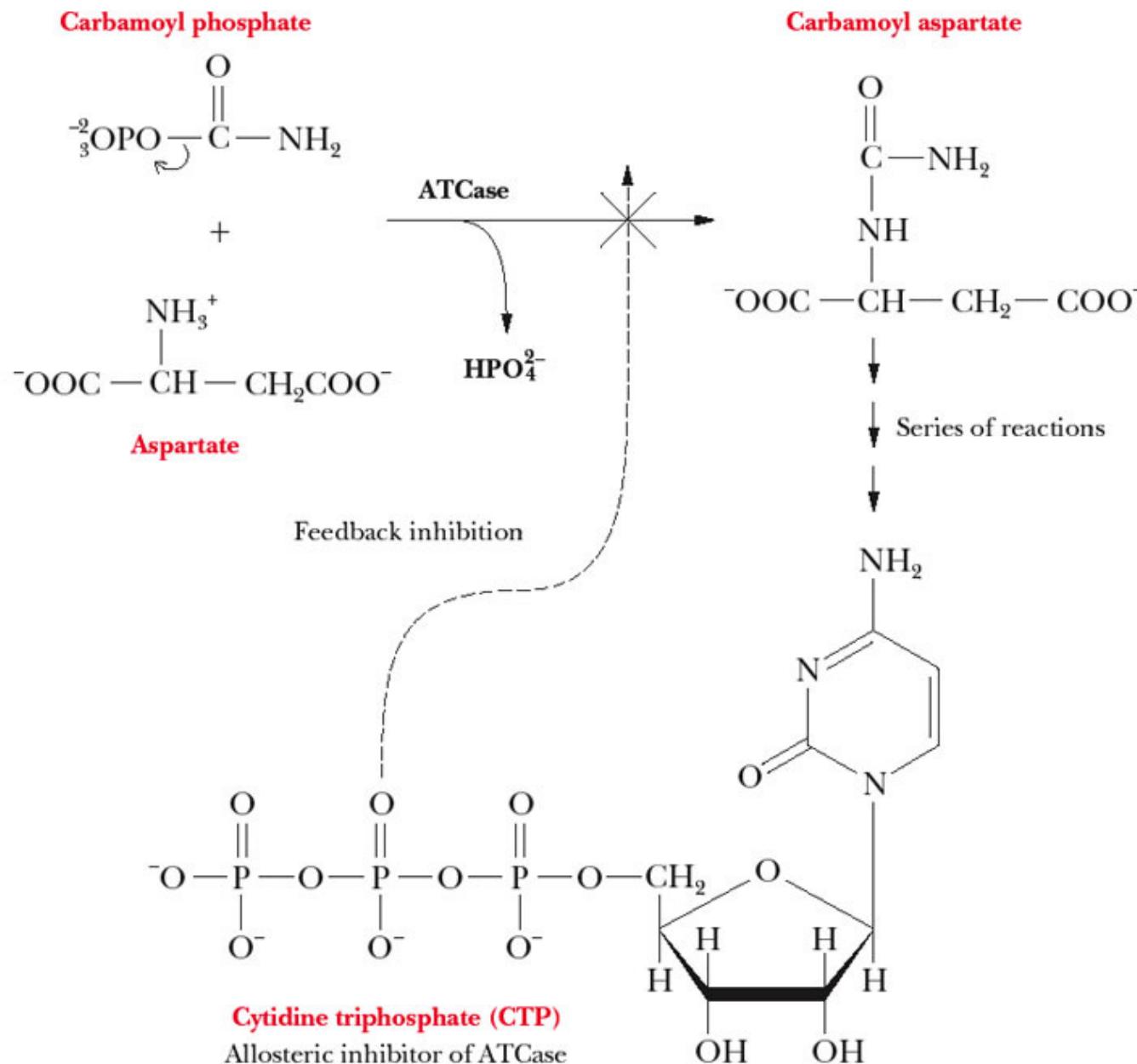
Allosteric Enzymes

- **Allosteric proteins:** have quaternary structure arrangement which results from noncovalent interaction among subunits.
 - Hb and ATCase are examples of allosteric proteins where they exhibits cooperative effect
 - Positive cooperative refers to the fact that binding of low level of substrate facilitate the action of the protein at higher level of substrate

Allosteric Enzymes

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 - Hb and ATCase are examples of allosteric proteins where they exhibits cooperative effect
 - Positive cooperative refers to the fact that binding of low level of substrate facilitate the action of the protein at higher level of substrate
- **Allosteric effector:** a substance that modifies the behavior of an allosteric enzyme as well as modifies the **quaternary structure**
 - may be an
 - Substrate
 - allosteric inhibitor
 - allosteric activator

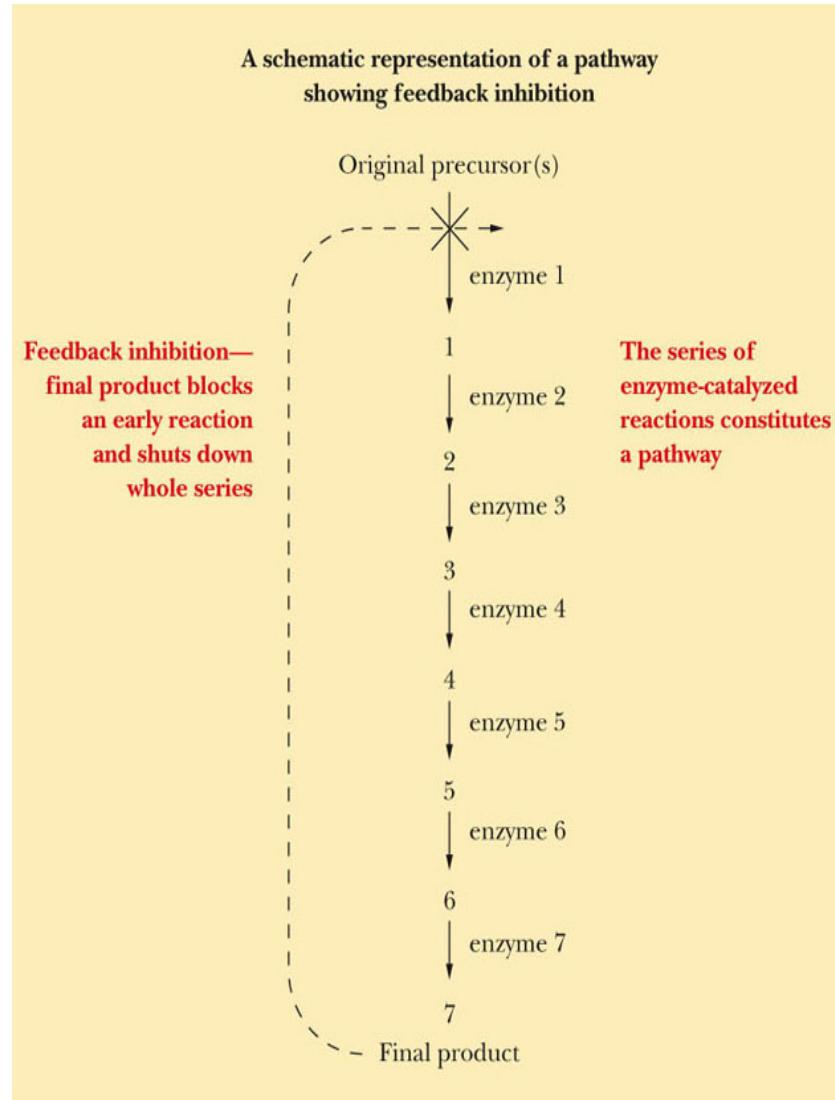
The reaction catalyzed by ATCase
leads eventually to the production of CTP

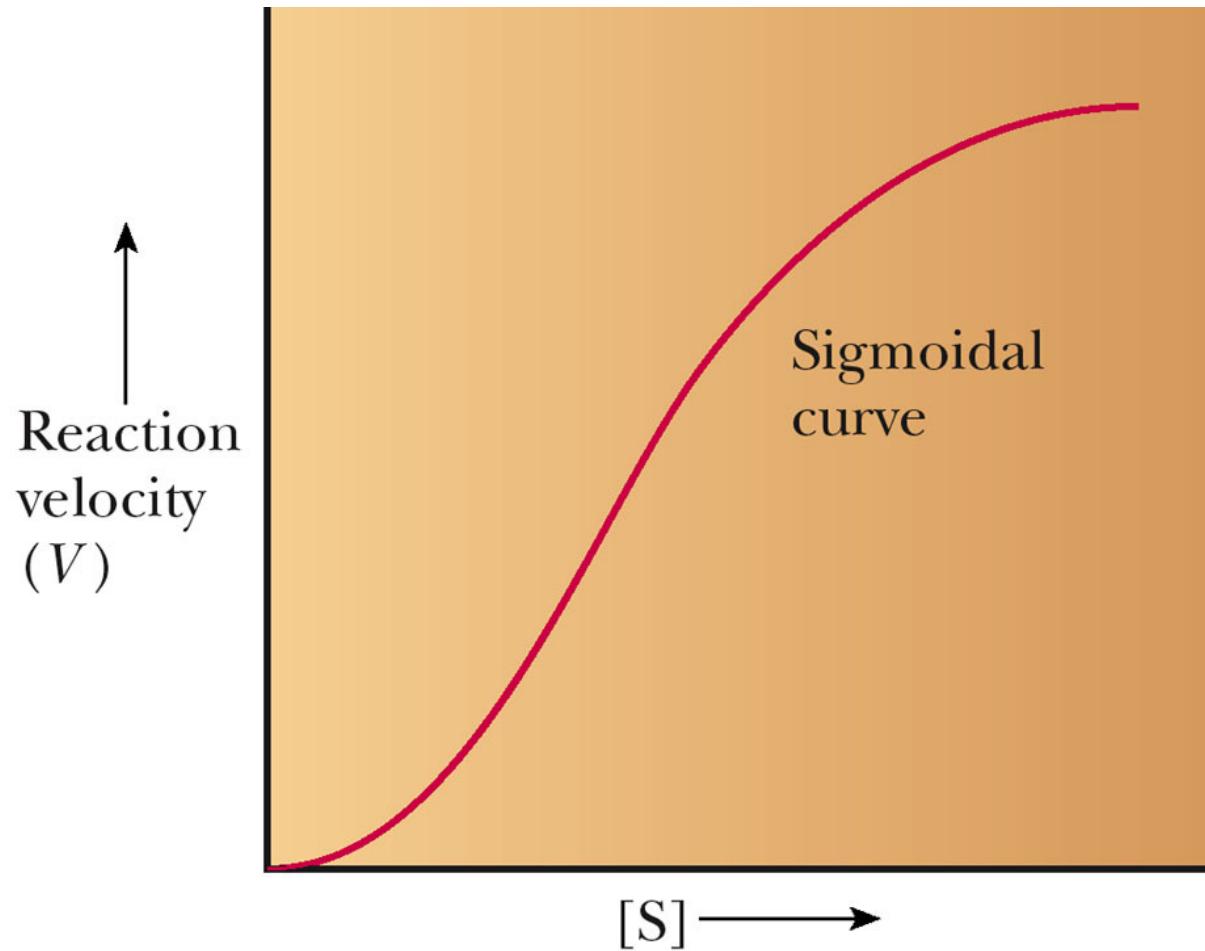


Control of allosteric enzymes

Feedback inhibition (end – product inhibition)

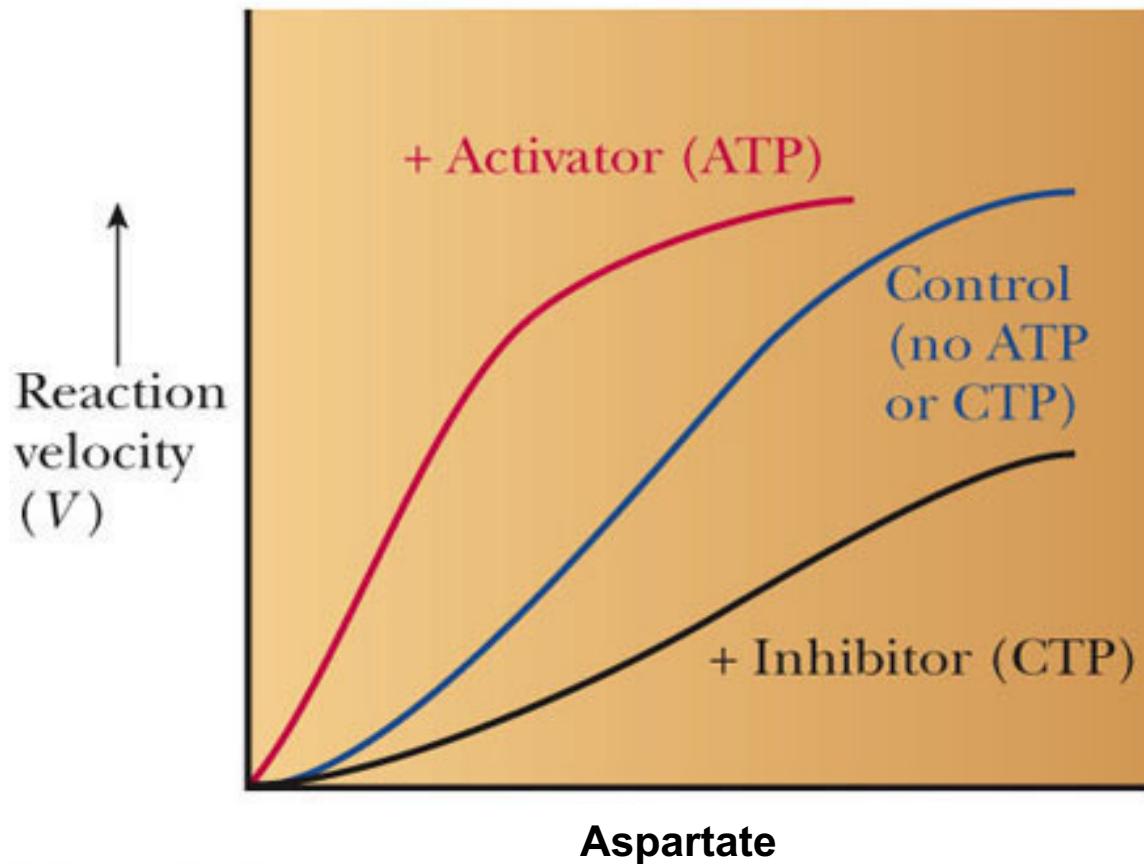
- Aspartate transcarbamoylase (ATCase) feedback inhibition
- The end product in the sequence of rxn inhibits the first step in the series
- This is an efficient system because the entire series of reactions can be shut down when excess of final product exists and preventing accumulation of intermediates.





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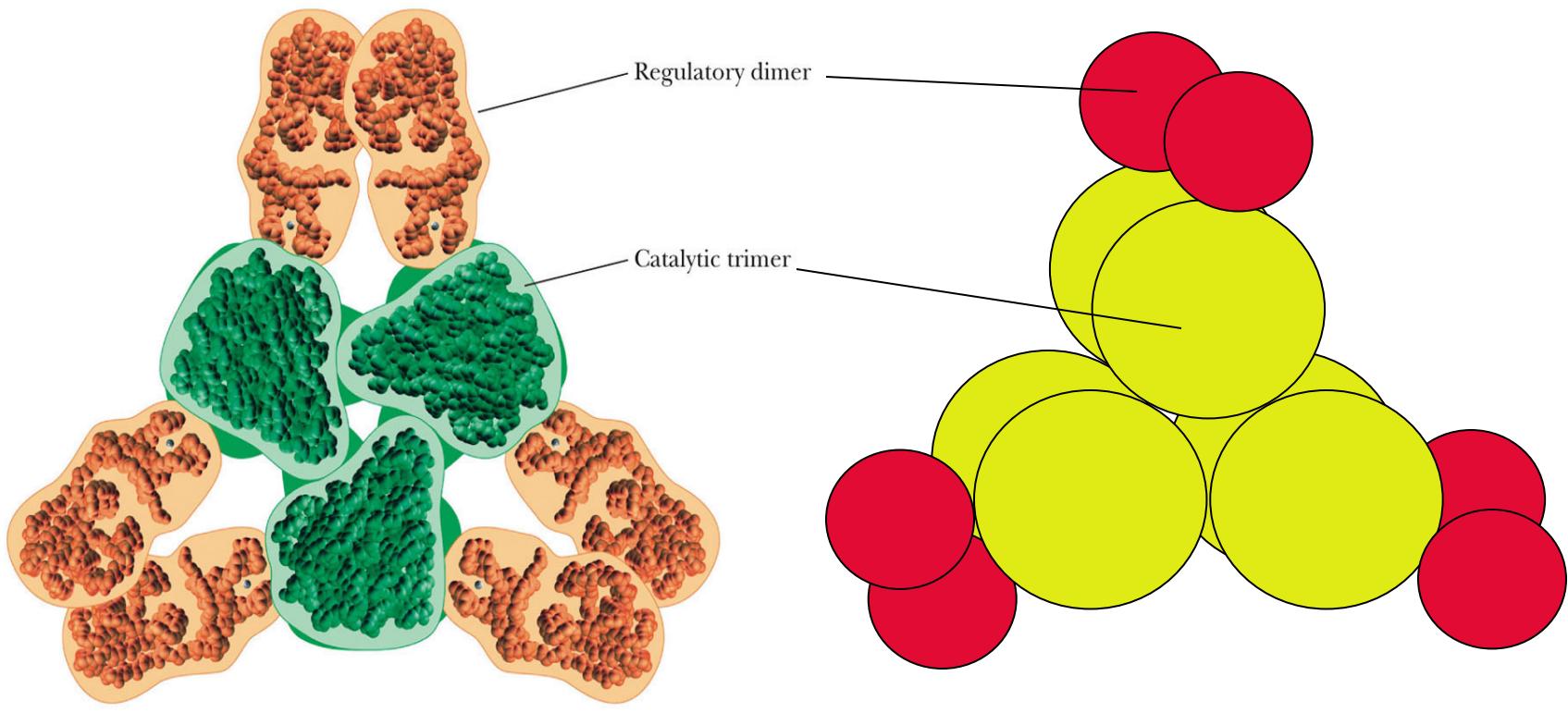
Rate of ATCase catalysis give Sigmoid shape which describes allosteric behavior (cooperative)



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ATCase catalysis in presence of CTP (inhibitor) and ATP (activator) (both have similar structure)

ATCase: composed of 2 different types of subunits



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- Catalytic subunits can be separated from regulatory subunits by a compound that reacts with cysteines in the protein (**p-hydroxymercuribenzoate**)
- ATCase still catalyze the rxn but loses its allosteric control

Allosteric Enzymes

- Two types of allosteric enzyme systems exist
- K system: an enzyme for which an inhibitor or activator alters $K_{0.5}$
- Substrate concentration to reach V_{max} change ATCase is an example
- V system: an enzyme for which an inhibitor or activator alters V_{max} but not $K_{0.5}$

Allosteric Effectors

- **Allosteric effector:** a substance (substrate, inhibitor, or activator) that modifies the quaternary structure and thus the behavior of an Allosteric protein
- **Homotropic effects:** allosteric interactions that occur when several identical molecules are bound to the protein;
 - Binding of aspartate to ATCase

Allosteric Effectors

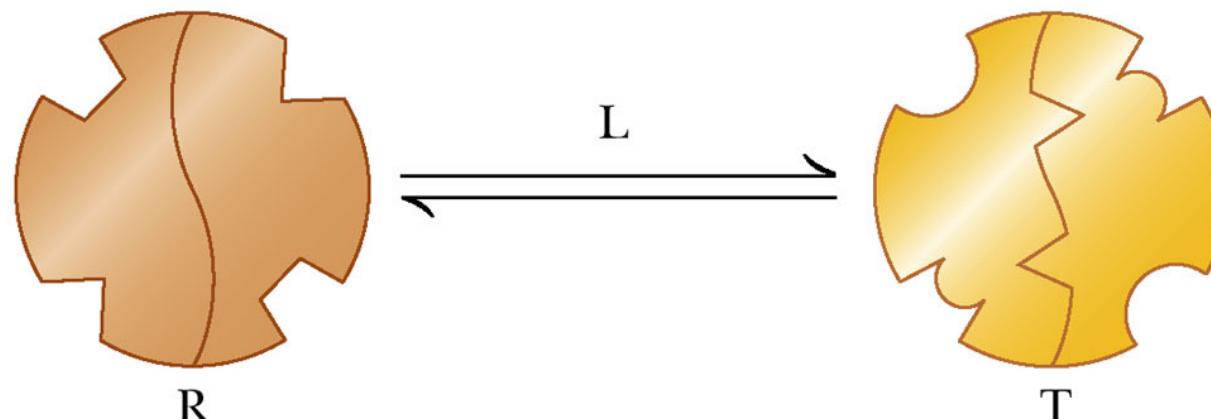
- **Allosteric effector:** a substance (substrate, inhibitor, or activator) that modifies the quaternary structure and thus the behavior of an Allosteric protein
- **Homotropic effects:** allosteric interactions that occur when several identical molecules are bound to the protein;
 - Binding of aspartate to ATCase
- **Heterotropic effects:** allosteric interactions that occur when different substances are bound to the protein (such as inhibitor and substrate)
 - ATCase inhibition by CTP and activation by ATP are heterotropic effectors
 - Positive heterotropic effectors or allosteric activators
 - Negative heterotropic effectors or allosteric inhibitors

There are two models to describe allosteric behavior

- **The concerted model:** The enzyme has two conformations
- **R conformation (relaxed)** binds substrate tightly; the **active** form
- **T conformation (tight)** binds substrate less tightly; **inactive** form

There are two models to describe allosteric behavior

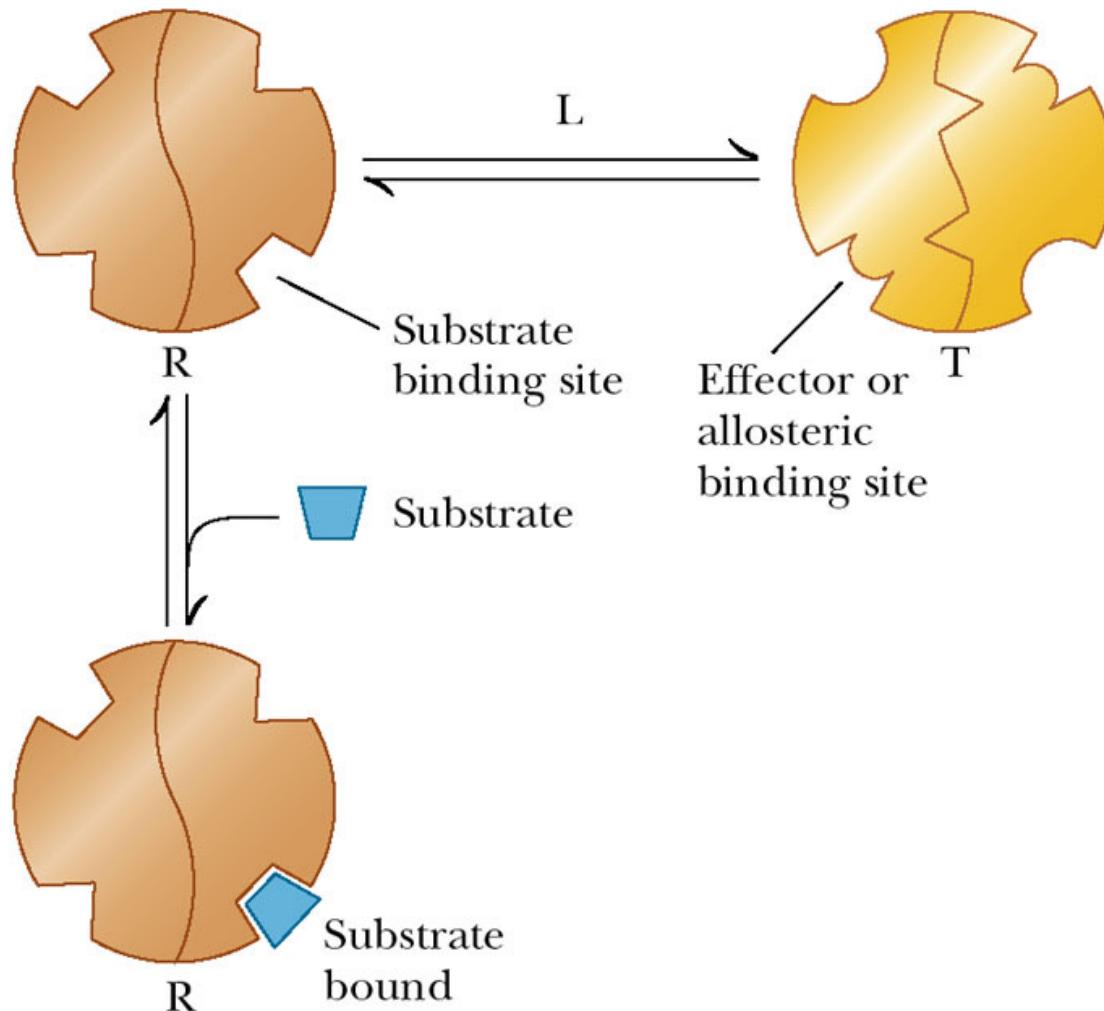
- **The concerted model:** The enzyme has two conformations
 - **R conformation (relaxed)** binds substrate tightly; the **active** form
 - **T conformation (tight)** binds substrate less tightly; **inactive** form
- (a) A dimeric protein can exist in either of two conformational states at equilibrium.



$$L = \frac{T}{R} \quad L \text{ is large. } (T \gg R)$$

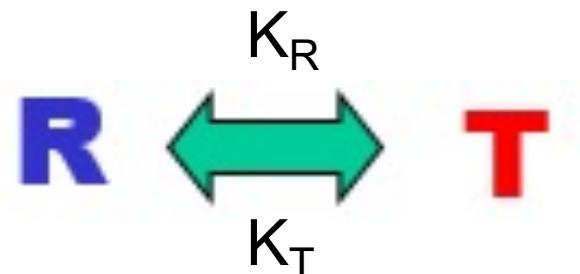
Concerted Model

(b) Substrate binding shifts equilibrium in favor of R.



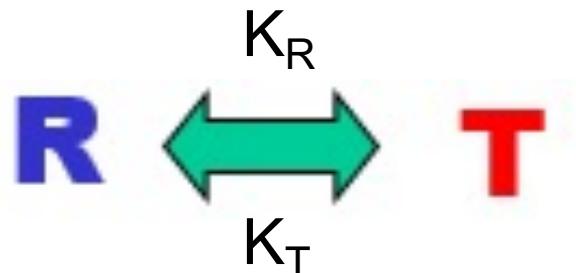
The Concerted Model

- The dissociation constant for enzyme –substrate complex is K_R for the relaxed form K_T for the tight form



The Concerted Model

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$$L = \frac{[T]}{[R]}$$

$$K_R = \frac{[E_R][S]}{[E_RS]} \quad K_T = \frac{[E_T][S]}{[E_TS]}$$

The Concerted Model

- The dissociation constant for enzyme –substrate complex is K_R for the relaxed form K_T for the tight form
- The affinity for substrate is higher in R than T ($K_R \lll K_T$)
- The ratio of $K_R/K_T = c$
- If the K_T is infinity greater than K_R then $c = 0$
- (Substrate will not bind to the T form at all time)

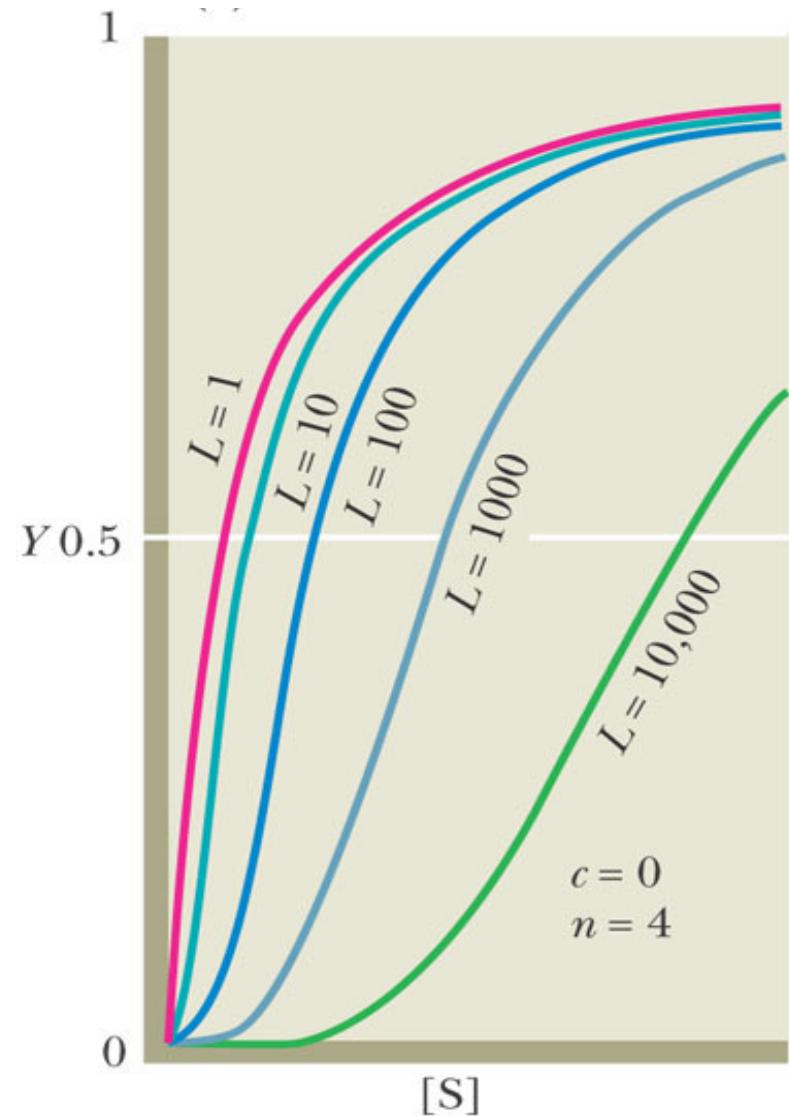
Ratio of dissociation constants

- The shape of the curve is dependent on the value of L and c

$$L = \frac{[T]}{[R]}$$

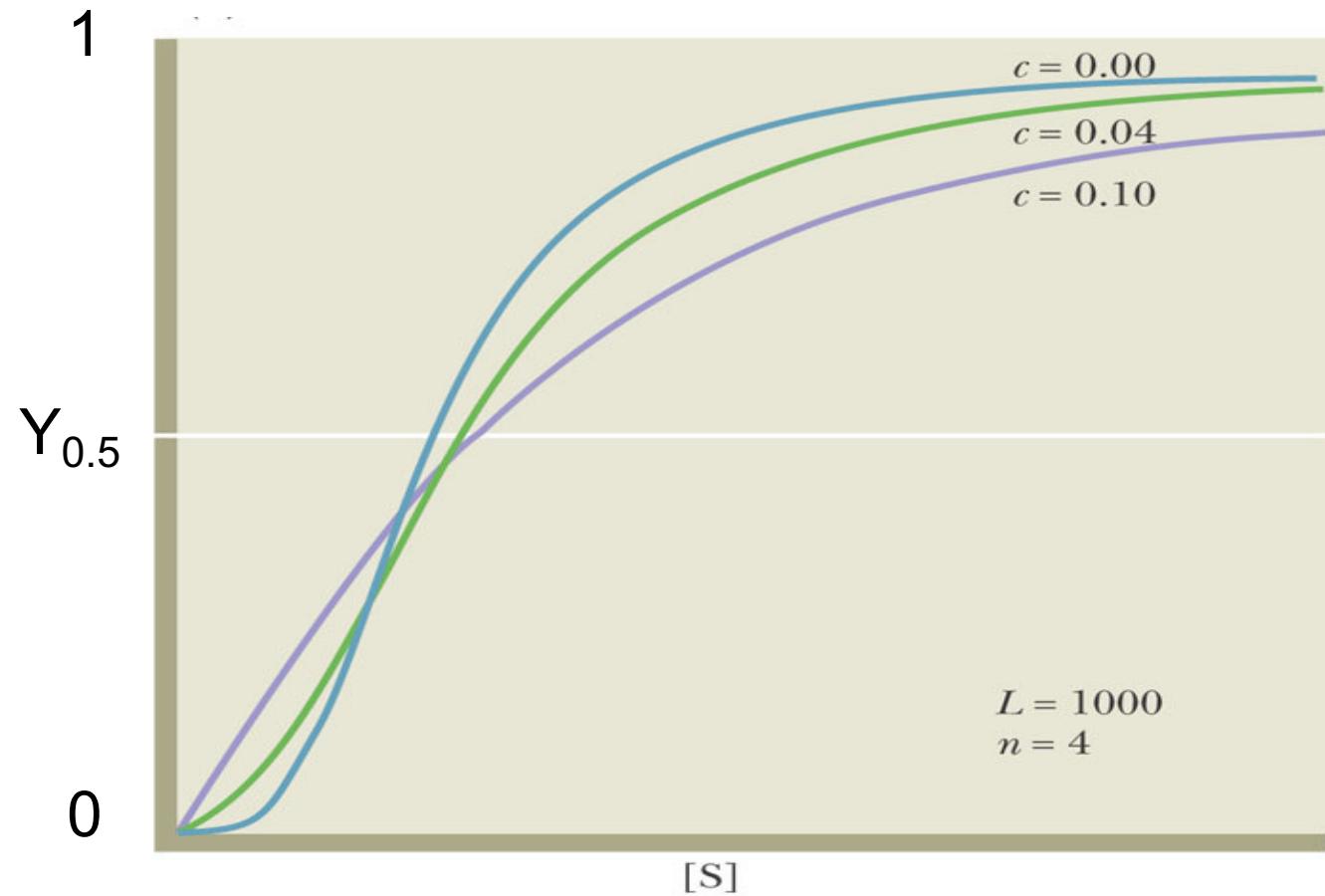
- As L increases (T/R) the shape become more sigmoidal

(T form is highly favored)



Ratio of dissociation constants

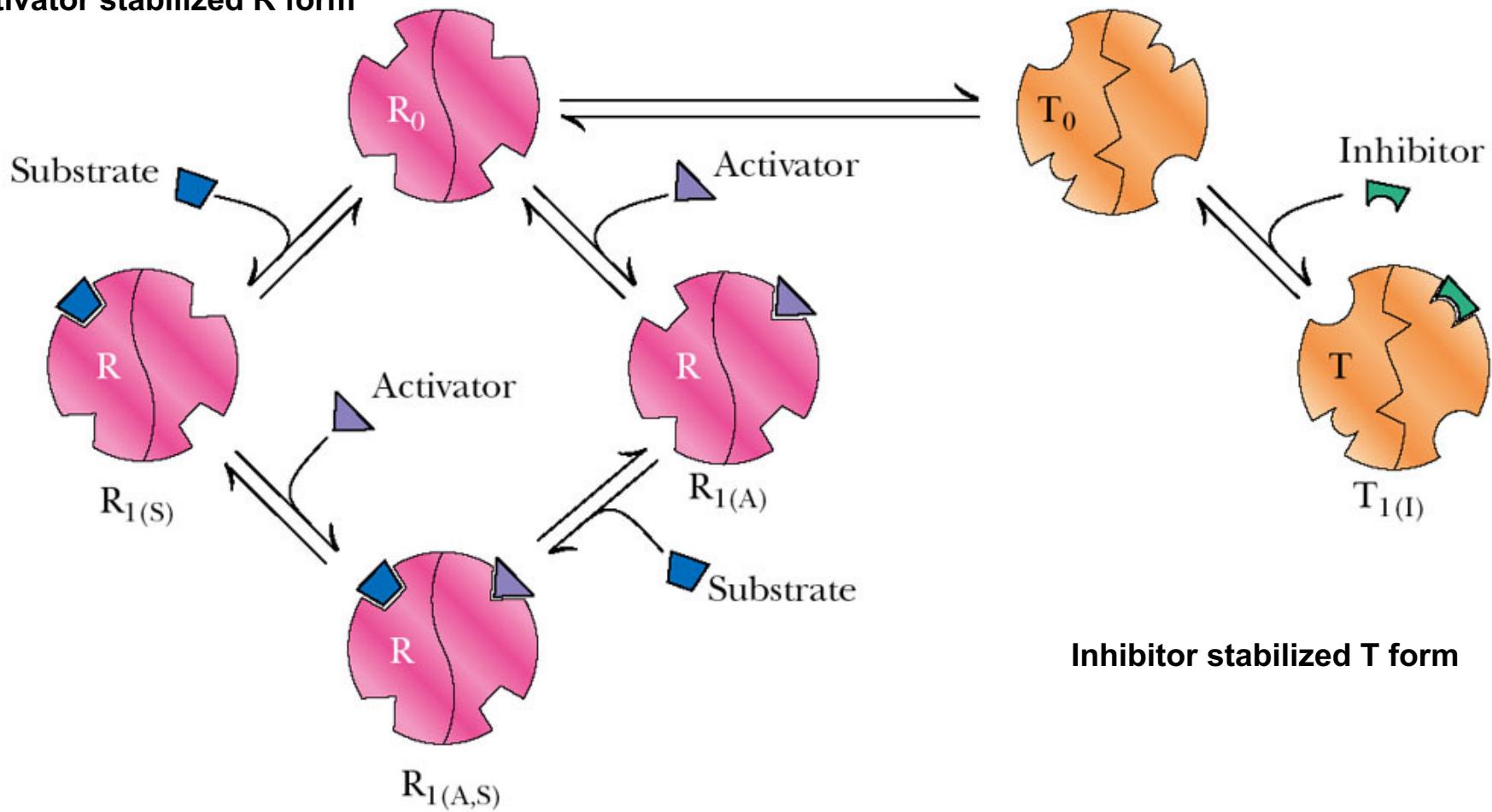
$$C = \frac{K_R}{K_T}$$



- As C decreases (K_R/K_T) the shape becomes sigmoidal
- (higher affinity between substrate and R form as compared to T form)

Inhibitors and activators can shift equilibrium between T and R forms

Activator stabilized R form

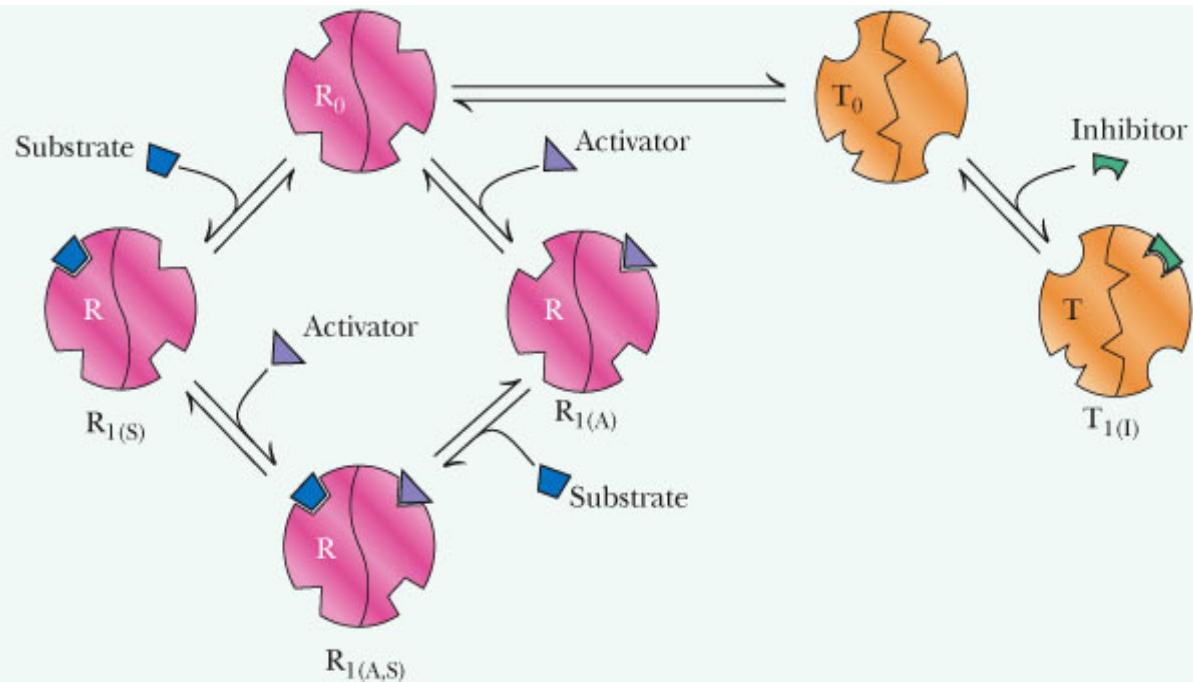
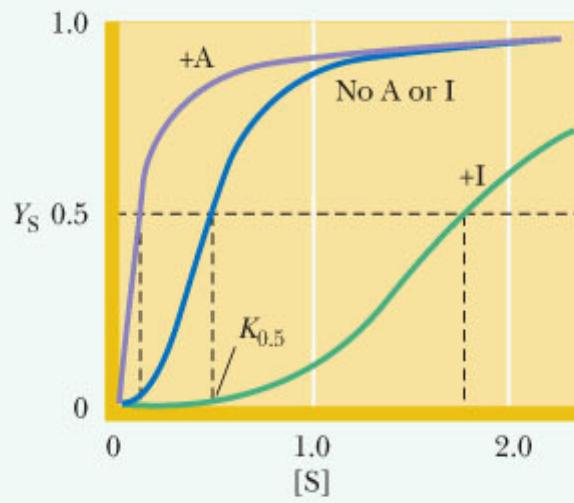


Inhibitor stabilized T form

Inhibitors and activators can shift equilibrium between T and R forms

A dimeric protein that can exist in either of two states: R_0 or T_0 . This protein can bind three ligands:

- 1) Substrate (S) : A positive homotropic effector that binds only to R at site S
- 2) Activator (A) : A positive heterotropic effector that binds only to R at site F
- 3) Inhibitor (I) : A negative heterotropic effector that binds only to T at site F



Effects of A:

$A + R_0 \rightarrow R_{1(A)}$
 Increase in number of R-conformers shifts $R_0 \rightleftharpoons T_0$ so that $T_0 \rightarrow R_0$

- (1) More binding sites for S made available.
- (2) Decrease in cooperativity of substrate saturation curve. Effector A lowers the apparent value of L .

Effects of I:

$I + T_0 \rightarrow T_{1(I)}$
 Increase in number of T-conformers (decrease in R_0 as $R_0 \rightarrow T_0$ to restore equilibrium)

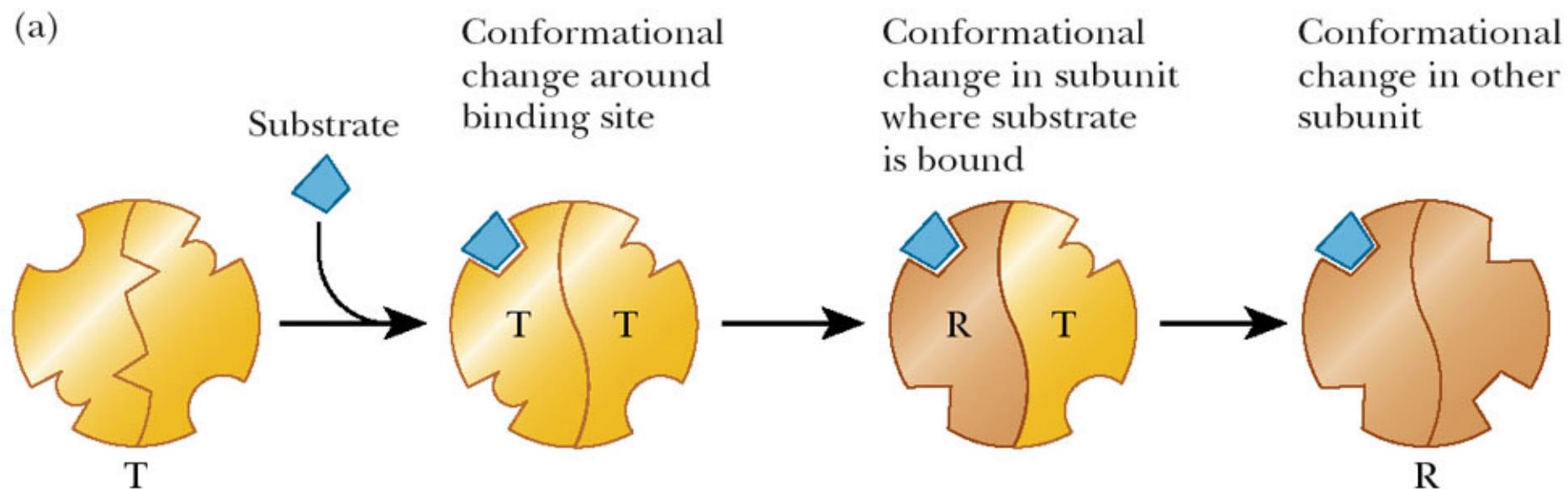
Thus, I inhibits association of S and A with R by lowering R_0 level. I increases cooperativity of substrate saturation curve. I raises the apparent value of L .

Sequential Model

- The binding of substrate induces a conformational change from the **T** form to the **R** form by induced fit mechanism (induced-fit model of substrate binding)
- **A conformational change from T to R in one subunit makes the same conformational change easier in another subunit (cooperative binding).**
- Binding of activator and inhibitor also take place by induced fit mechanism
- R form is favored when the activator is present
- T form is favored when the inhibitor is present

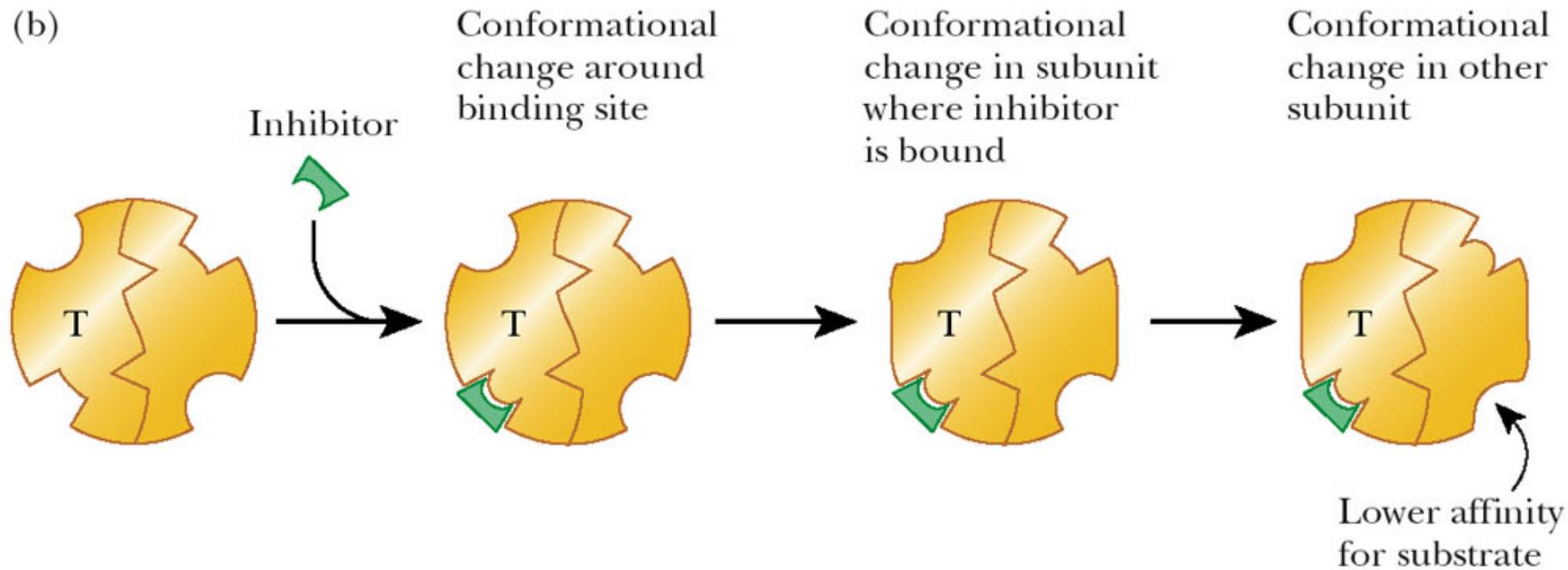
Sequential Model

(a)



Sequential Model

(b)



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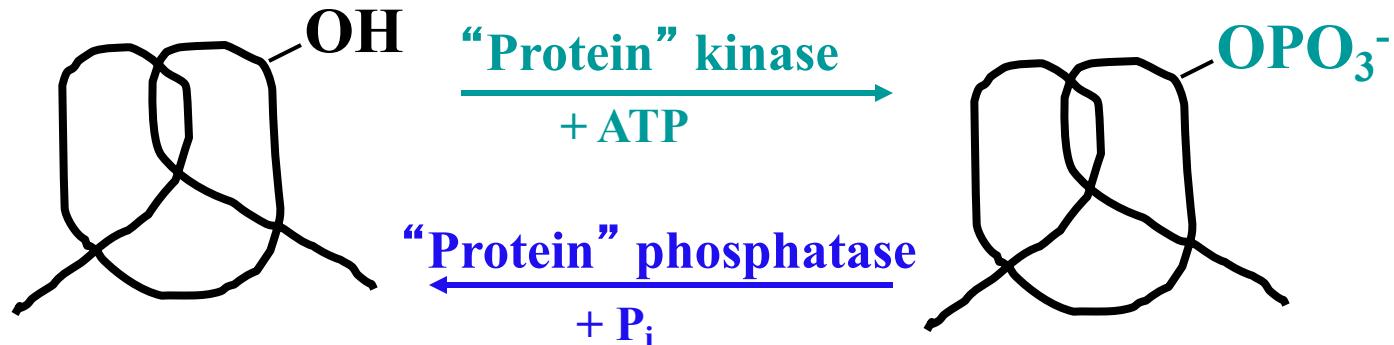
- Binding of inhibitor causes a conformational change that passes from one subunit to another making them more likely to bind inhibitor and less likely to bind substrate (cooperative effect)

Covalent Modifications

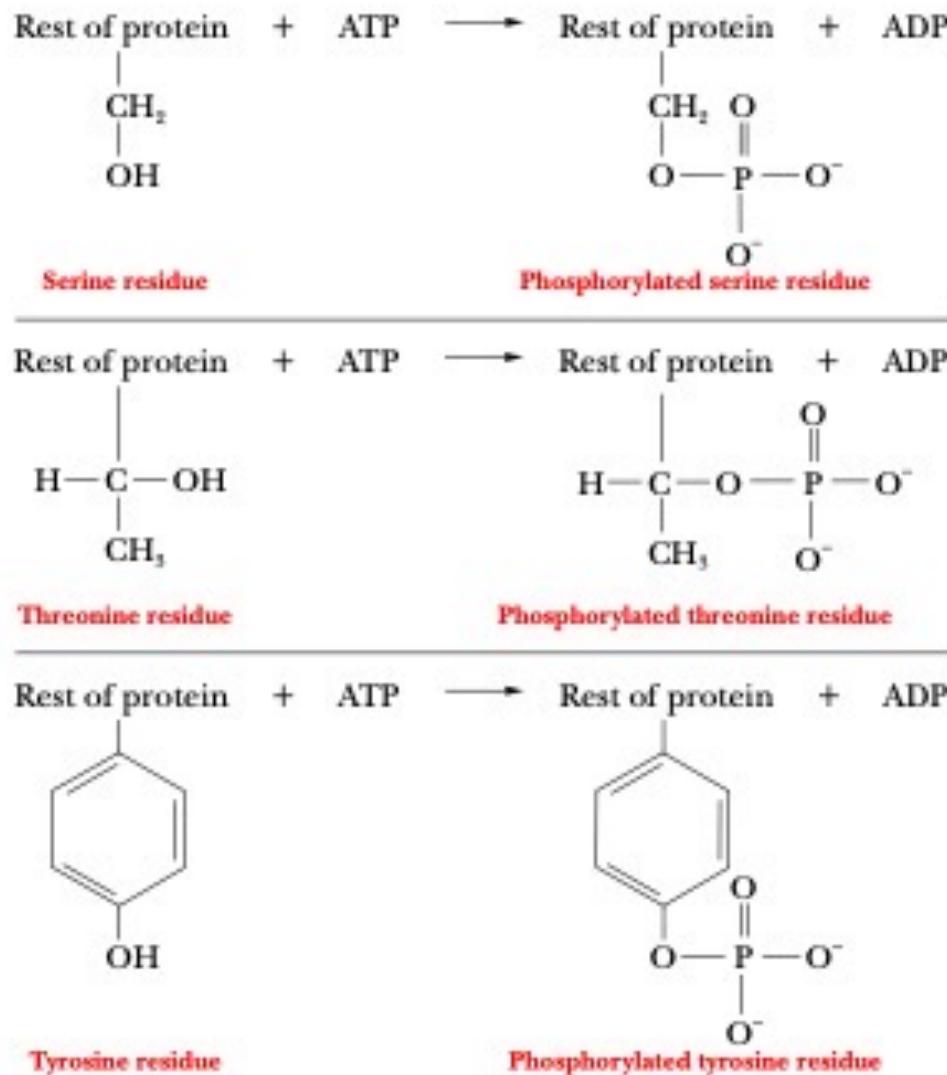
- There are many enzymes in cells can modify other enzymes:
- Phosphorylation catalyzed by protein kinases, (PK) or dephosphorylation catalyzed by phosphoprotein phosphatase, (PP) of various amino acid side chains (e.g., **serine**, **threonine**, **tyrosine**, and **histidine**).
- Proteolytic cleavage (by proteases), e.g. activation of zymogens

Phosphorylation

- Enzyme has active & inactive forms
 - Transformation due to “phosphorylation” of side Chain
 - Protein Kinase catalyzes Phosphoryl transfer (ATP donor)
 - Phosphatase reverses
- Phosphorylation = ? Activation
- Dephosphorylation = ? inhibition

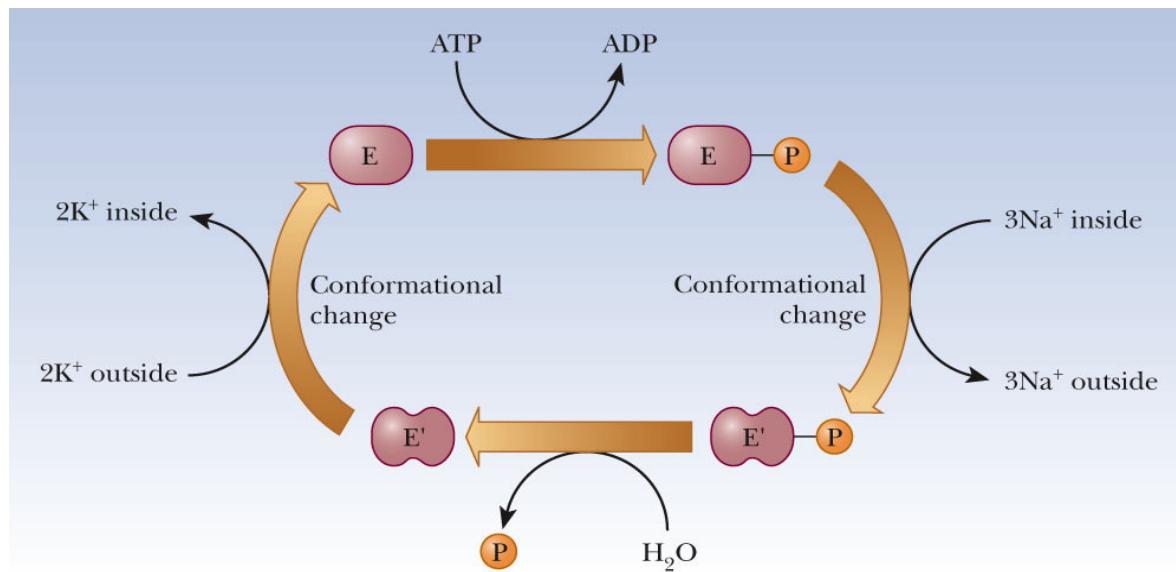


Control of Enzyme Activity via Phosphorylation



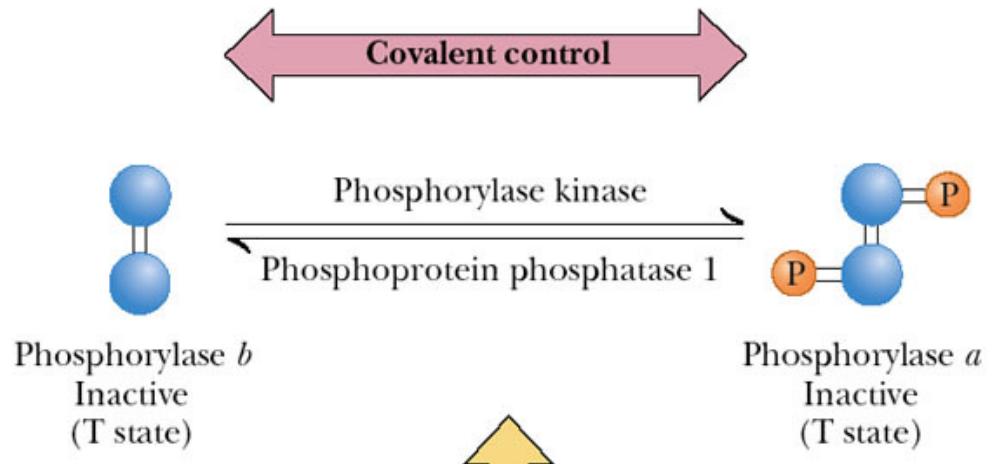
Membrane Transport

- Na/K pump is activated by phosphorylation
- Source of phosphate is ATP
- When ATP is hydrolyzed, energy released that drives other energetically unfavorable reactions to take place
- Phosphate is donated from ATP to aspartate 369 residue and causing a conformational change in the enzyme



- Covalent modifications

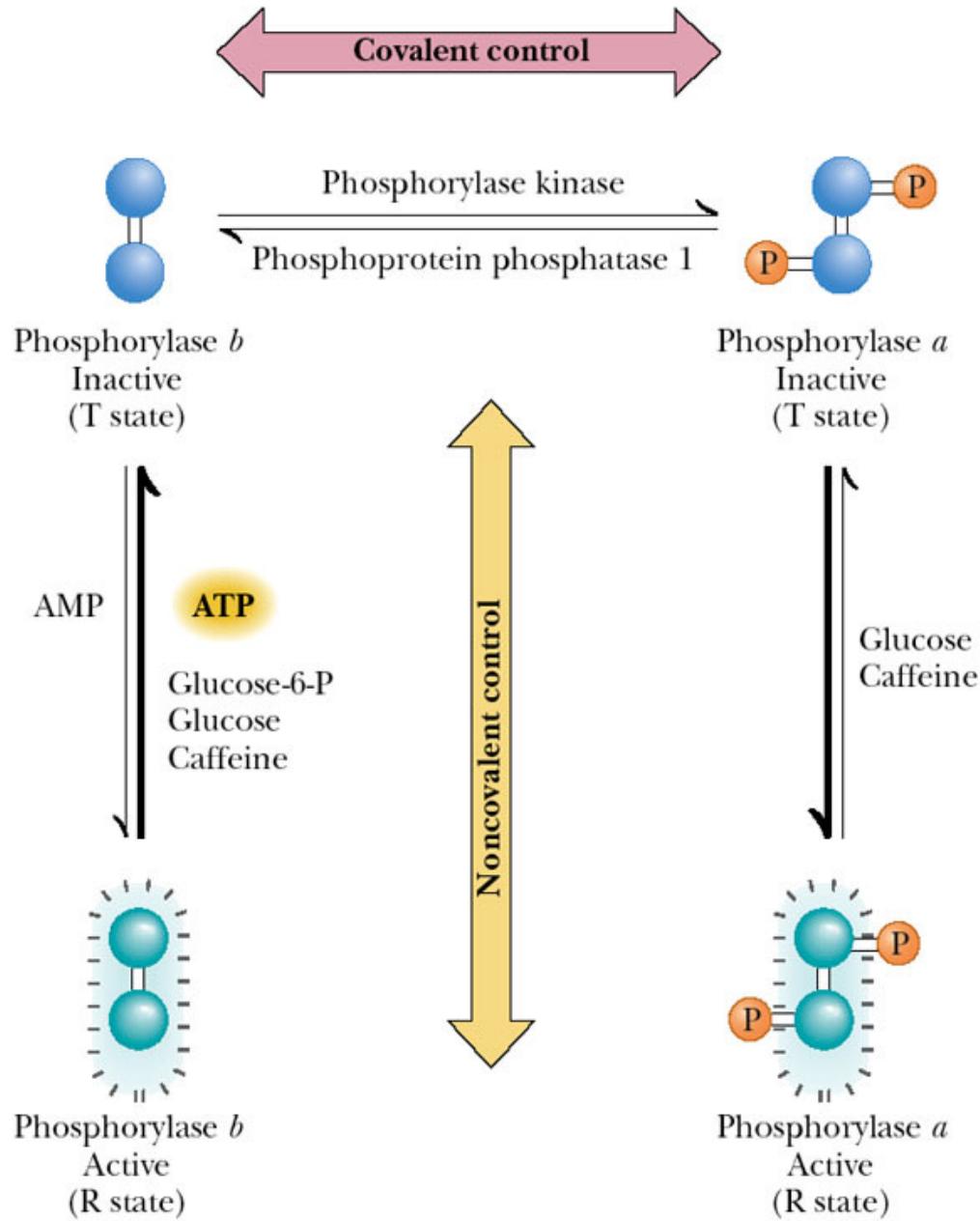
- Phosphorylase a has 2 subunits each with specific serine residue that is phosphorylated at its hydroxyl group.



- Covalent modifications

- Phosphorylase a has 2 subunits each with specific serine residue that is phosphorylated at its hydroxyl group.

- Allosteric modification



Zymogens

- **Zymogens:** Inactive enzyme precursor can be irreversibly transformed into an active enzyme by cleaving of covalent bonds.
- **Chymotrypsinogen and trypsinogen are examples of zymogens**
 - Synthesized and stored in the pancreas (inactive)
 - Chymotrypsin is a single polypeptide chain of 245 amino acid residues cross linked by 5 disulfide bonds
 - When secreted into the small intestine, the digestive enzyme trypsin cleaves a 15 unit polypeptide from the N-terminal end to give **Chymotrypsin**

Activation of chymotrypsin

Yellow lines represents the 5 disulfide bonds that hold the chymotrypsin together

Chymotrypsinogen (inactive zymogen)



Cleavage at Arg¹⁵
by trypsin

π -Chymotrypsin (active enzyme)

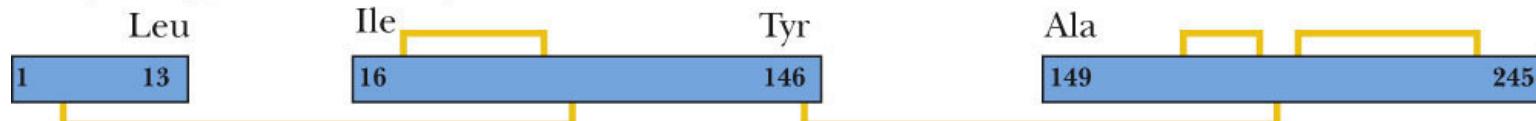


Self-digestion at Leu¹³,
Tyr¹⁴⁶, and Asn¹⁴⁸ by
 π -chymotrypsin

14 15
Ser Arg

147 148
Thr Asn

α -Chymotrypsin (active enzyme)



The Nature of The Active Site

- Important questions to ask about enzyme mode of action:
 - Which amino acid residues on the enzyme are in the active site and catalyze the reaction?
 - What is the spatial relationship of the essential amino acids residues in the active site?
 - What is the mechanism by which the essential amino acid residues catalyze the reaction?

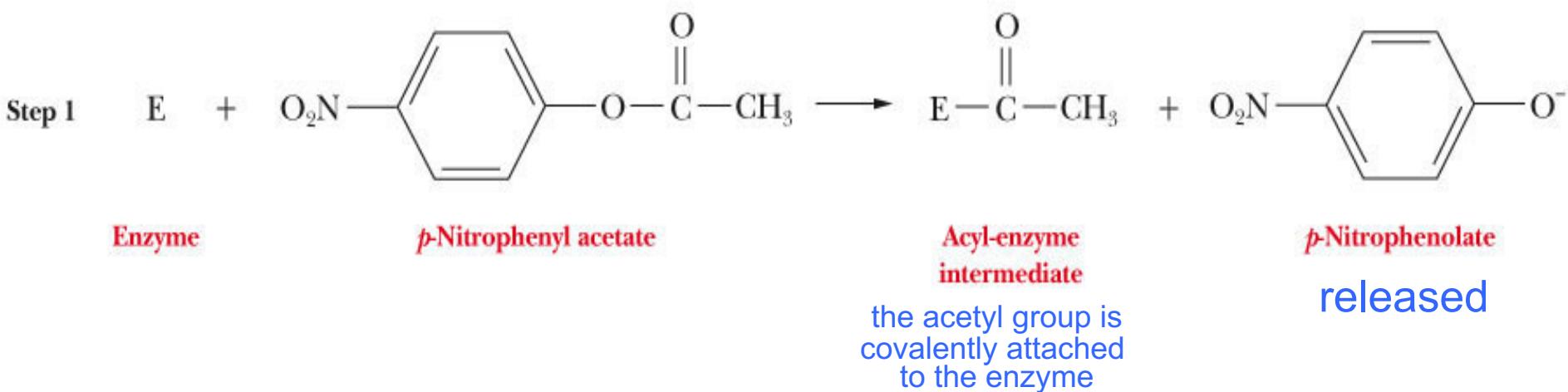
The Active Site

- Enzyme catalyze reaction required some **reactive groups** on the enzyme to interact with substrate
- Functional groups play catalytic role include:
 - Imidazole group of histidine
 - OH of serine
 - COO of aspartate and glutamate
 - Sulfhydryl group of cysteine
 - Amino chain of lysine
 - Phenol of tyrosine

Kinetics of Chymotrypsin Reaction

Chymotrypsin catalyzes the hydrolysis of: Peptide bond
adjacent to aromatic AA residues and Ester bond

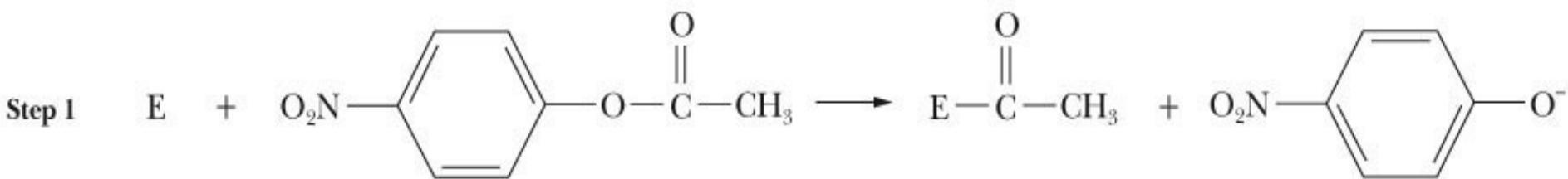
p-nitrophenyl acetate is hydrolyzed by chymotrypsin in 2 stages



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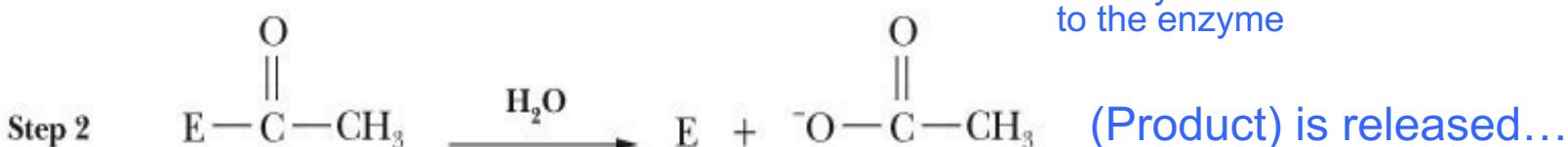
Enzyme

p-Nitrophenyl acetate

Acyl-enzyme
intermediate

p-Nitrophenolate
released

the acetyl group is
covalently attached
to the enzyme

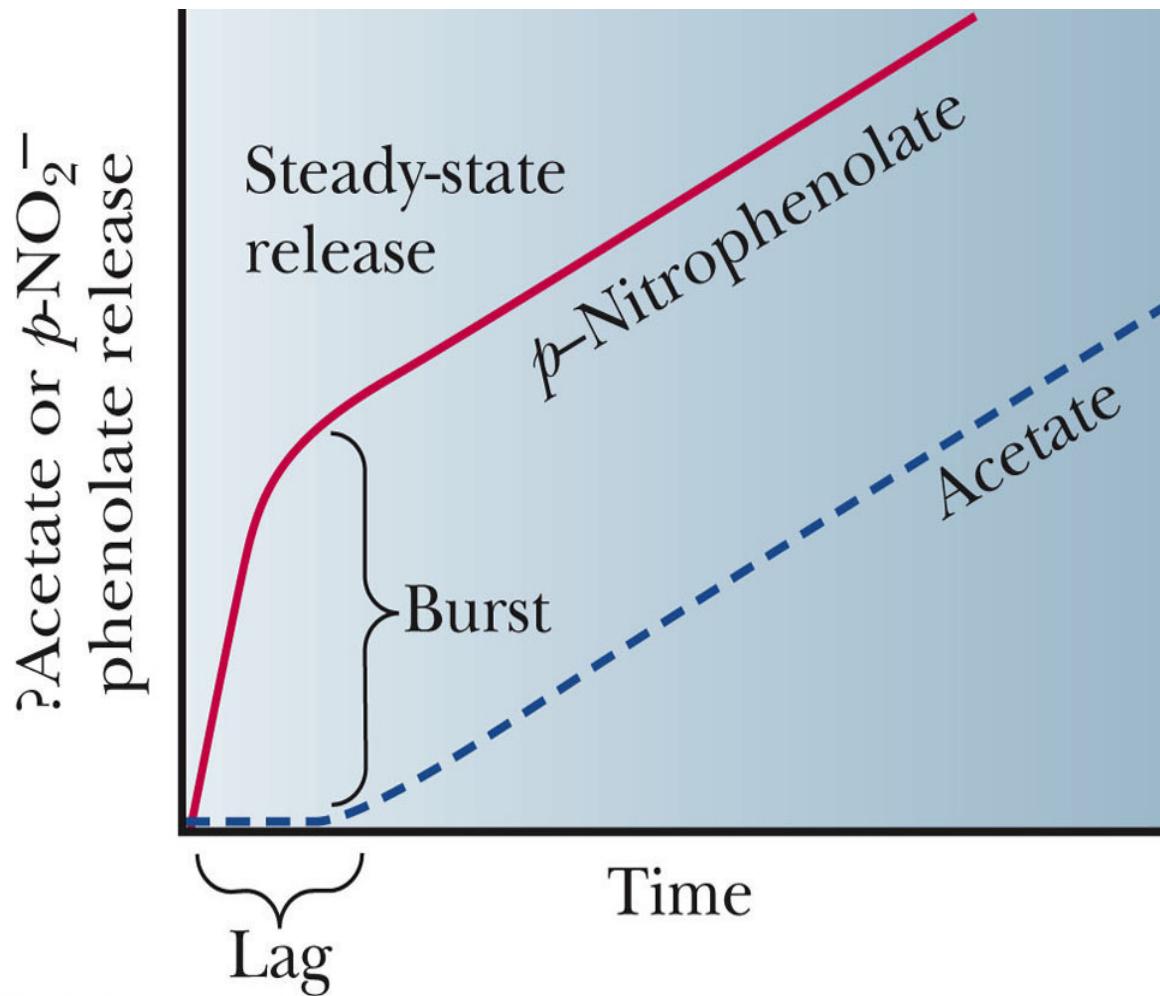


Acyl-enzyme
intermediate is hydrolyzed

Acetate

Chymotrypsin

Burst phase indicates a covalent intermediate is formed and free enzyme is regenerated

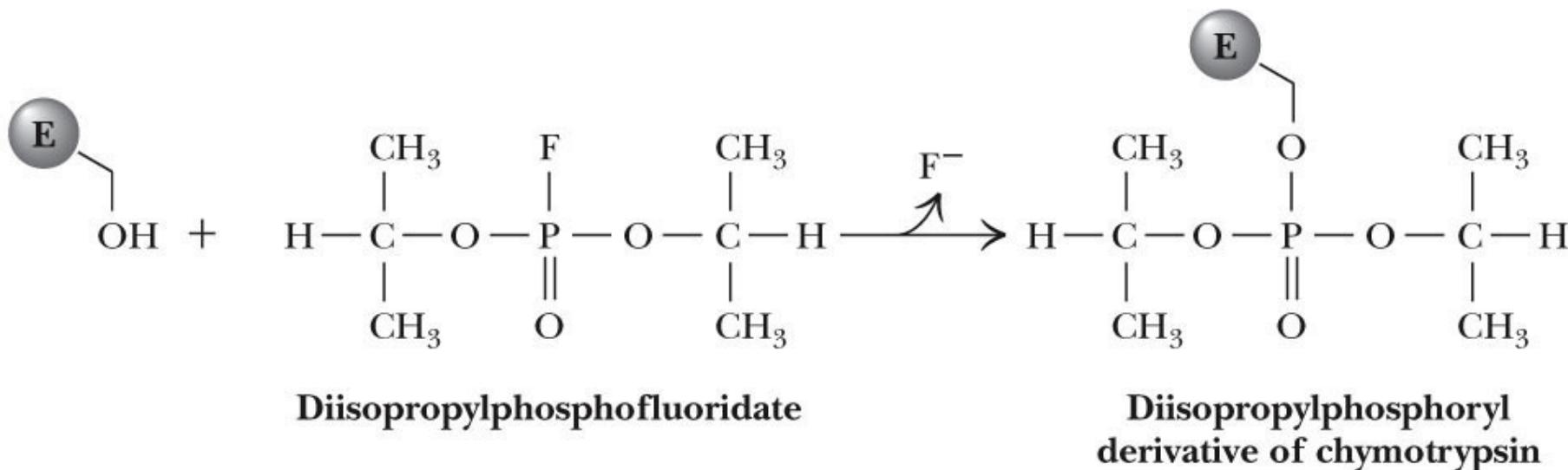


How to determine the essential amino acid residues for enzyme activity?

- Chymotrypsin is a serine protease
 - Same as Trypsin and thrombin
- The enzyme is completely inactivated when DIPF react with serine-195
- This covalent modification called labeling
 - Other serine are less reactive and do not bind DIPF

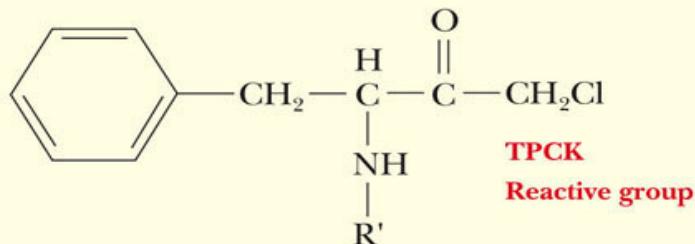
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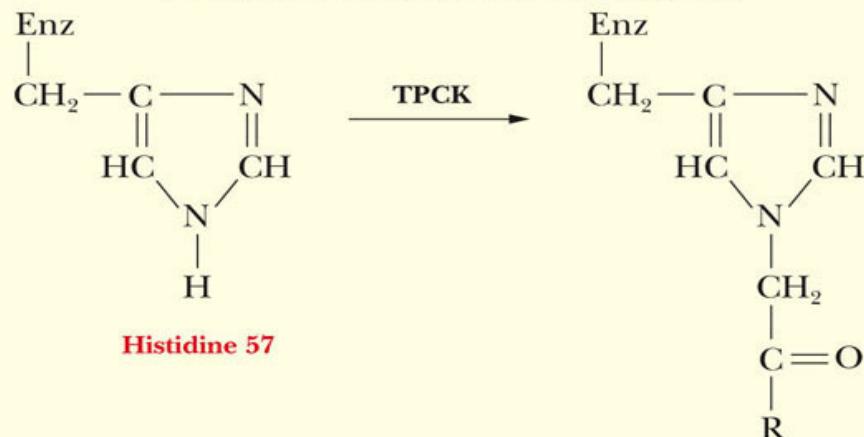


The labeling of the active-site histidine of chymotrypsin by TPCK

Phenylalanyl moiety chosen because of specificity of chymotrypsin for aromatic amino acid residues



Structure of N-tosylamido-L-phenylethyl chloromethyl ketone (TPCK), a labeling reagent for chymotrypsin
[R' represents a tosyl (toluenesulfonyl) group]



His 57 also critical for enzyme activity, Can be chemically labeled by **TPCK**

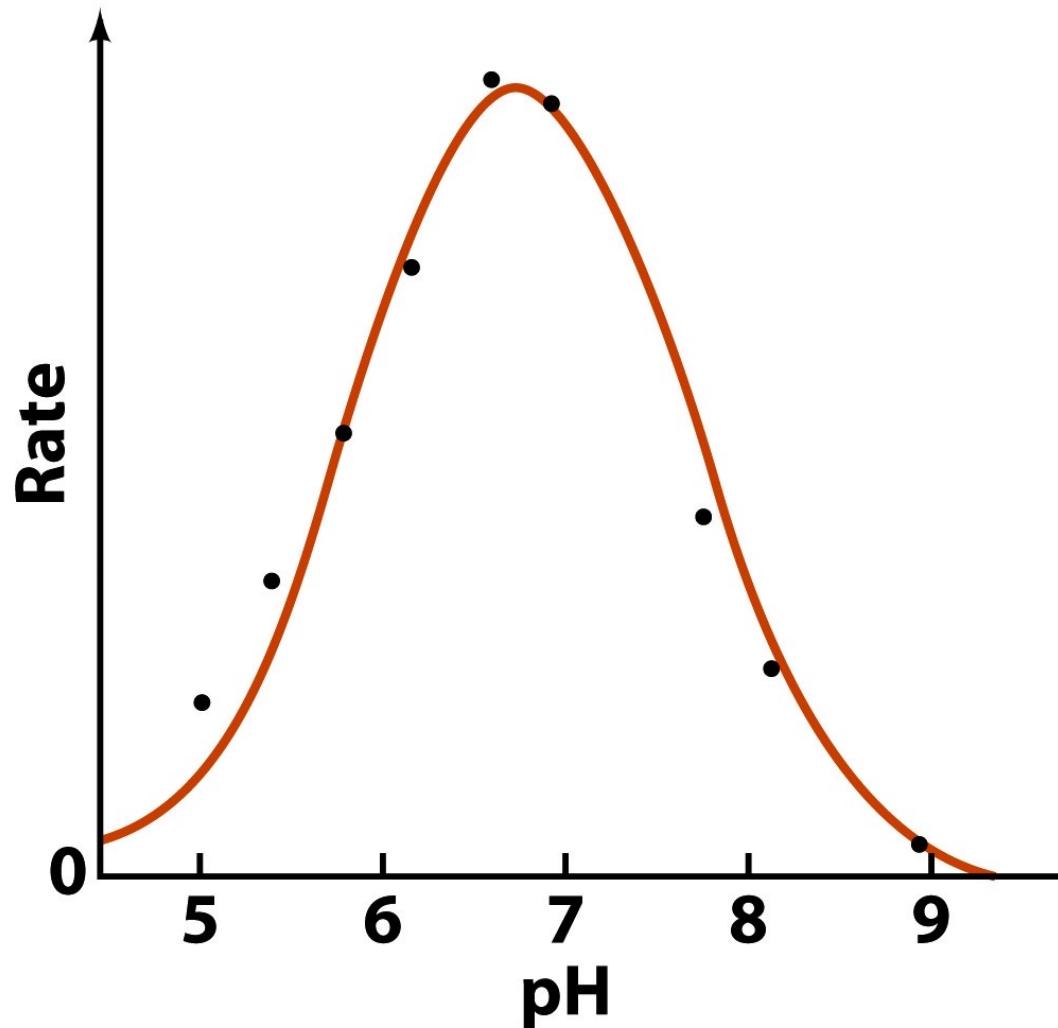
How the architecture of active site affect catalysis?

- Because Ser-195 and His-57 are required for activity, they must be close to each other in the active site
- The folding of the Chymotrypsin backbone, positions the essential amino acids around the active-site pocket

Enzymatic catalysis and mechanisms

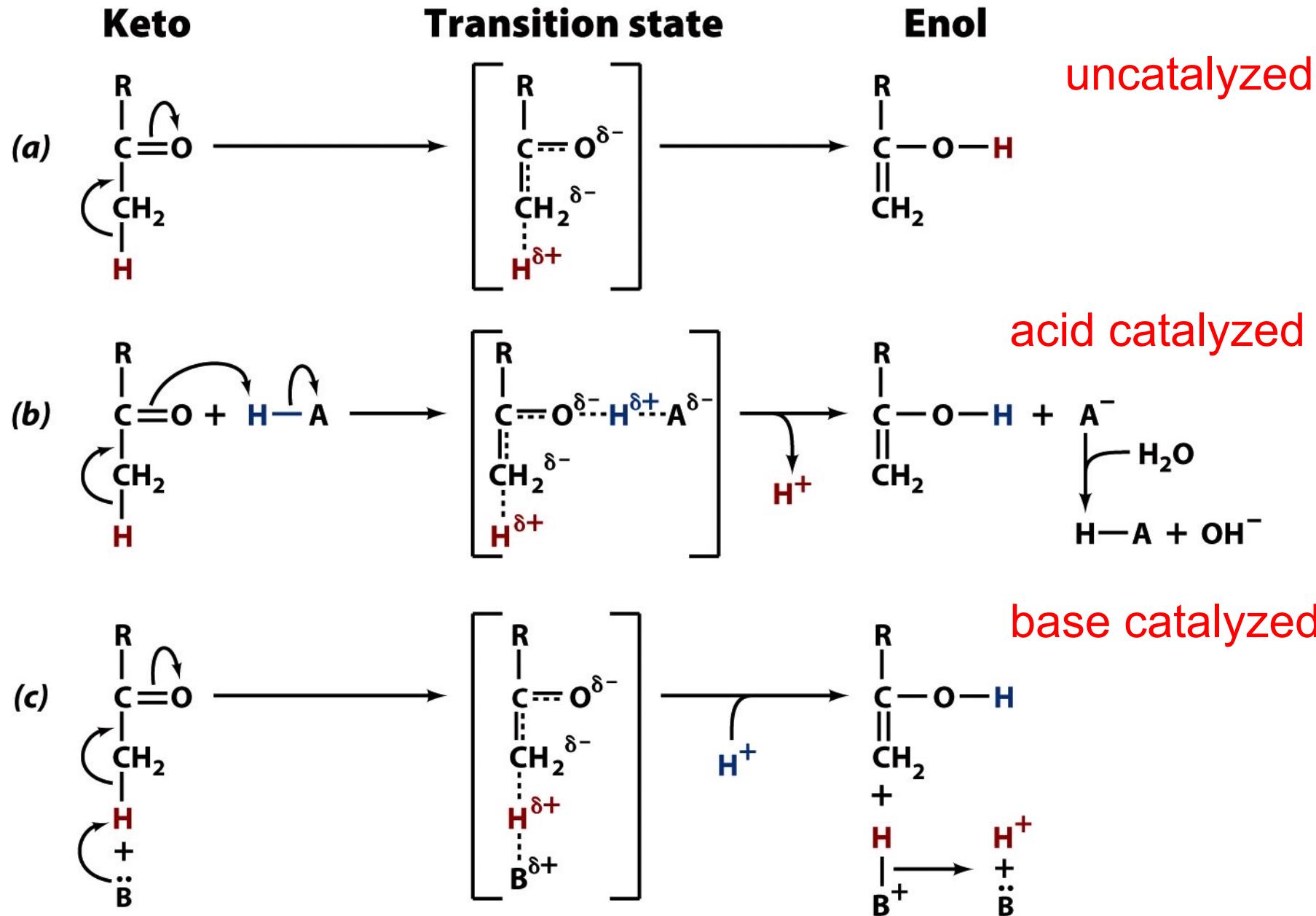
- Acid - Base catalysis
- Covalent catalysis
- Metal ion aided catalysis
- Electrostatic interactions
- Orientation and Proximity effects
- Transition state binding

Effect of pH on catalytic activity.



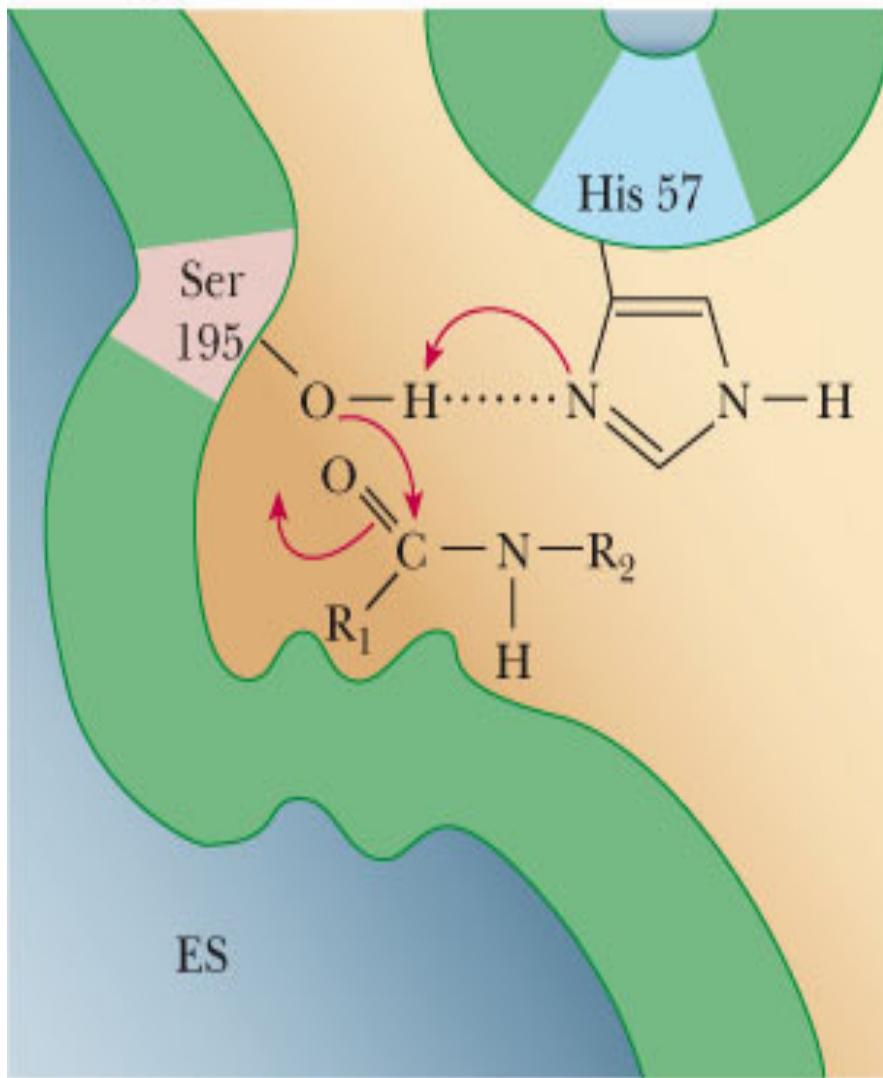
Acid-base Catalysis Mechanisms

- Depends on donation and acceptance of proton by groups such as imidazole, hydroxyl, carboxyl, sulfhydryl and phenolic side chains of Amino acids.
- If the enzyme mechanism involves an amino acid **donating H⁺**
 - General acid catalyst
- If the enzyme mechanism involves an amino acid **accepting H⁺**
 - General base catalyst

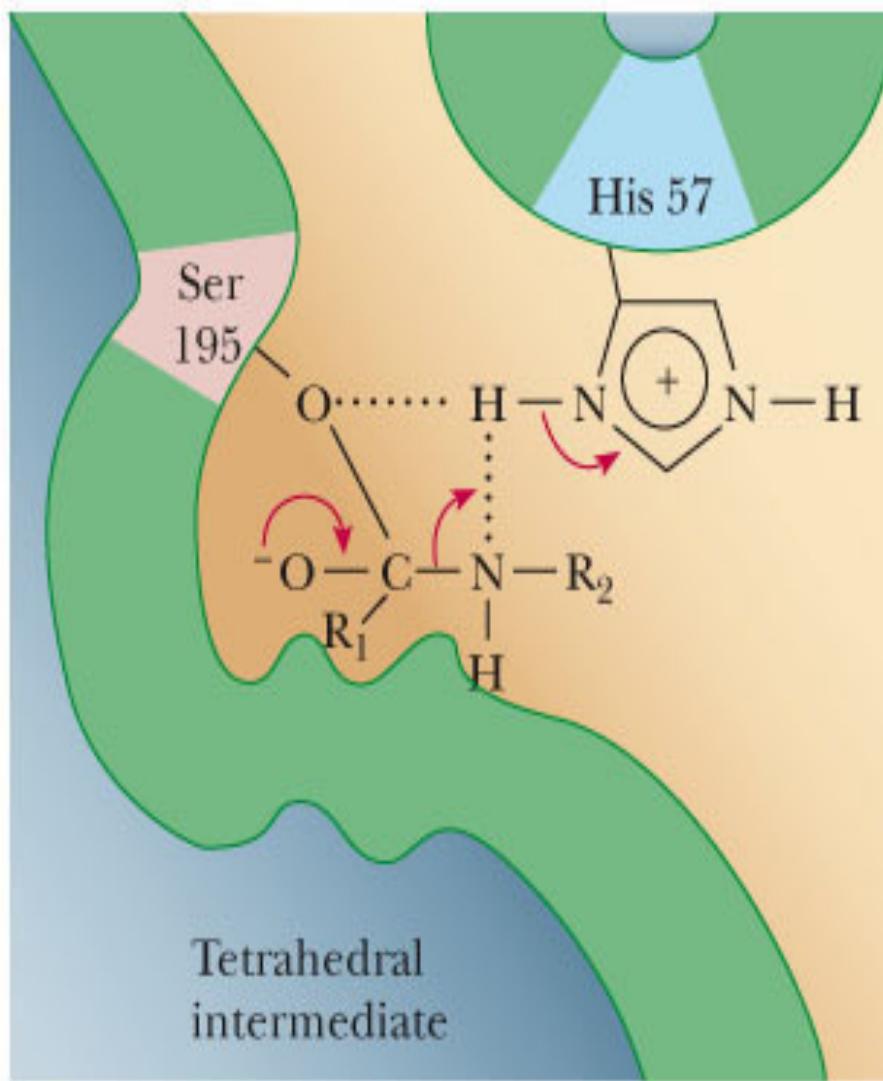


Mechanism of Action of Critical Amino Acids in Chymotrypsin

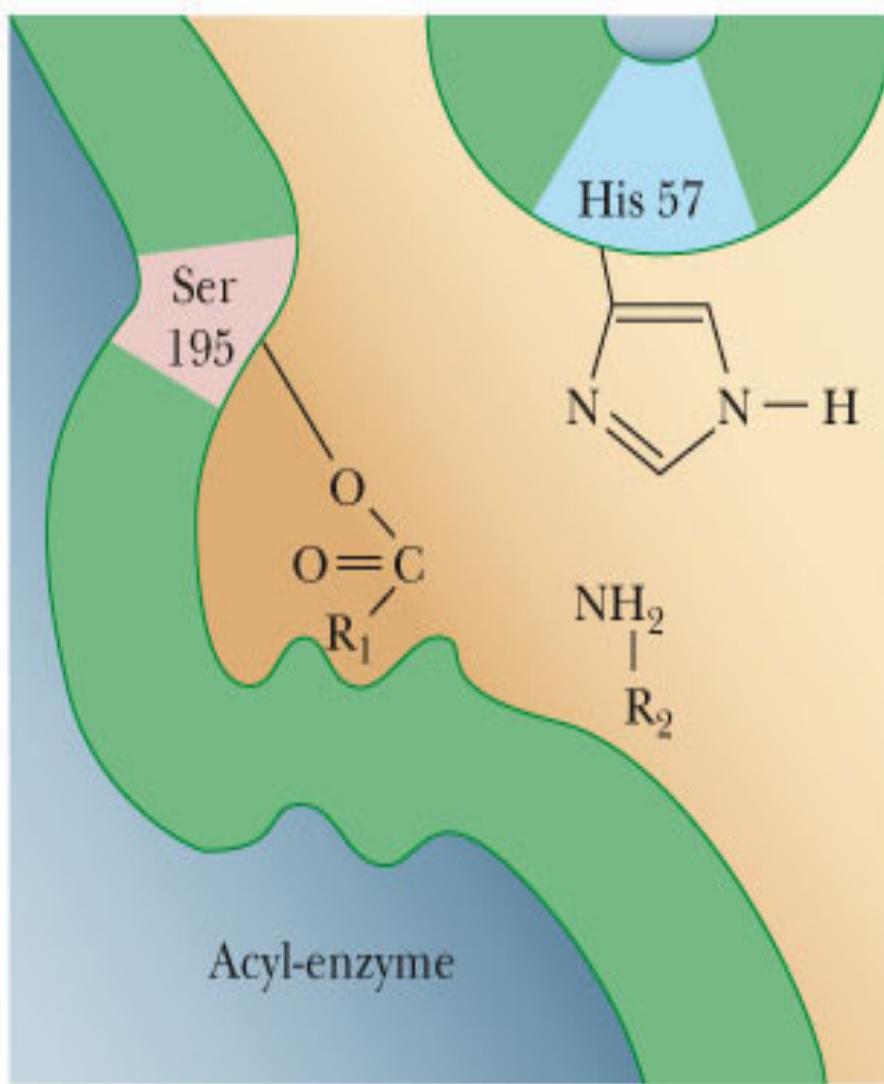
1st stage reaction



Mechanism of Action of Critical Amino Acids in Chymotrypsin



Mechanism of Action of Critical Amino Acids in Chymotrypsin



Mechanism of Action of Critical Amino Acids in Chymotrypsin

Nucleophile is a nucleus-seeking substance which tend to bond to a site of +ve charge

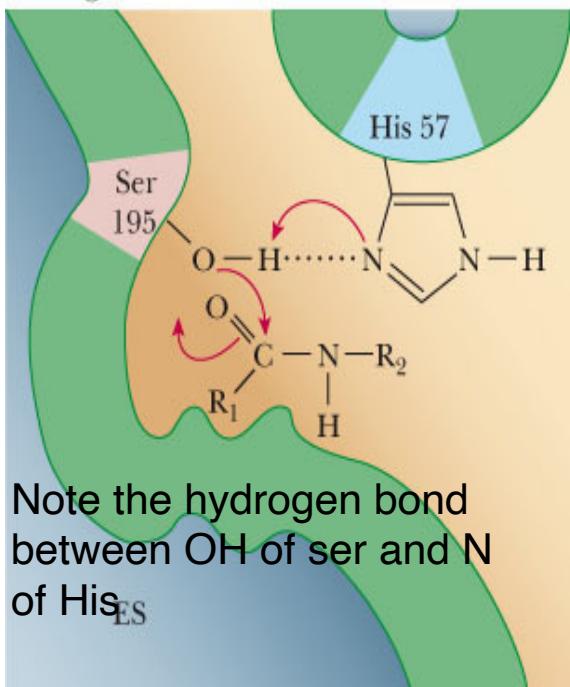
Electrophile is electron-seeking substance and tend to bond to a site of negative charge.

The nucleophilic oxygen of Ser attacks carbonyl carbon of the peptide group

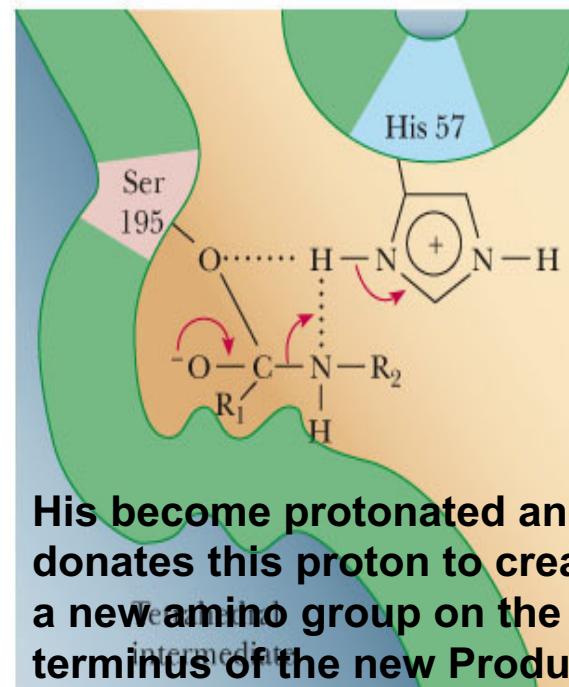
The carbon now has four single bonds and **tetrahedral intermediate** is formed. The –C=O become single bond and carbonyl oxygen become oxyanion

The original C-N bond is broken leaving Acyl-enzyme intermediate formed

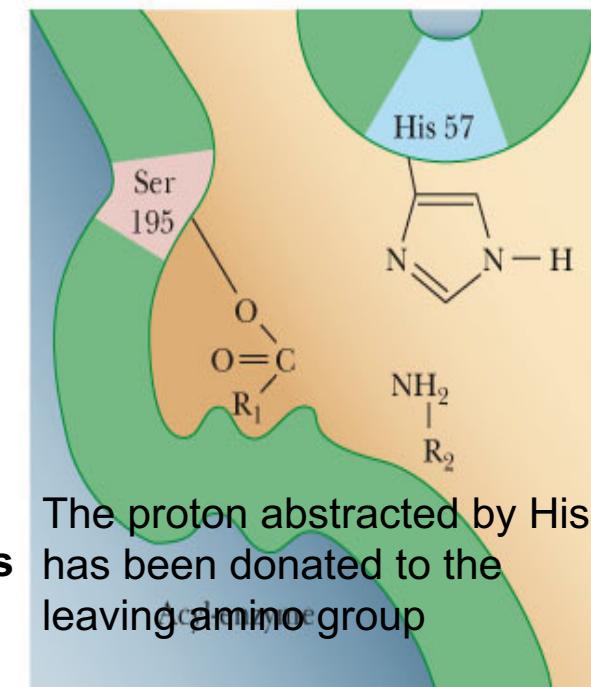
1st stage reaction



Note the hydrogen bond between OH of ser and N of His



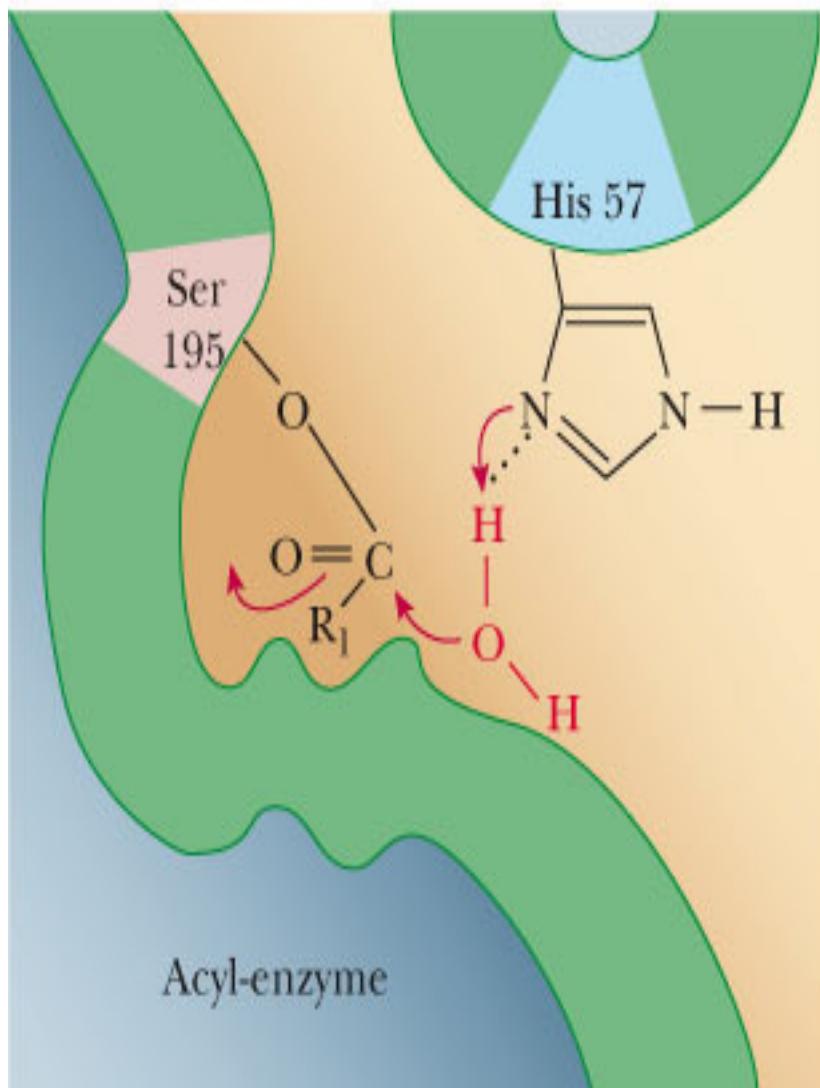
His become protonated and donates this proton to creates a new amino group on the terminus of the new Product



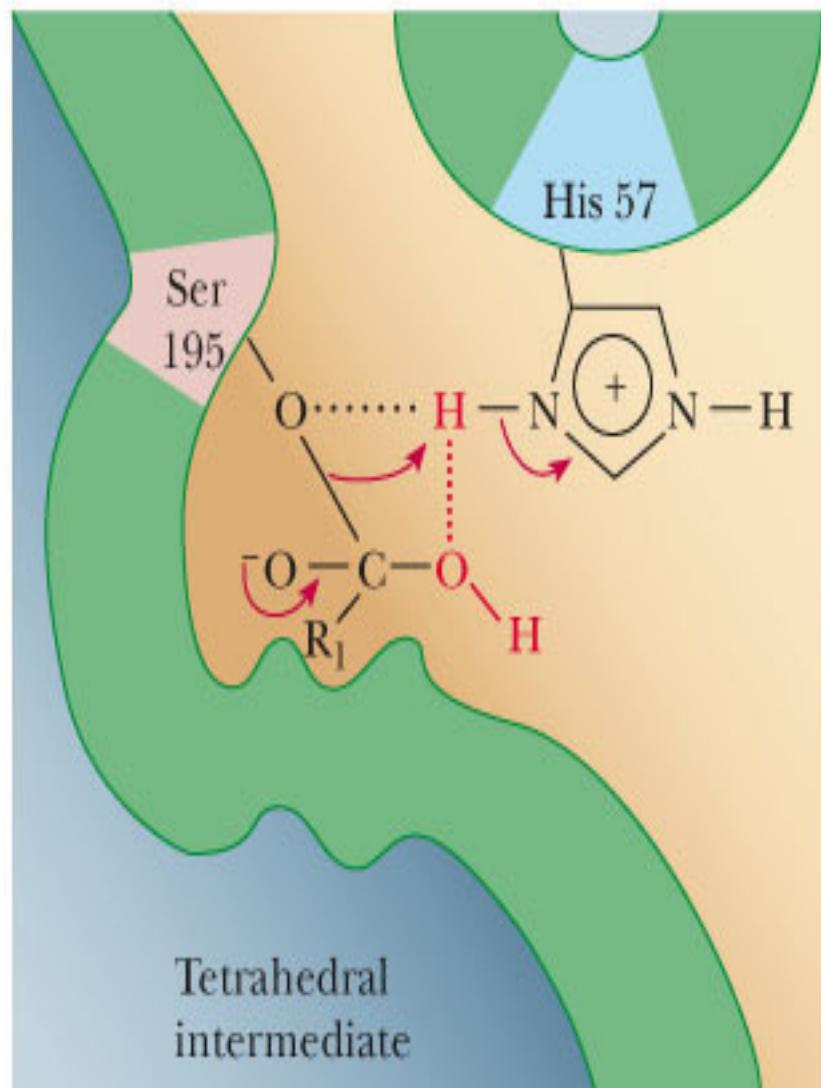
The proton abstracted by His has been donated to the leaving amino group

The deacylation phase the last 2 steps are reversed

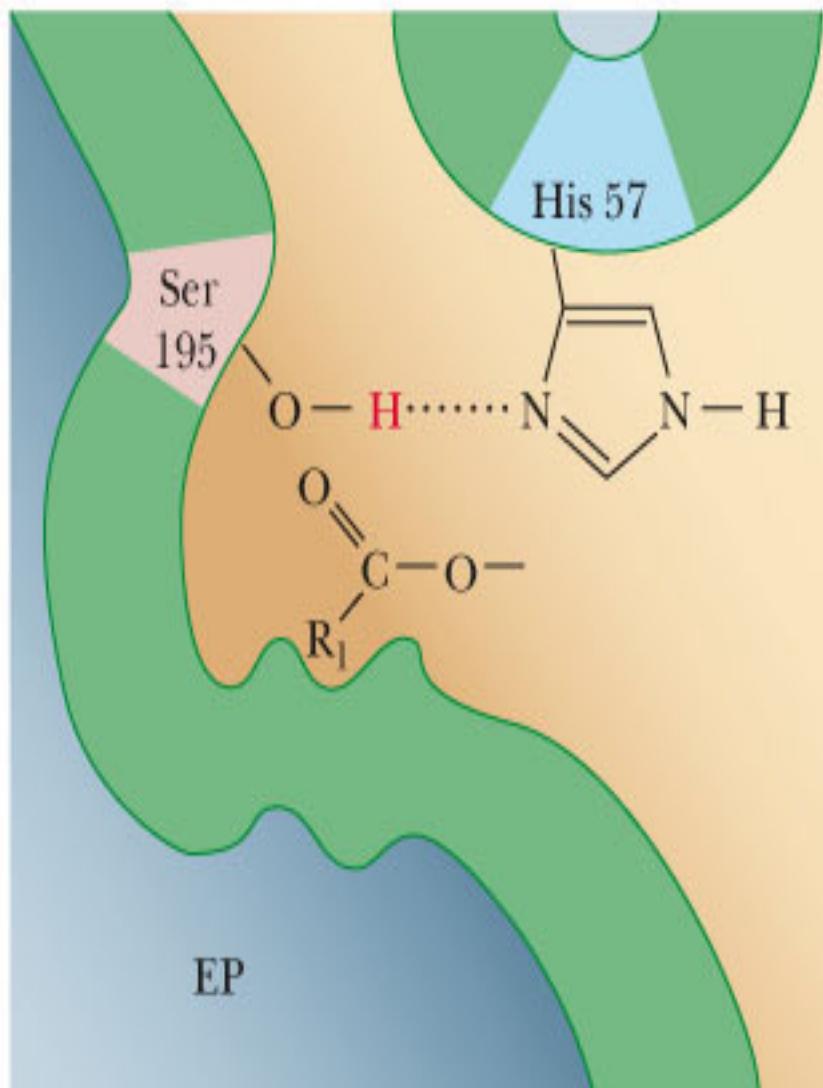
2nd stage reaction



The deacylation phase the last 2 steps are reversed



The deacylation phase the last 2 steps are reversed

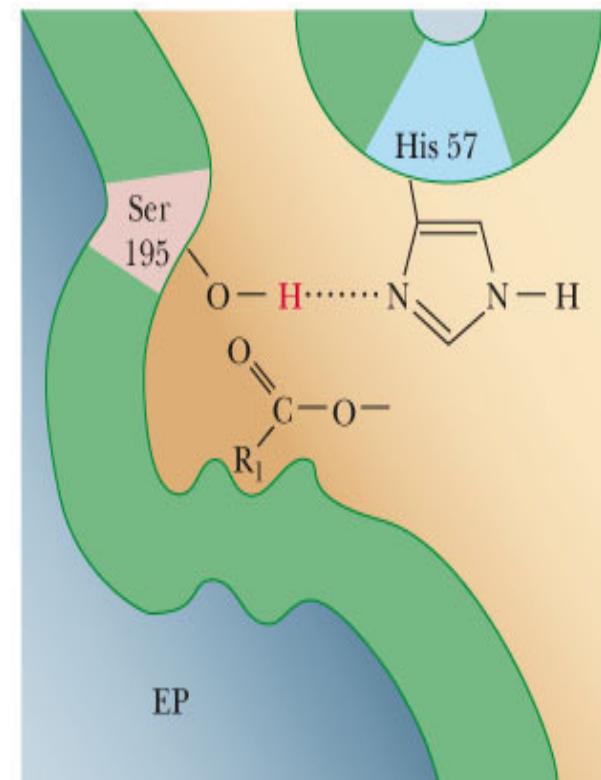
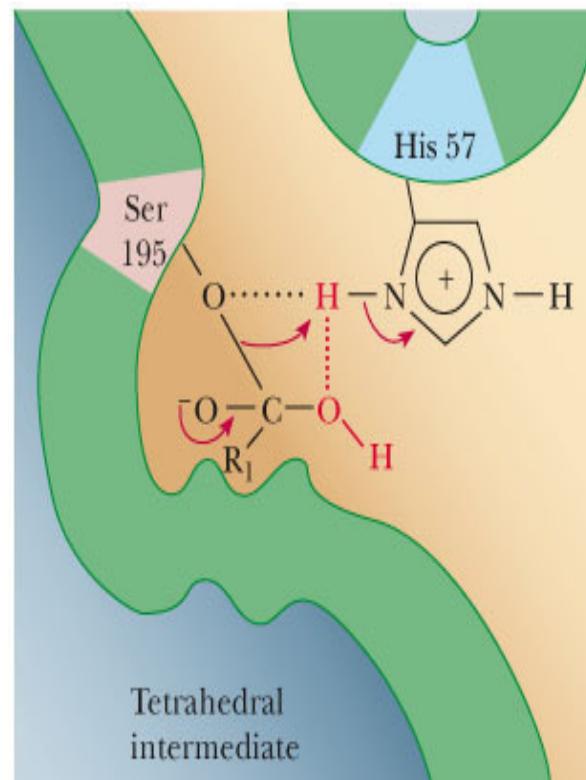
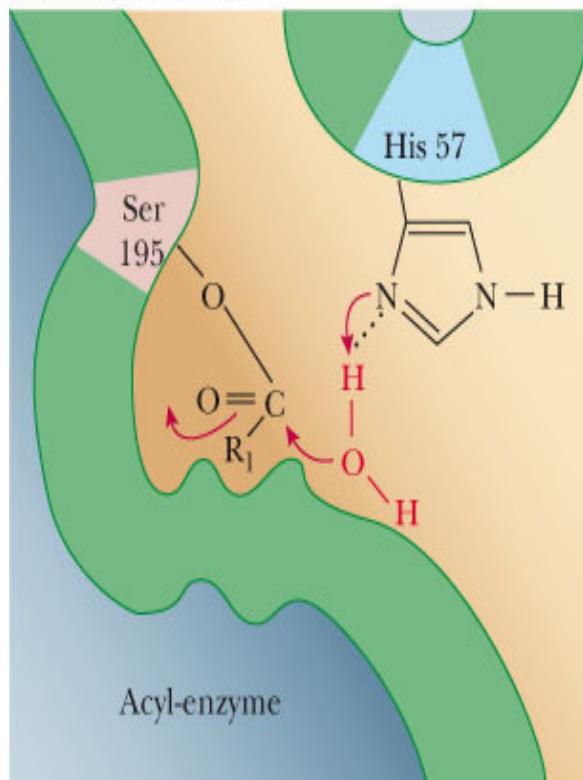


The deacylation phase the last 2 steps are reversed

Water is acting as attacking nucleophilic on acyl carbon of original peptide
H of H₂O is hydrogen bonded to His

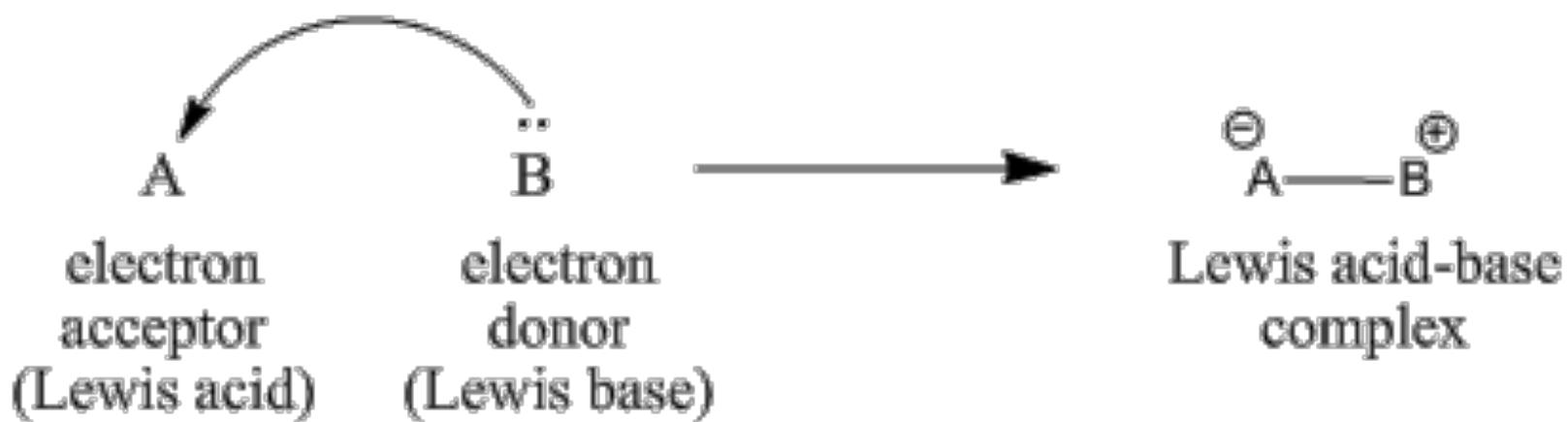
The bond between the ser oxygen and carbonyl carbon breaks and Product released

2nd stage reaction



Metal ion catalysis

- Lewis acid/base reactions:
 - **Lewis acid:** an electron pair acceptor (electrophile)
 - **Lewis base:** an electron pair donor (Nucleophile)
- Lewis acids such as Mn^{2+} , Mg^{2+} , and Zn^{2+} are essential components of many enzymes

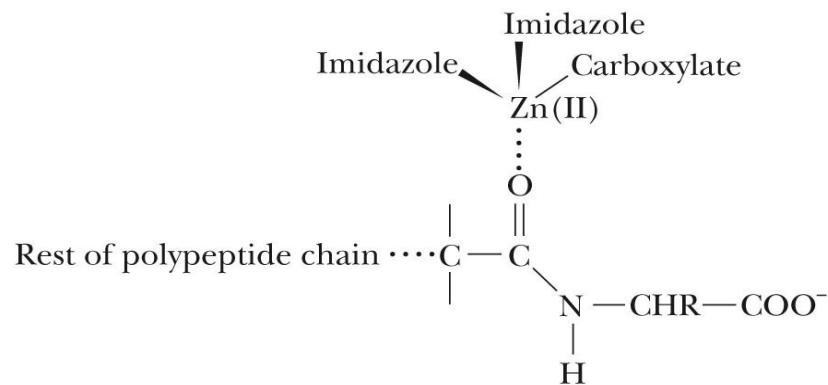


Metal ion catalysis

- Activity of carboxypeptidase A requires Zn^{2+} which make complexes with the imidazole side chains of enzymes His-69 and 196 and carboxylate side chain of Glu-72.
- Zn is also complexed to carbonyl group of the substrate which polarizes the carbonyl group and make it susceptible to be attacked by water

Metal ion catalysis

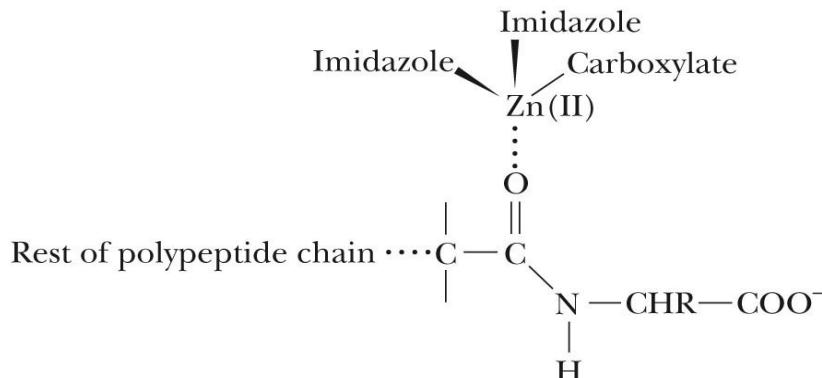
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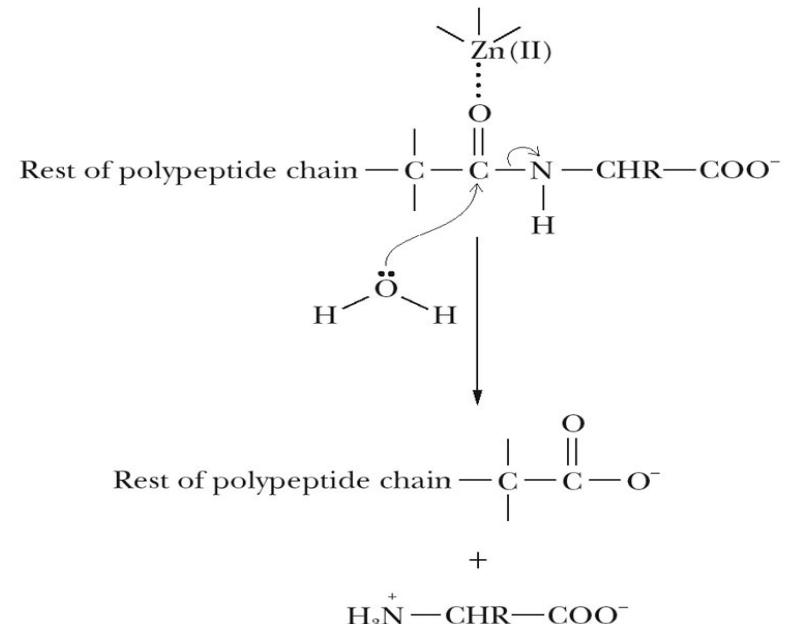
A zinc ion is complexed to three
side chains of carboxypeptidase
and to a carbonyl group on the substrate.

Metal ion catalysis

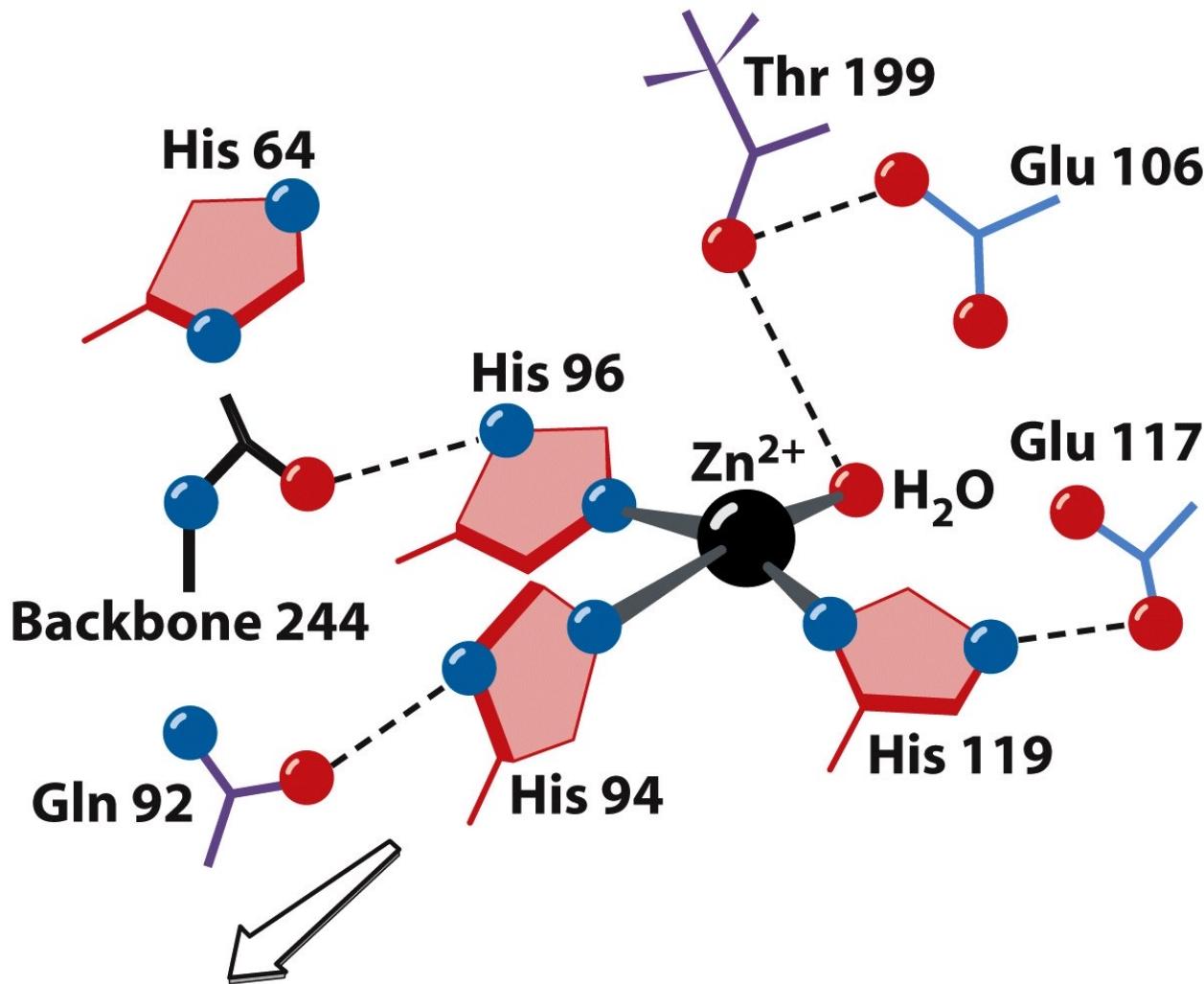
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- Zn is also complexed to carbonyl group of the substrate which polarizes the carbonyl group and make it susceptible to be attacked by water



A zinc ion is complexed to three side chains of carboxypeptidase and to a carbonyl group on the substrate.

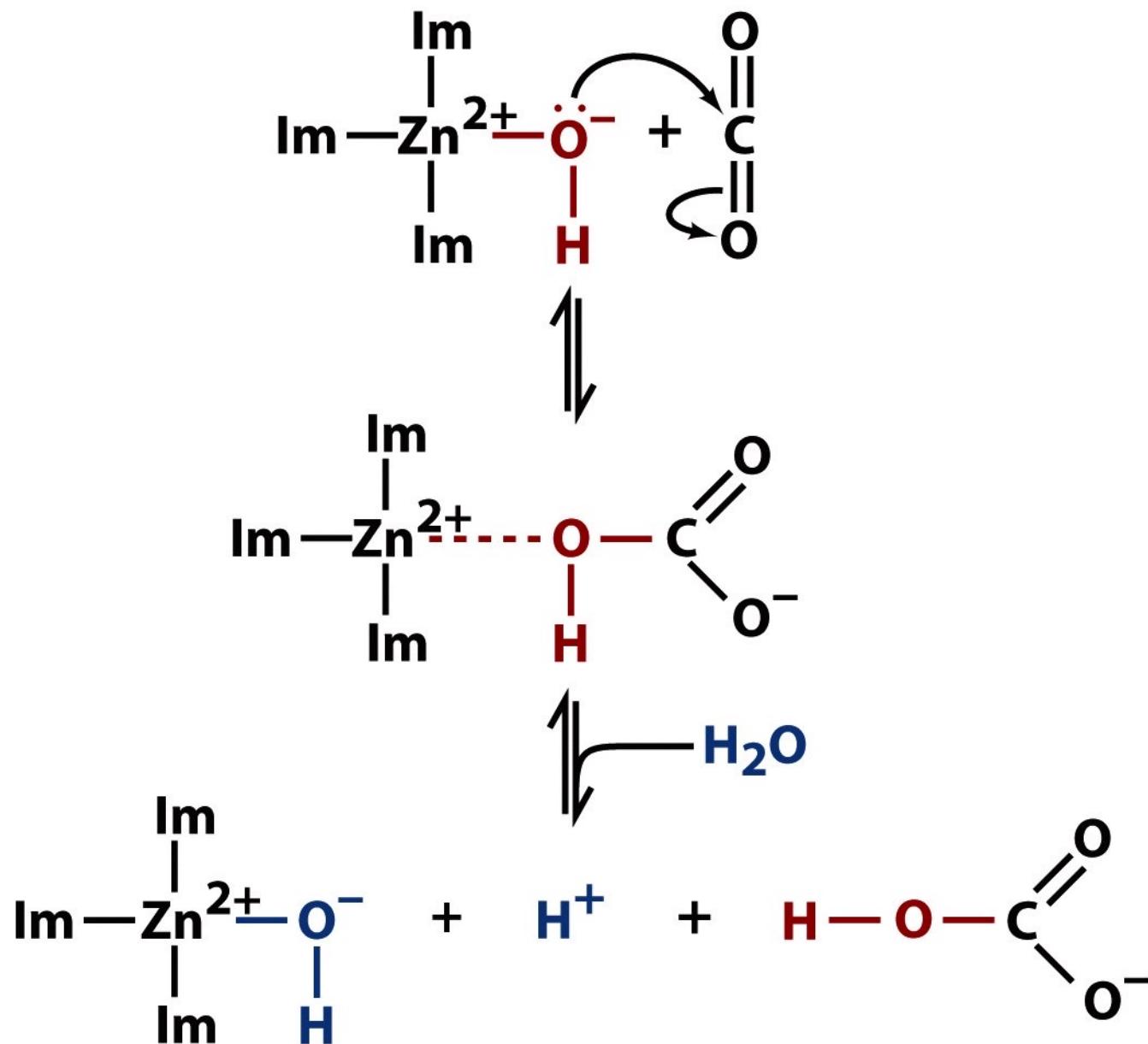


Metal ions in catalysis



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Figure 11-13a

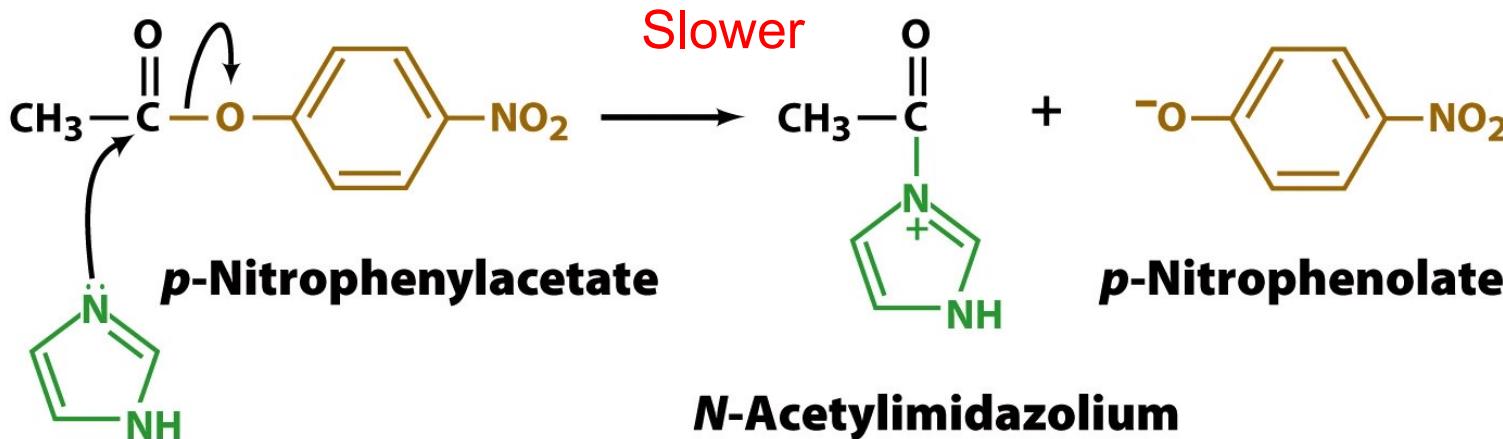


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Figure 11-13b

Proximity and Orientation: Entropy Trapping

(i) Proximity increases reaction rates

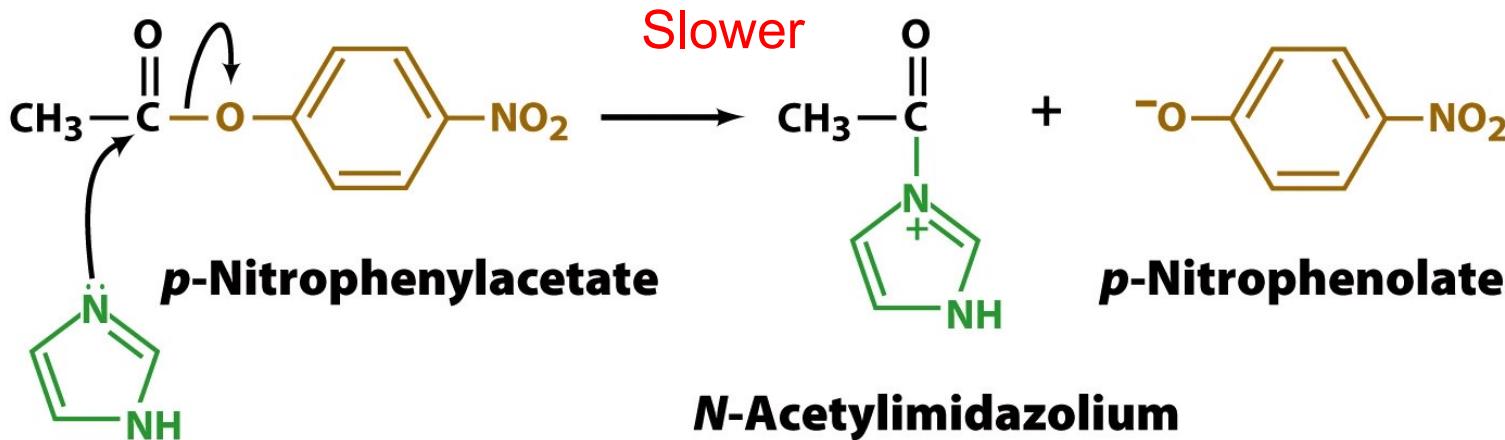


Imidazole

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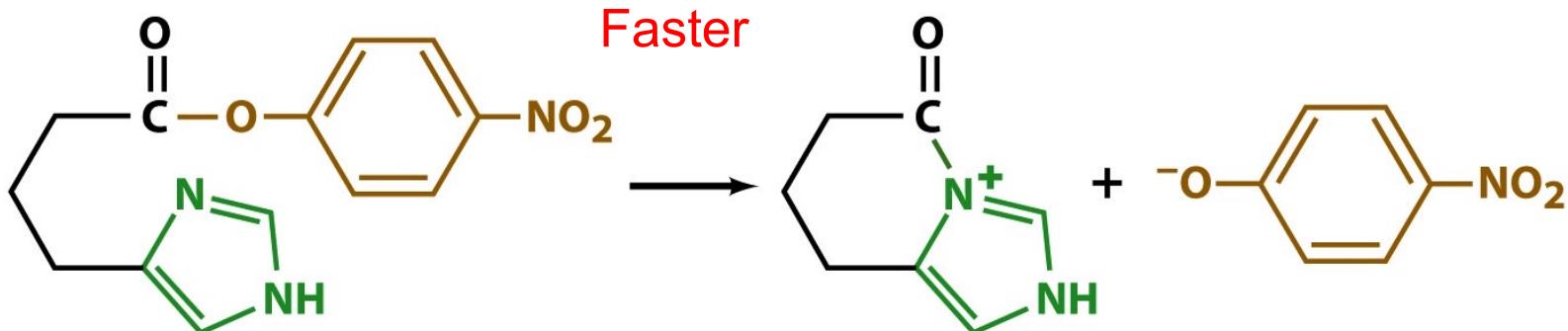
Proximity and Orientation: Entropy Trapping

(i) Proximity increases reaction rates



Imidazole

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Enzyme Specificity

- **Absolute specificity:** catalyzes the reaction of one unique substrate to a particular product (active site is rigid and best described by key and lock model)

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- **Absolute specificity:** catalyzes the reaction of one unique substrate to a particular product (active site is rigid and best described by key and lock model)
- **Relative specificity:** catalyzes the reaction of structurally related substrates to give structurally related products (more flexible and best characterized by induced fit model)

Enzyme Specificity

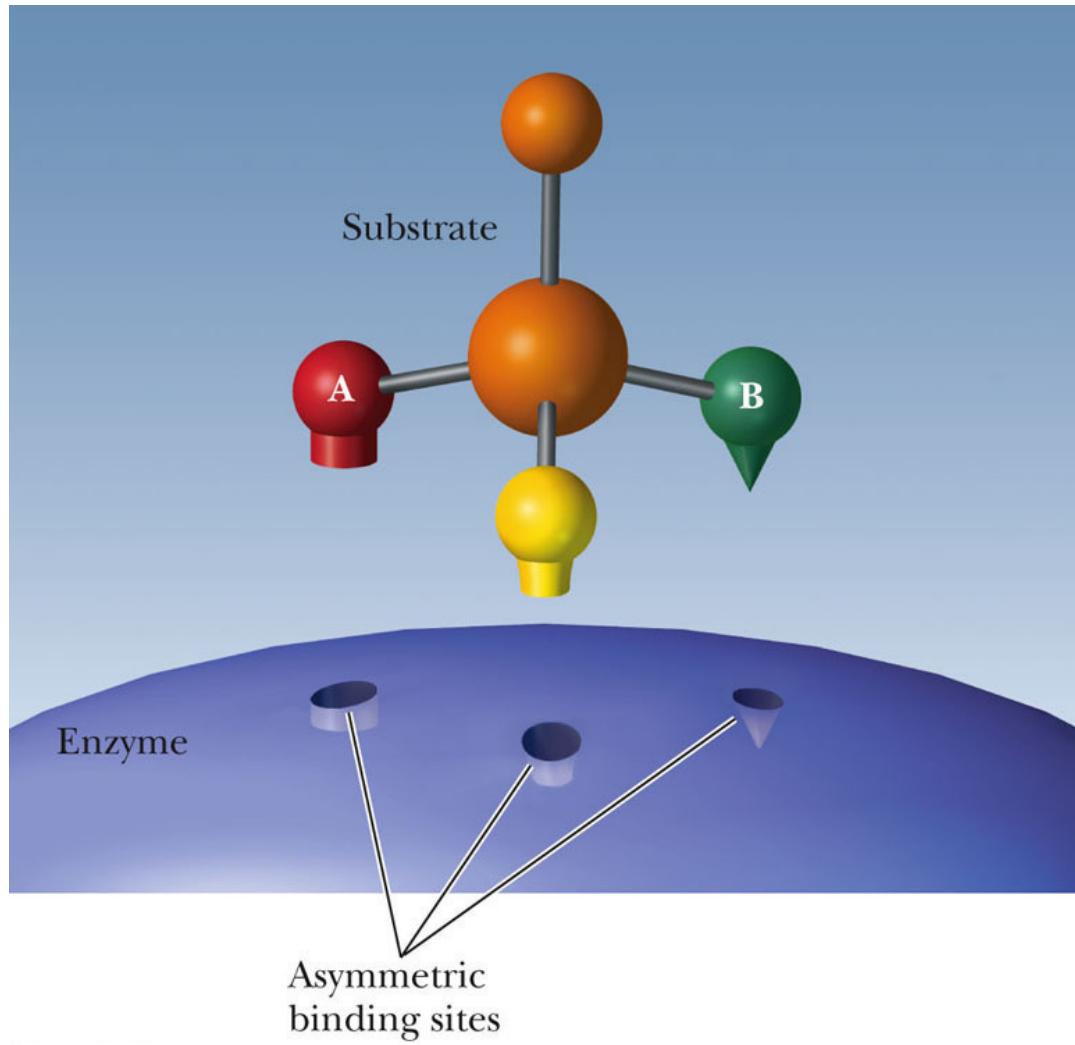
- **Absolute specificity:** catalyzes the reaction of one unique substrate to a particular product (active site is rigid and best described by key and lock model)
- **Relative specificity:** catalyzes the reaction of structurally related substrates to give structurally related products (more flexible and best characterized by induced fit model)
- **Stereospecific enzyme:** catalyzes a reaction in which one stereoisomer is reacted or formed in preference to all others that might be reacted or formed
 - Having the same shape not the mirror image

Asymmetric binding

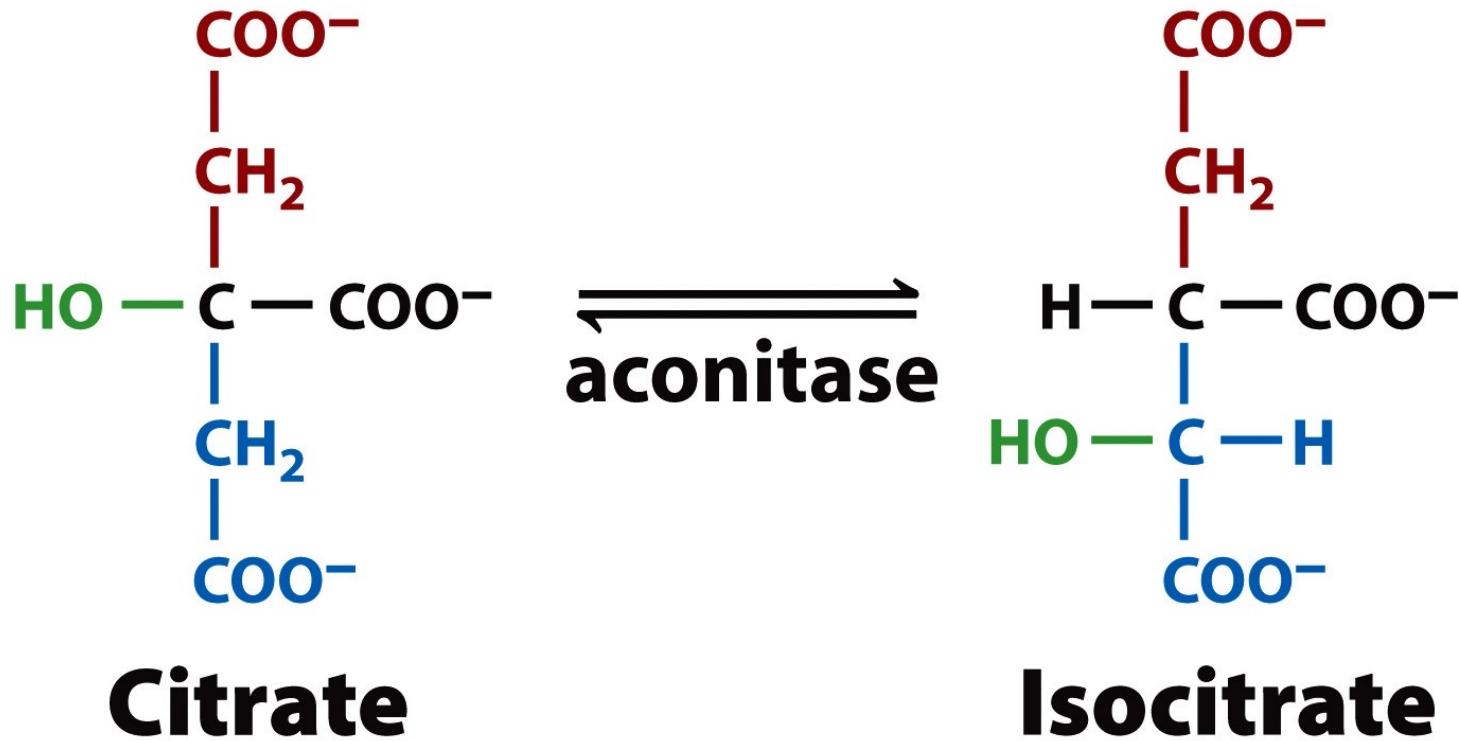
Enzymes can be
Stereospecific

(Specificity where optical
activity may play a role)

Binding sites on enzymes
must be **of the same
shape**



Enzymes are stereospecific



Enzymes are stereospecific

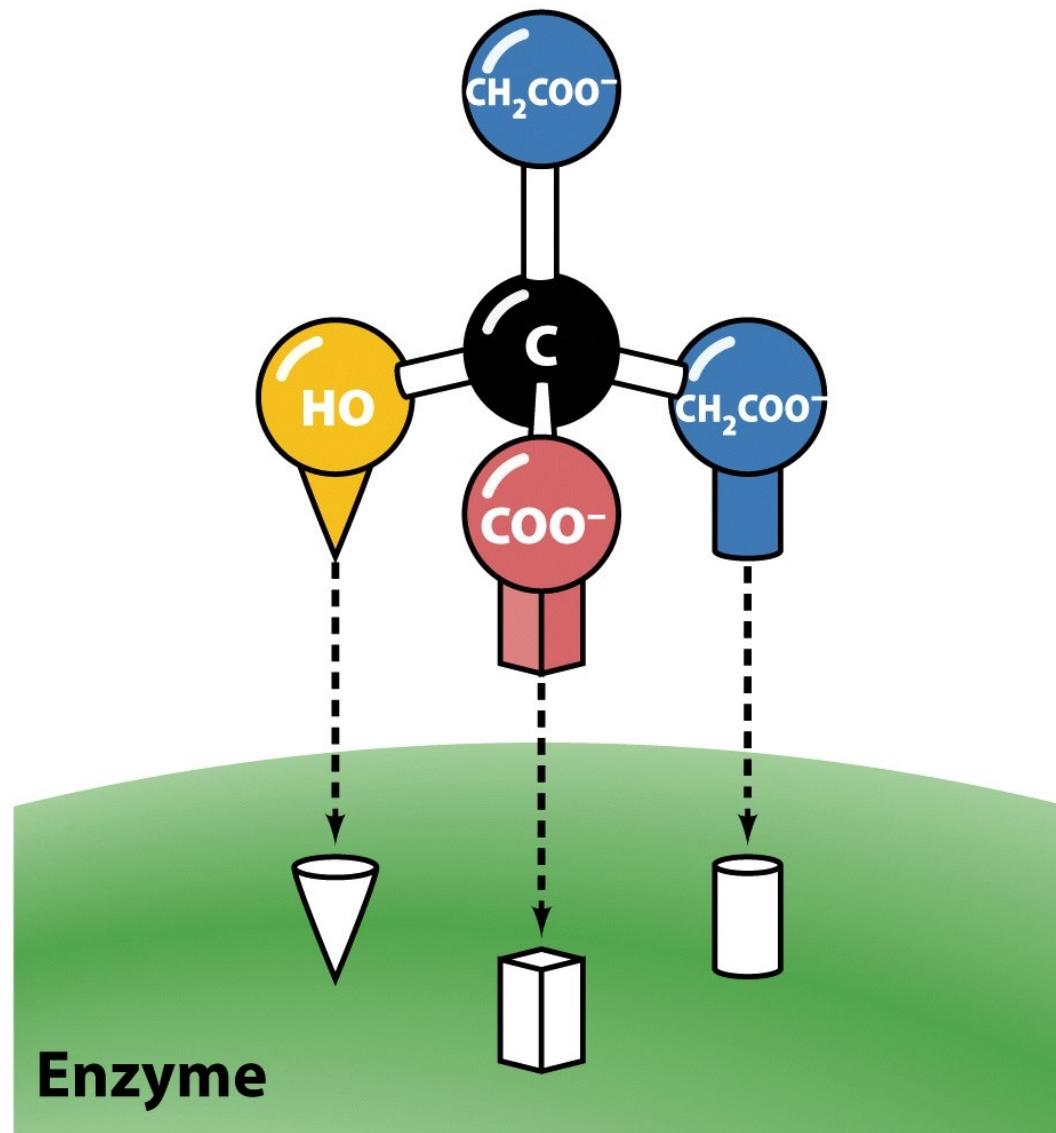
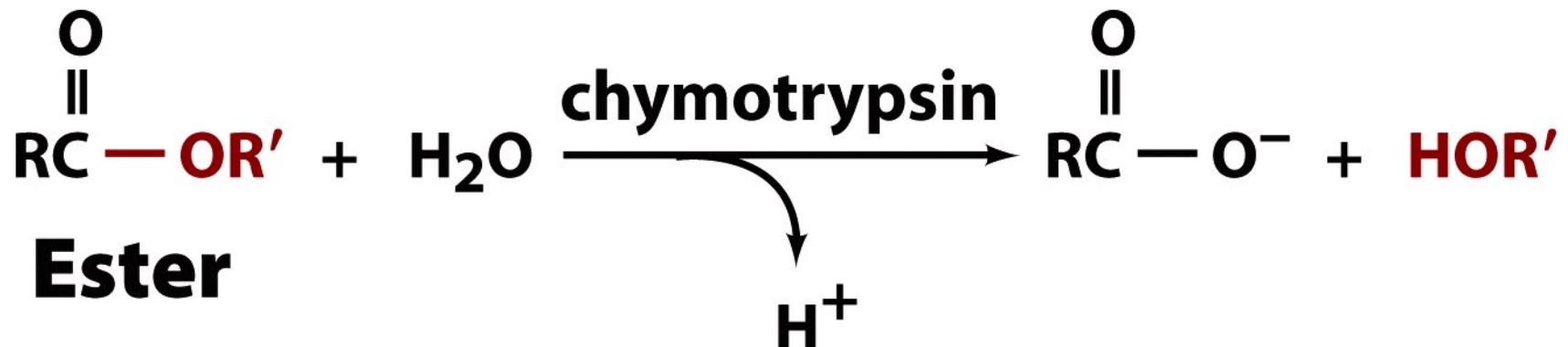


Figure 11-2

Some enzymes are not too specific



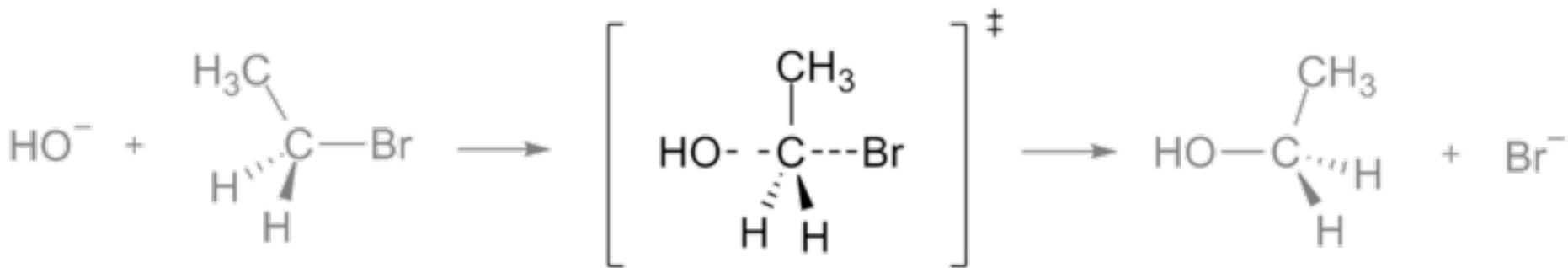
Peptide



Ester

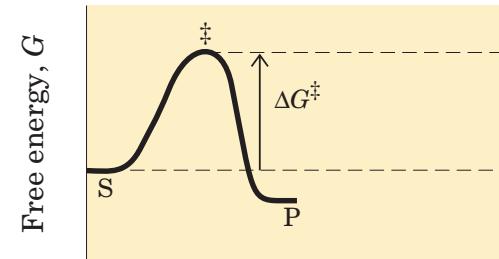
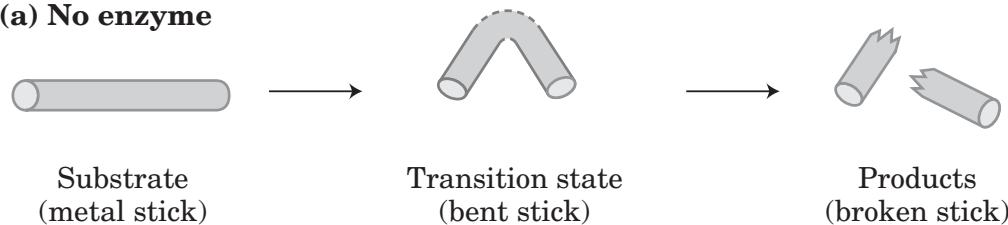
Transition state

The highest energy species (most unstable species) along the reaction coordinate.



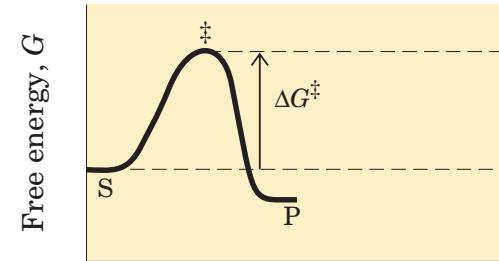
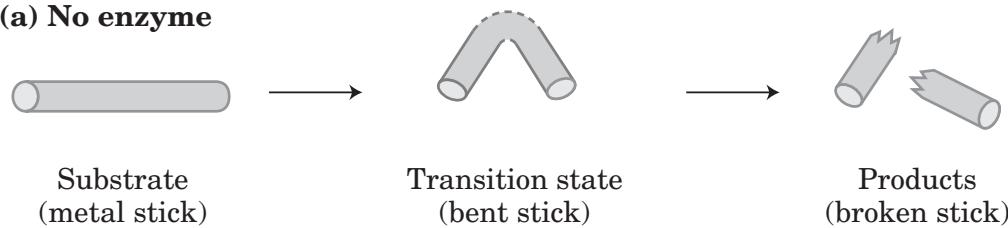
Enzymes stabilize transition state

(a) No enzyme

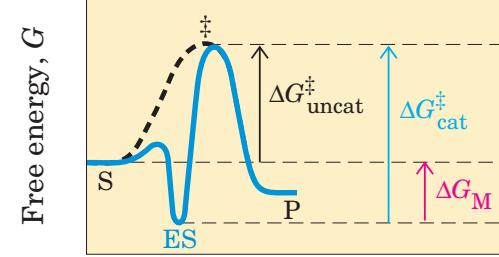
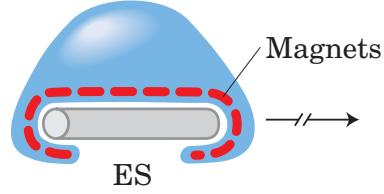


Enzymes stabilize transition state

(a) No enzyme

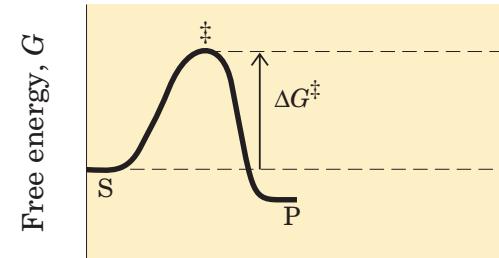
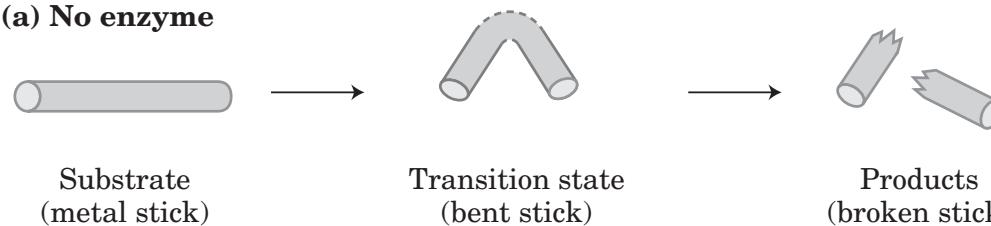


(b) Enzyme complementary to substrate

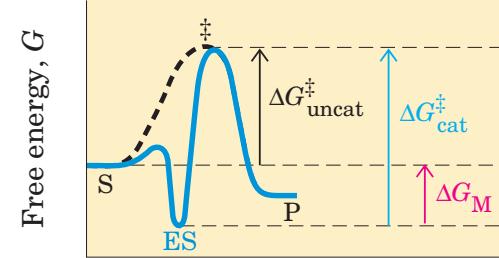
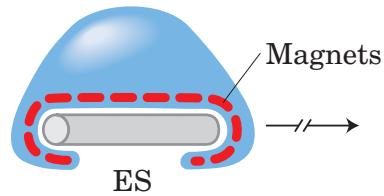


Enzymes stabilize transition state

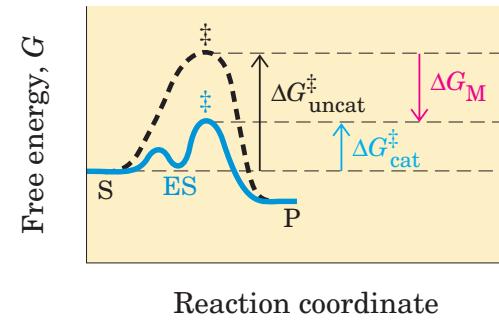
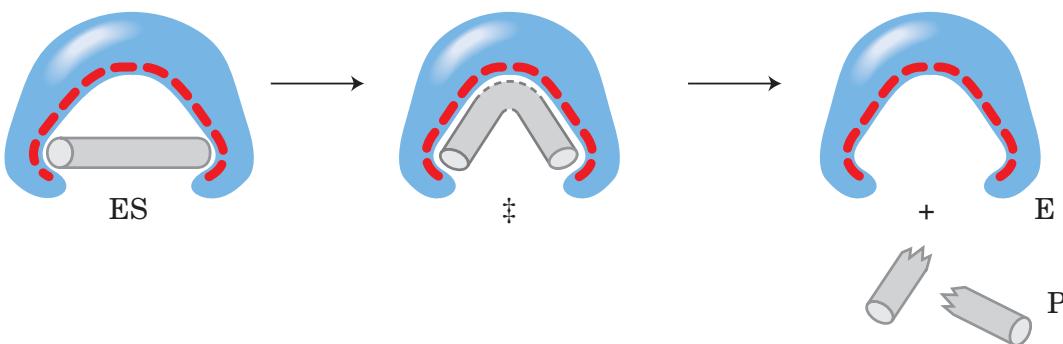
(a) No enzyme



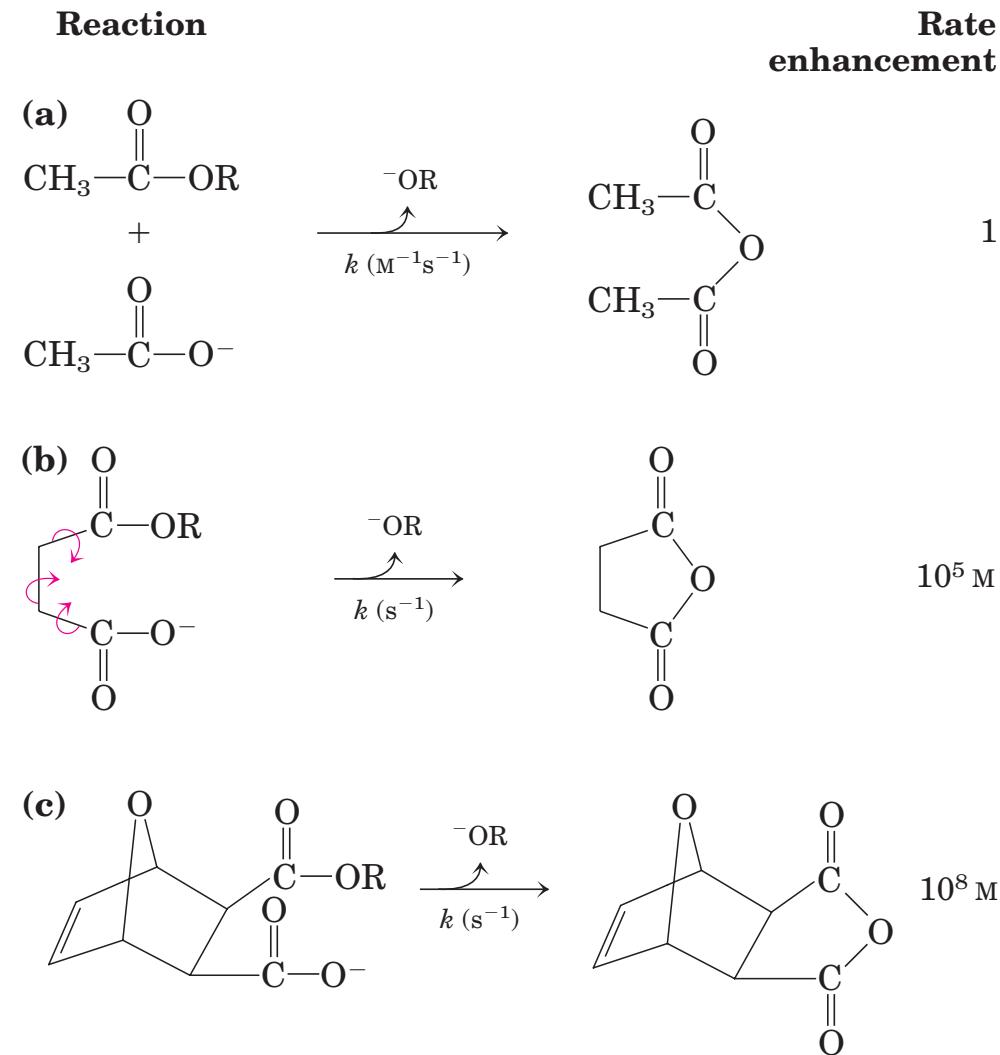
(b) Enzyme complementary to substrate



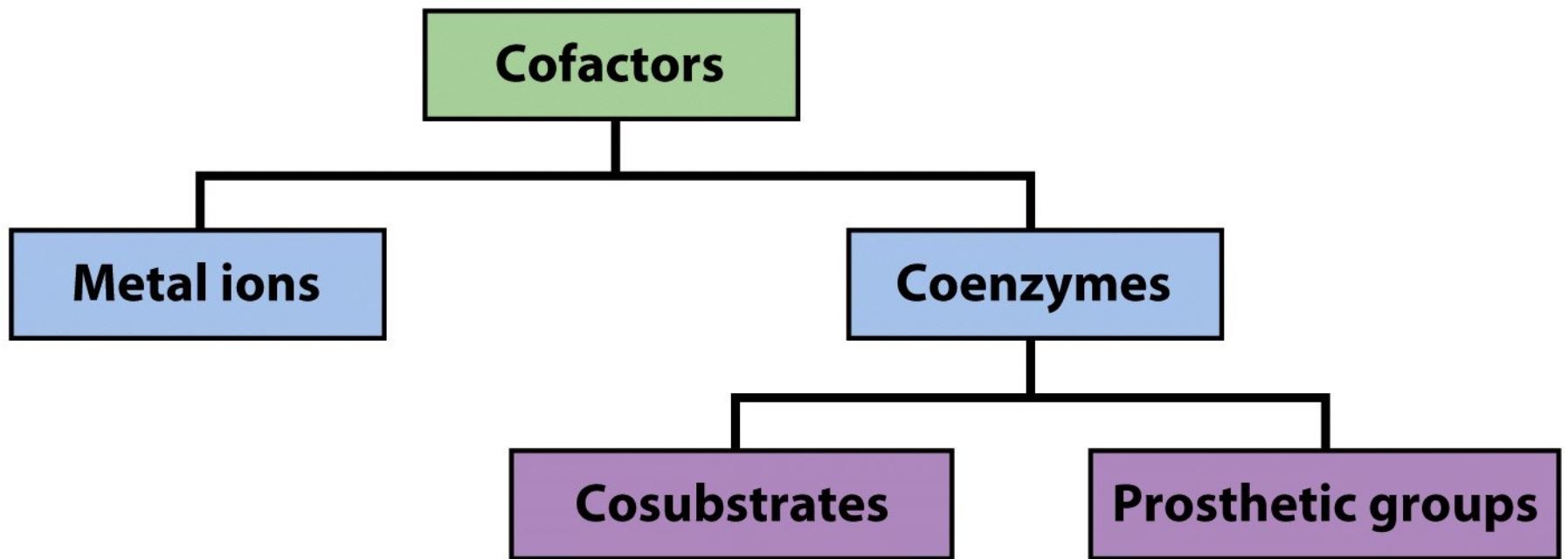
(c) Enzyme complementary to transition state



Binding energy contributes to reaction specificity and catalysis



Some enzymes need help

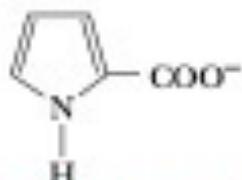
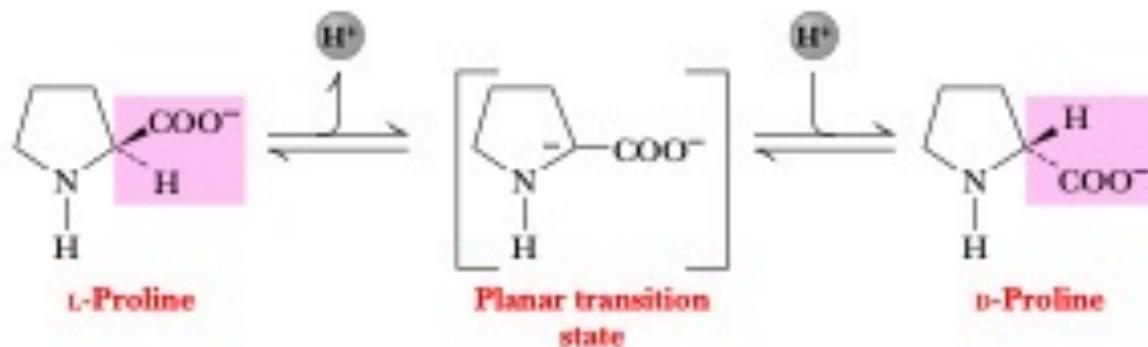


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Transition-state analogs

- Molecules with a shape that mimics the transition state of the substrate.

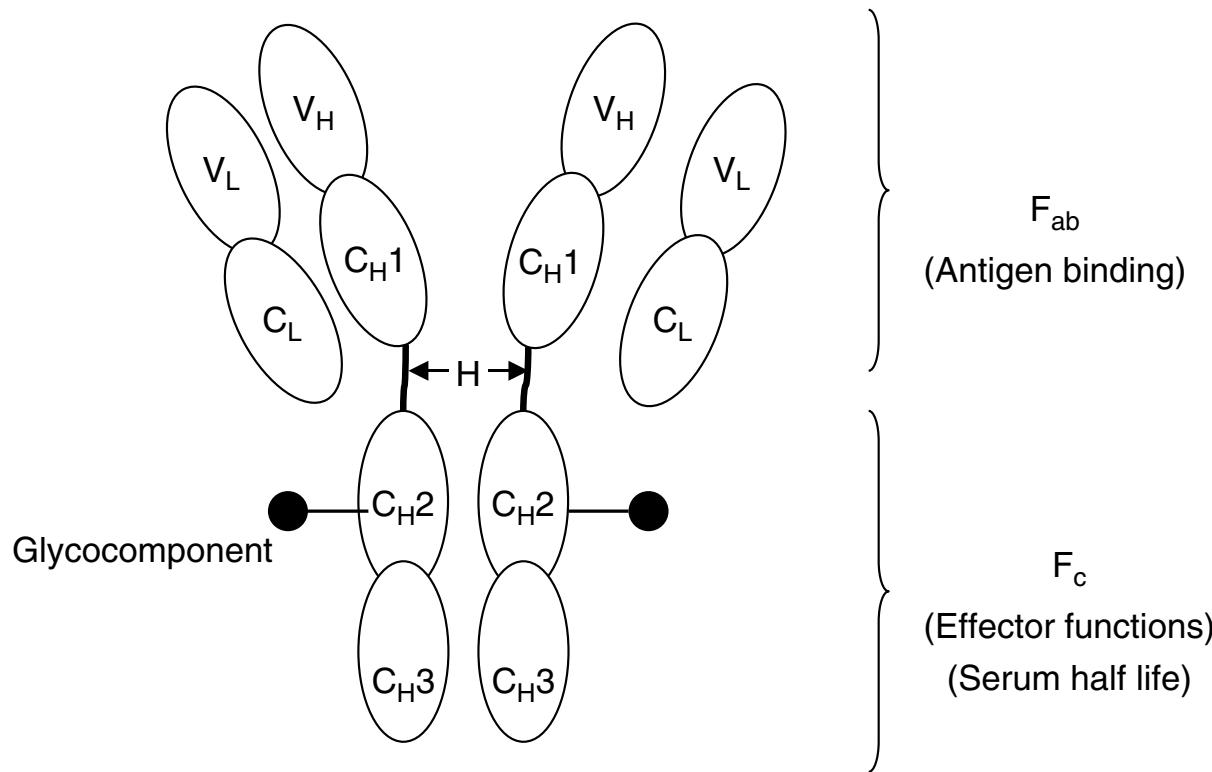
Proline racemase reaction



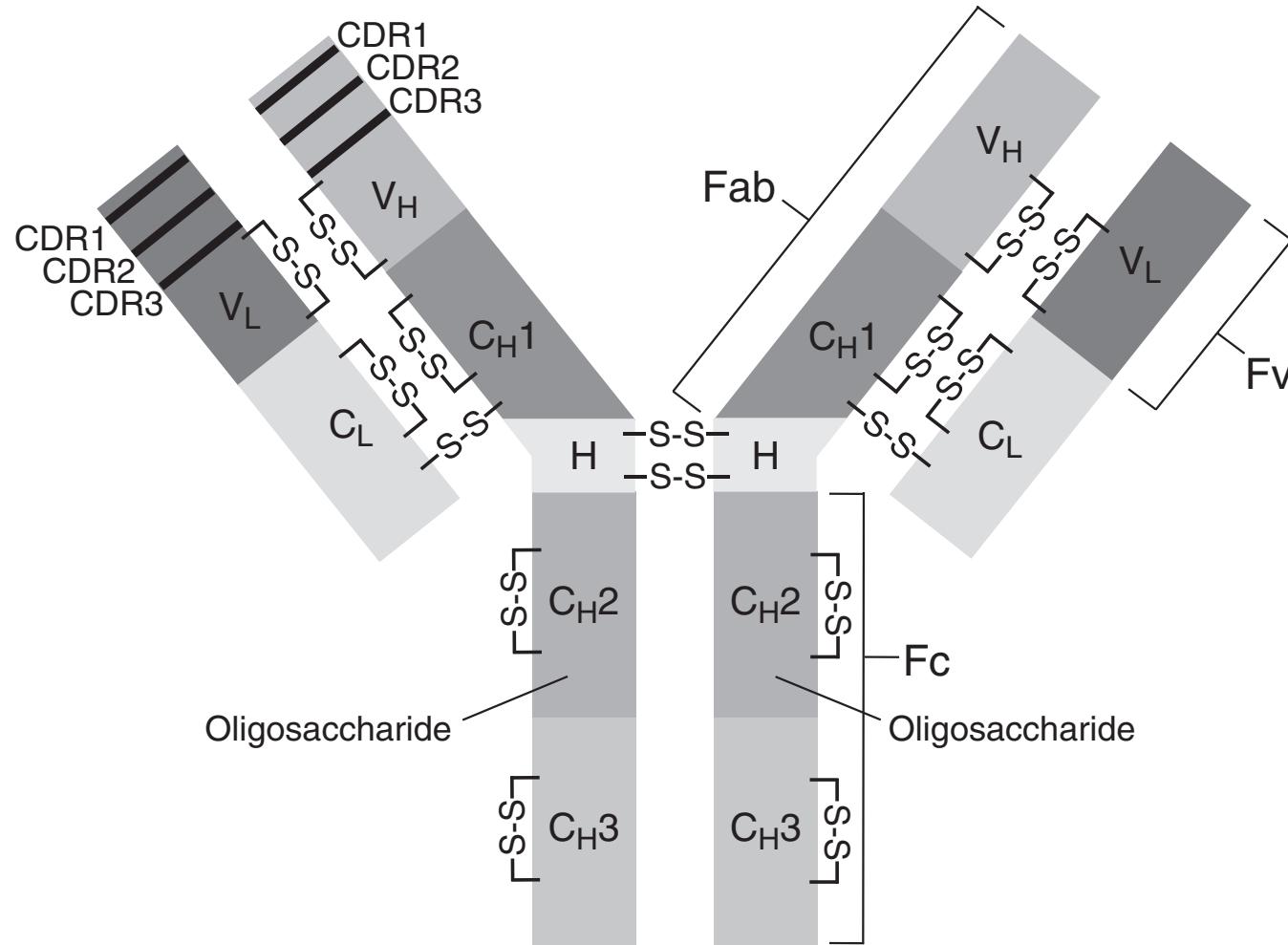
Pyrrole-2-carboxylate
(inhibitor and transition state analog)

Abzymes (Catalytic antibodies)

- Antibodies that are produced against a transition-state analog and that have catalytic activity similar to that of a naturally occurring enzyme



Antibody Structure



Antibody Structure

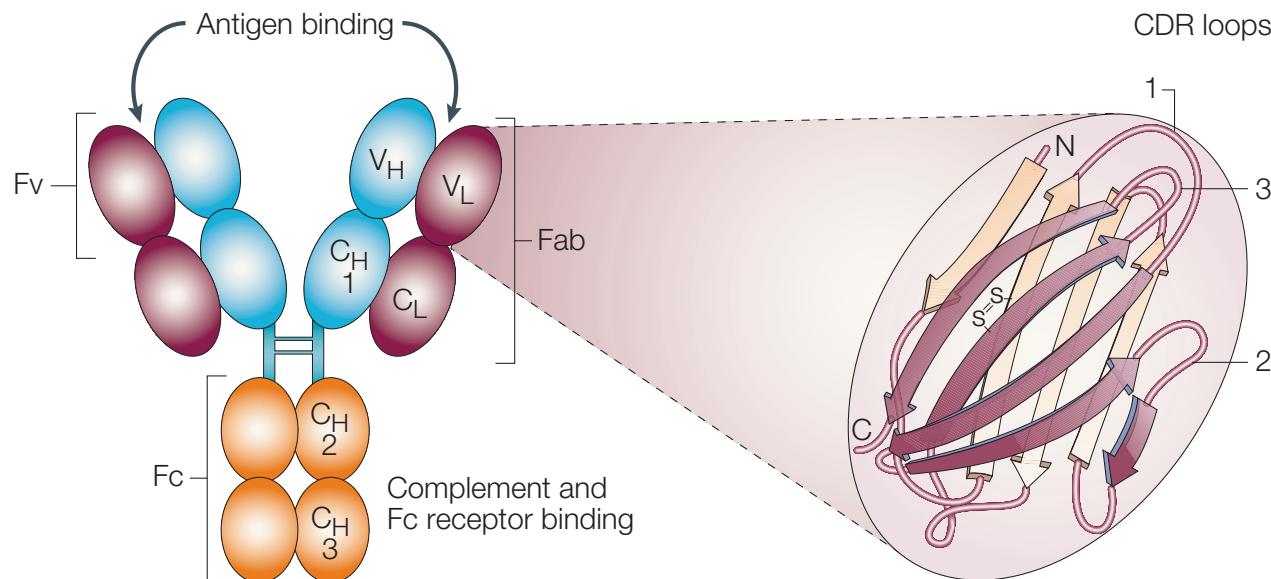
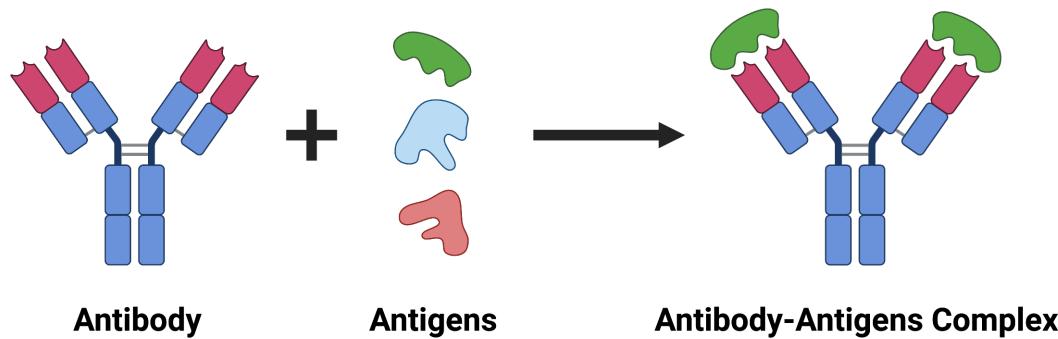


Figure 1 | The modular structure of immunoglobulins. This figure shows a single immunoglobulin (Ig) molecule. All immunoglobulin monomers are composed of two identical light (L) chains and two identical heavy (H) chains. Light chains are composed of one constant domain (C_L) and one variable domain (V_L), whereas heavy chains are composed of three constant domains ($C_H 1$, $C_H 2$ and $C_H 3$) and one variable domain (V_H). The heavy chains are covalently linked in the hinge region and the light chains are covalently linked to the heavy chain. The variable domains of both the heavy and light chains compose the antigen-binding part of the molecule, termed Fv. Within the variable domains there are three loops designated complementarity-determining regions (CDRs) 1, 2 and 3, which confer the highest diversity and define the specificity of antibody binding. The Fc portion is glycosylated and contains the sites for interaction with effector molecules, such as the C1 complex of the complement system and a variety of Fc receptors including the neonatal Fc receptor (FcRn).

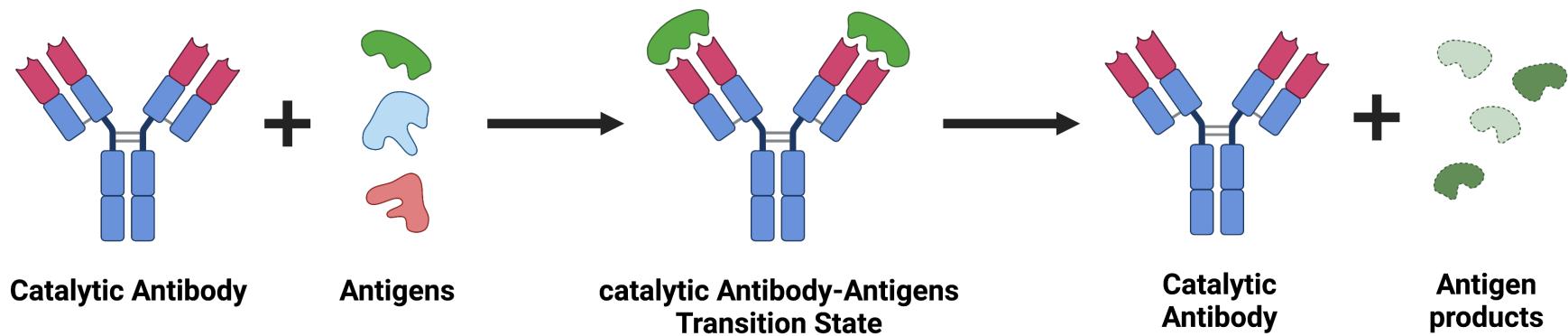
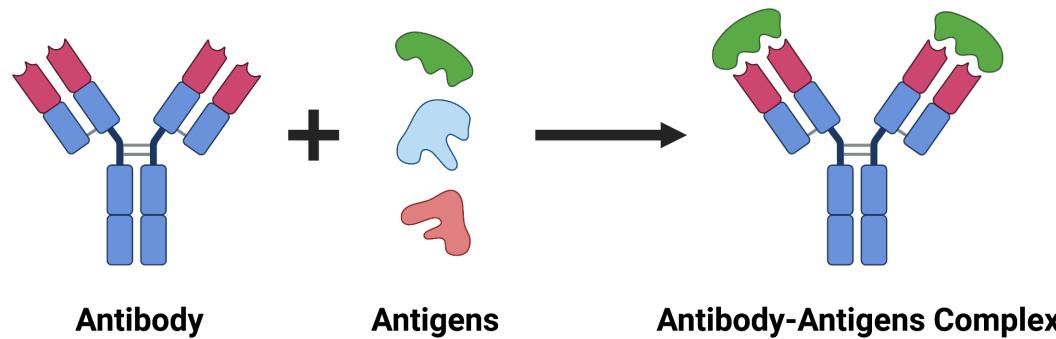
Abzymes (Catalytic antibodies)

- Antibodies that are produced against a transition-state analog and that have catalytic activity similar to that of a naturally occurring enzyme



Abzymes (Catalytic antibodies)

- Antibodies that are produced against a transition-state analog and that have catalytic activity similar to that of a naturally occurring enzyme



Coenzymes

- **Coenzyme:** a non protein substance that takes part in an enzymatic reaction and is regenerated for further reaction
 - **Metal ions** are Lewis acid (electron pair acceptor) and can behave as coordination compounds. (Zn^{2+} , fe^{2+})
 - Aid in positioning the group involved in reaction for optimal catalysis
 - **Organic compounds**, many of which are vitamins or are metabolically related to vitamins

Coenzymes

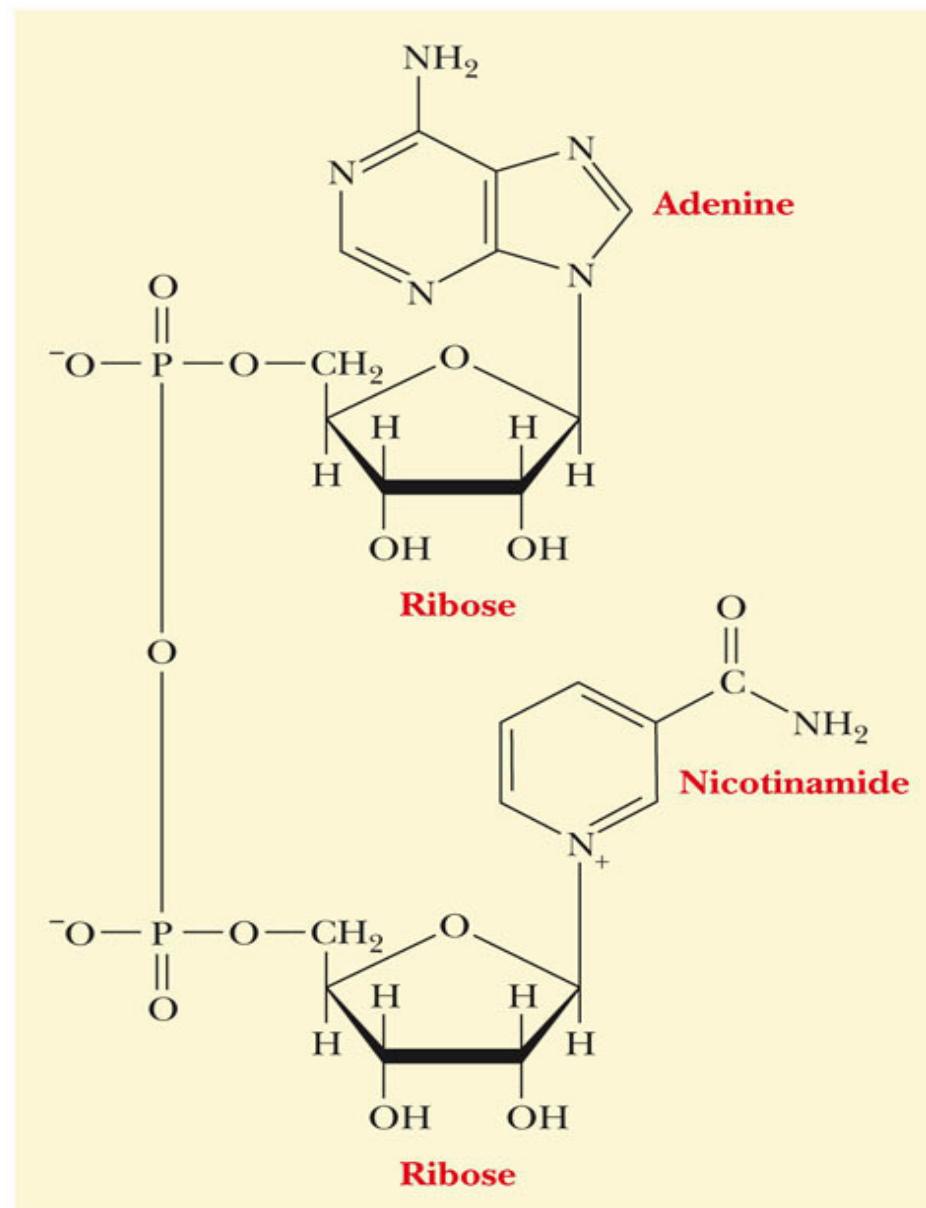
Table 7.1

Coenzymes, Their Reactions, and Their Vitamin Precursors

Coenzyme	Reaction Type	Vitamin Precursor	See Section
Biotin	Carboxylation	Biotin	18.2, 21.6
Coenzyme A	Acyl transfer	Pantothenic acid	15.7, 19.3, 21.6
Flavin coenzymes	Oxidation-reduction	Riboflavin (B ₂)	15.7, 19.3
Lipoic acid	Acyl transfer	—	19.3
Nicotinamide adenine coenzymes	Oxidation-reduction	Niacin	15.7, 17.3, 19.3
Pyridoxal phosphate	Transamination	Pyridoxine (B ₆)	23.4
Tetrahydrofolic acid	Transfer of one-carbon units	Folic acid	23.4
Thiamine pyrophosphate	Aldehyde transfer	Thiamine (B ₁)	17.4, 18.4

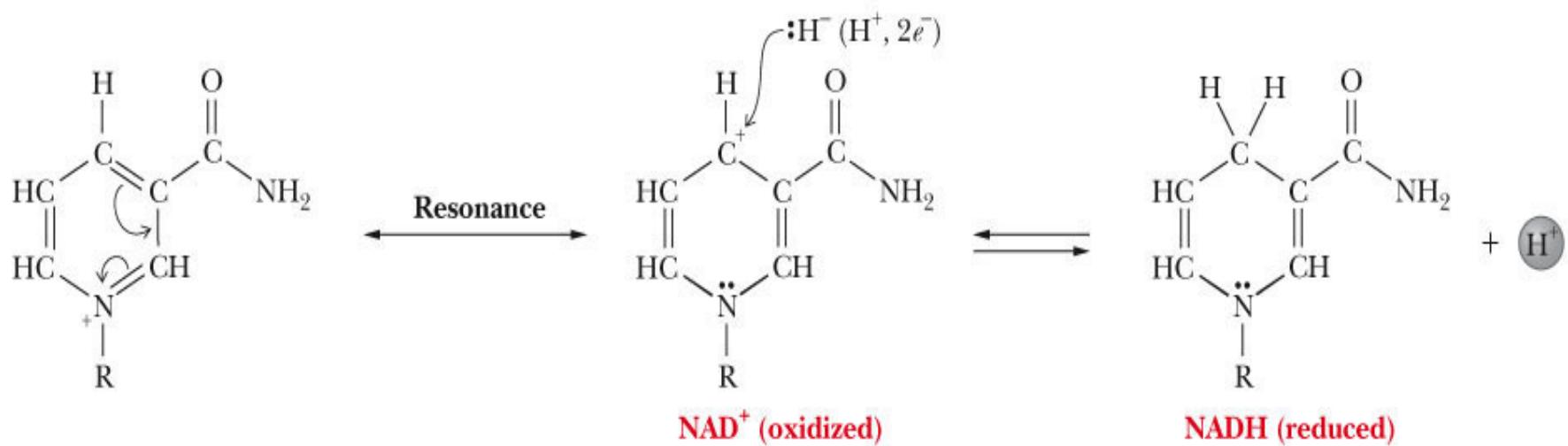
NAD+/NADH

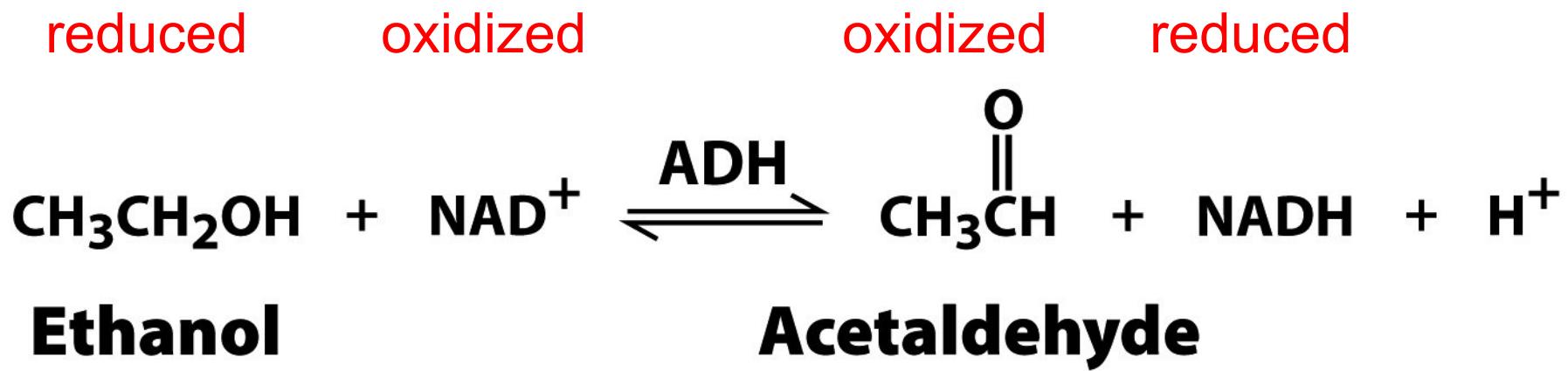
- Niacin vitamin or nicotinic acid
- Nicotinamide adenine dinucleotide (NAD⁺) involved in oxidation reduction reactions
- **Contains:**
 - Nicotinamide ring
 - Adenine ring
 - 2 sugar-phosphate groups



NAD⁺/NADH

- NAD⁺ is a two-electron oxidizing agent, and is reduced to NADH

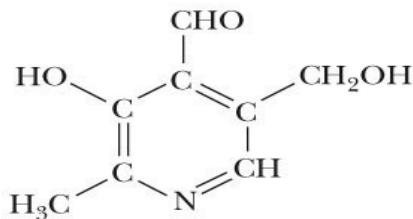




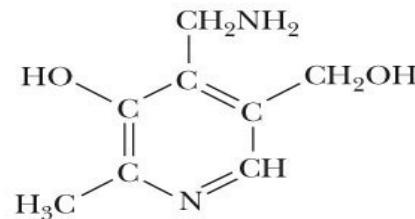
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B6 Vitamins

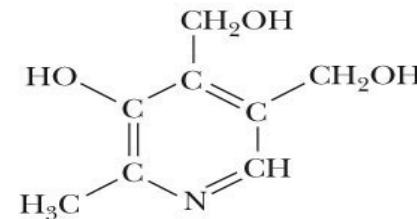
- **B6 vitamins** are coenzymes involved in (**transamination**) transfer of amino group from one molecule to another.
- From donor to coenzyme then from coenzyme to acceptor
- Important in amino acid biosynthesis



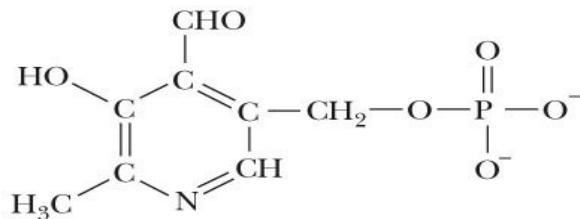
Pyridoxal



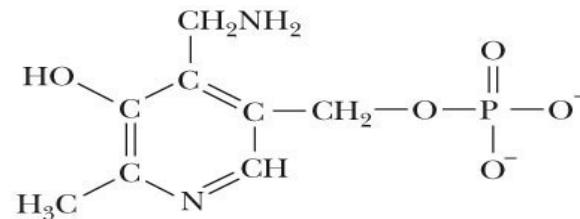
Pyridoxamine



Pyridoxine



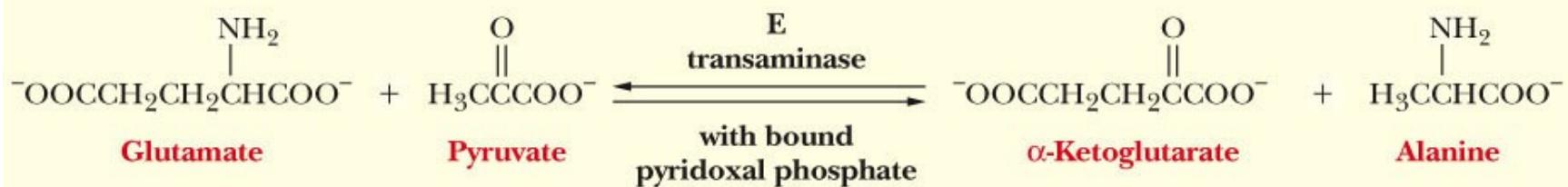
Pyridoxal phosphate



Pyridoxamine phosphate

Pyridoxal Phosphate

Pyridoxal and pyridoxamine phosphates are involved in the transfer of amino group in a reaction called **transamination**



This amino (NH_2) group transfer reaction occurs in two stages:

