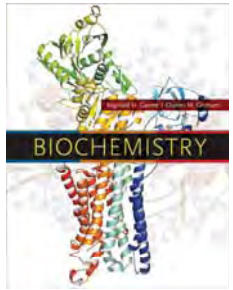
Chapter 14



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Mechanisms of Enzyme Action

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Essential Questions

- Before this class, ask your self the following questions:
  - How fast an enzyme could accelerate a reaction?
  - Why enzyme could accelerate a reaction?
  - How enzymes could accelerate a reaction?
  - What are the universal chemical principles that influence the mechanisms of enzymes and allow us to understand their enormous catalytic power?

Outline

- Part I: The general concepts of enzyme catalysis
  - What are the magnitudes of enzyme-induced rate accelerations?
  - What role does transition-state stabilization play in enzyme catalysis?
  - How does destabilization of ES affect enzyme catalysis?
  - How tightly do transition-state analogs bind to the active site?
- Part II: The mechanisms of catalysis
- Part III: What can be learned from typical enzyme mechanisms?
  - 3 examples: serine protease, aspartic protease and chorismate mutase!

How Much Enzyme-Induced Rate Accelerations?

- Typically $10^7 – 10^{14}$ greater
- Why? 2 reasons….
  - Stabilization of transition state
  - Destabilization of ES
    - due to strain, desolvation or electrostatic effects
- How? 5 catalytic mechanisms….
  - covalent catalysis
  - general acid or base catalysis
  - Low-Barrier H-bonds
  - metal ion catalysis
  - proximity/orientation (Near-Attack Conformation).

14.1 What Are the Magnitudes of Enzyme-Induced Rate Accelerations?

TABLE 14.1 A Comparison of Enzyme-Catalyzed Reactions and Their Uncatalyzed Counterparts

Reaction	Enzyme	Uncatalyzed Rate, v_u (sec^{-1})	Catalyzed Rate, v_e (sec^{-1})	v_e/v_u
Fructose-1,6-bisP ⟶ fructose-6-P + P_i	Fructose-1,6-bisphosphatase	2×10^{-20}	21	1.05×10^{21}
$(Glucose)_n + H_2O$ ⟶ $(glucose)_{n-2}$ + maltose	β-amylase	1.9×10^{-15}	1.4×10^{3}	7.2×10^{17}
DNA, RNA cleavage	Staphylococcal nuclease	7×10^{-16}	95	1.4×10^{17}
$CH_3-O-PO_3^{2-} + H_2O$ ⟶ $CH_3OH + HPO_4^{2-}$	Alkaline phosphatase	1×10^{-15}	14	1.4×10^{16}
$H_2N-C(=O)-NH_2 + 2\,H_2O + H^+$ ⟶ $2\,NH_4^+ + HCO_3^-$	Urease	3×10^{-10}	3×10^{4}	1×10^{14}
$R-C(=O)-O-CH_2CH_3 + H_2O$ ⟶ $RCOOH + HOCH_2CH_3$	Chymotrypsin	1×10^{-10}	1×10^{2}	1×10^{12}
Glucose + ATP ⟶ Glucose-6-P + ADP	Hexokinase	$<1 \times 10^{-13}$	1.3×10^{-5}	$>1.3 \times 10^{10}$
$CH_3CH_2OH + NAD^+$ ⟶ $CH_3C(=O)H + NADH + H^+$	Alcohol dehydrogenase	$<6 \times 10^{-12}$	2.7×10^{-5}	$>4.5 \times 10^{6}$
$CO_2 + H_2O$ ⟶ $HCO_3^- + H^+$	Carbonic anhydrase	10^{-2}	10^{5}	1×10^{7}
Creatine + ATP ⟶ Co-P + ADP	Creatine kinase	$<3 \times 10^{-9}$	4×10^{-3}	$>1.33 \times 10^{6}$

What is transition state?

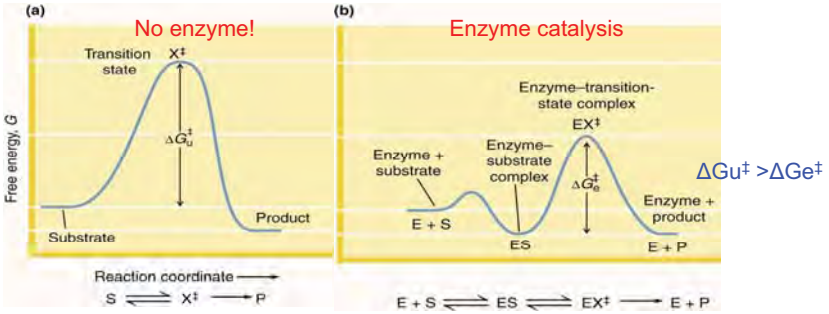
- The structure represents, as nearly as possible, the transition between the reactants and products, and it is known as the **transition state.**
- Transition =\= intermediates (*Ex*: ES or EP)
  - Intermediates are longer-lived, with lifetimes in the range of 10^{-13} sec to 10^{-3} sec.
  - A typical transition state has very short lifetime, typically 10^{-13} sec.

$$H\text{-}O\text{-}H + Cl^- \rightleftharpoons \left[H\text{-}O^{\delta-}\cdots H\cdots Cl^{\delta-}\right] \rightleftharpoons HO^- + H\text{-}Cl$$

Transition state existed even there is no enzyme

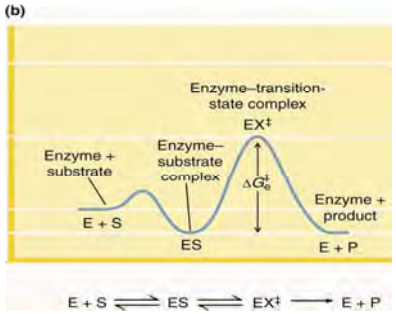
Reason 1: Transition-State Stabilization

- With enzyme, the transition state changed from $X^‡$ to $EX^‡$
- Activation energy difference ($\Delta G^‡$) between ES and $EX^‡$ is smaller than between S and $X^‡$, therefore the catalyzed rate of product formation will be faster.



ΔGu‡ >ΔGe‡

Reason 2: Destabilize ES complex



The dissociation constant for the enzyme –substrate complex

$$ES \xrightarrow{Ks} E+S$$

$$Ks = \frac{[E]\ [S]}{[ES]}$$

The dissociation constant for the transition-state complex

$$EX^‡ \xrightarrow{K_T} E+X^‡$$

$$K_T = \frac{[E]\ [X^‡]}{[EX^‡]}$$

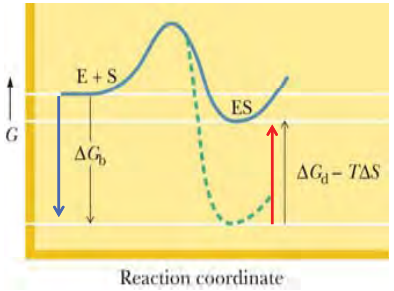
Enzyme catalysis requires To stabilize $EX^‡$ more! ⇒ $K_T < K_s$

Dissociation of ES facilitates reaction → $ke/ku \approx K_S/K_T$

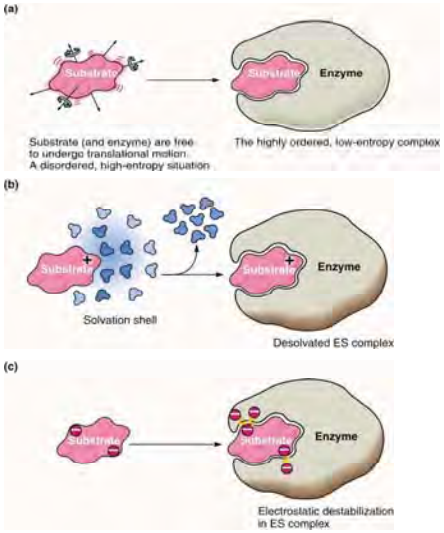
The binding of S to E must be favorable but not too favorable!

- ES cannot be "too tight" – The idea is to make the energy barrier between ES and EX‡ small!

- **Intrinsic binding energy** ΔG_b
  - Some amino acid of enzyme favor to bind substrate, making ΔG_b negative!
- **Compensation**
  - **Entropy loss** due to the binding of E and S ($T\,\Delta S$)
  - **Destabilization** of ES (ΔG_d) by strain, distortion, desolvation , and similar effects.



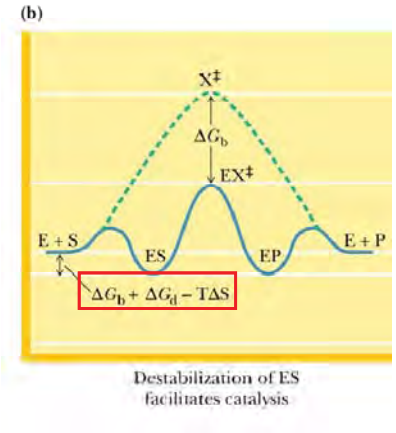
Destabilization of ES



Strain

Desolvation

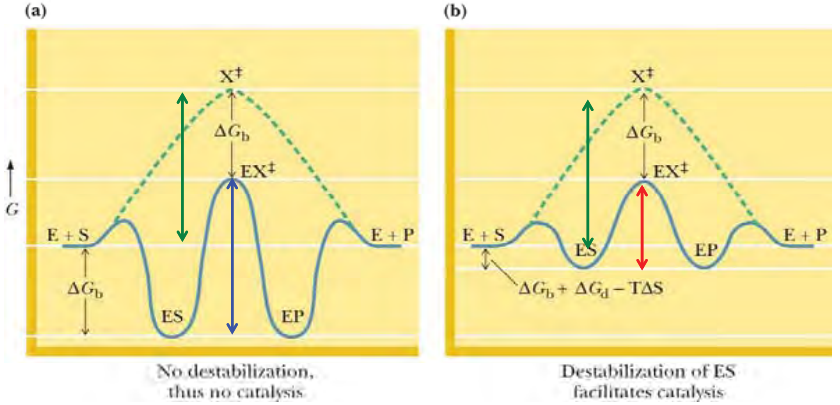
Distortion



- Net energy difference between E + S and the ES complex is the sum of ….
  1. intrinsic binding energy, ΔG_b
  2. the entropy loss on binding, $-T\,\Delta S$
  3. the distortion energy, ΔG_d.

No Destabilization of ES No Catalysis

- Raising the energy of ES raises the rate!

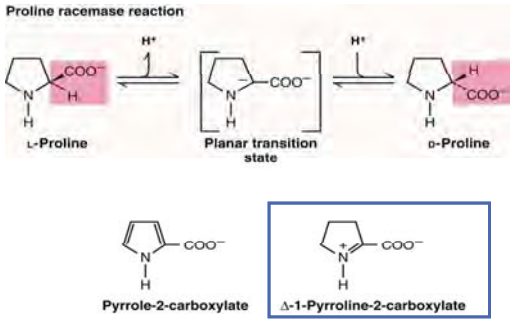


14.4 How Tightly Do Transition-State Analogs Bind to the Active Site?

- To have better ke/ku value, K_S/K_T should be big!
  - K_T should be small
  - The affinity of the enzyme for the transition state may be 10^{-20} to 10^{-26} M!
- Can we prove such kind of tight binding?
- Transition state analogs (TSAs)
  - are stable molecules that are chemically and structurally similar to the transition state
  - bind more strongly than a substrate
  - transition-state analogs are potent enzyme inhibitors

First TSA case: Proline racemase

- Proline racemase, a bacterial enzyme, catalyzes the interconversion of D and L-proline.
- The TSA, *pyrrole-2-carboxylate*, bound to the enzyme 160 times more tightly than L-proline



TSAs are also very strong inhibitors

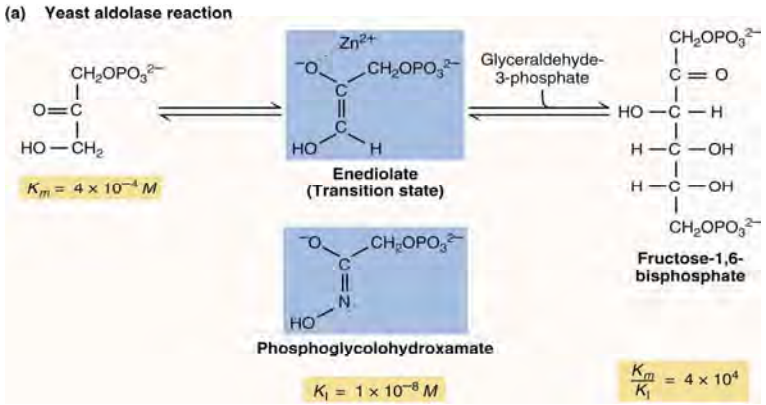
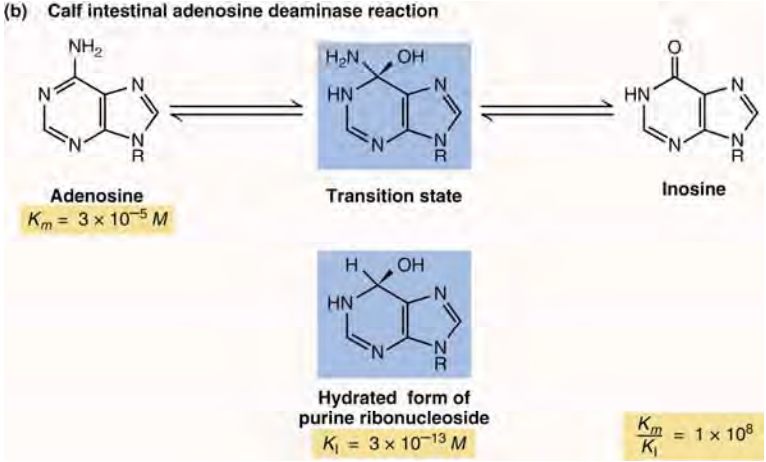


Figure 14.6 (a) Phosphoglycolohydroxamate is an analog of the enediolate transition state of the yeast aldolase reaction.



(b) Purine riboside inhibits adenosine deaminase. The hydrated form is an analog of the transition state of the reaction.

Transition-State Analogs Make Our World Better

- Enzymes are often targets for drugs and other beneficial agents
- Transition state analogs often make ideal enzyme inhibitors
  - Enalapril and Aliskiren lower blood pressure
  - Statins lower serum cholesterol
  - Protease inhibitors are AIDS drugs
  - Juvenile hormone esterase is a pesticide target
  - Tamiflu is a viral neuraminidase inhibitor

End of Part 1

- Ask yourself…
  - What is transition state?
  - What is intermediate?
  - What is the two reasons that enzyme could accelerate reaction?
  - What effects could destabilize ES complex?
  - What is TSA? What can TSA tell us?

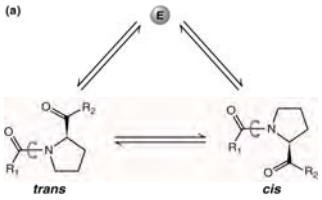
14.5 What Are the Mechanisms of Catalysis?

- Protein motions are essential to enzyme catalysis
- 5 major mechanisms:
  - Enzymes facilitate formation of near-attack complexes
  - Covalent catalysis
  - General acid-base catalysis
  - Low-barrier hydrogen bonds
  - Metal ion catalysis

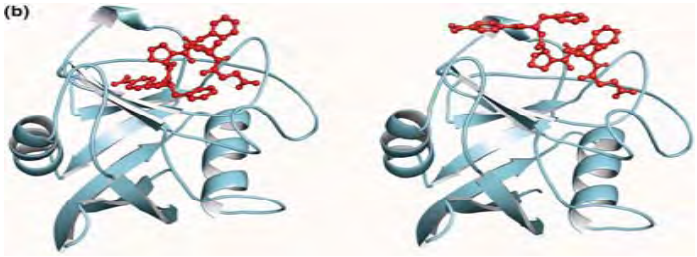
Protein Motions Are Essential to Enzyme Catalysis

- Proteins are constantly moving
  - bonds vibrate, side chains bend and rotate, backbone loops wiggle and sway, and whole domains move as a unit
- Protein motions support catalysis in several ways. Active site conformation changes can:
  - Assist substrate binding
  - Bring catalytic groups into position
  - Induce formation of NACs
  - Assist in bond making and bond breaking
  - Facilitate conversion of substrate to product

Example: Human cyclophilin A

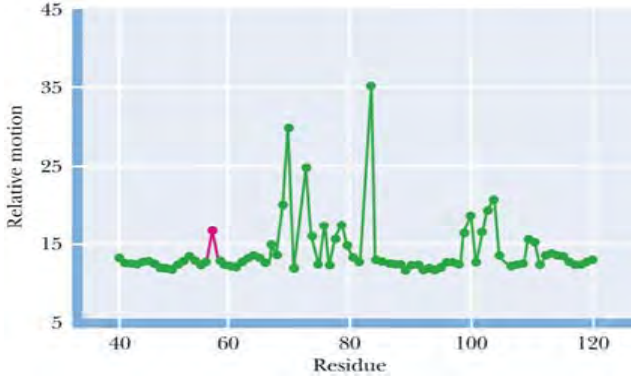


a **prolyl isomerase**, which catalyzes the interconversion between *trans* and *cis* conformations of proline in peptides.



Evident of enzyme movement

NMR showed that several active-site residues undergo greater motion during catalysis than residues elsewhere in the protein.



Some Review of General Chemistry

- The custom of writing chemical reaction by Gilbert Newton Lewis and Sir Robert Robinson
- Review of the concepts
  - Lewis dot structures
  - Valence electrons and formal charge
  - Formal charge = group number – nonbonding electrons – (1/2 shared electrons)
  - Electronegativity is also important:
  - F > O > N > C > H

How to read and write mechanisms Step 1

- In written mechanisms, a curved arrow shows the movement of an electron pair
- And thus the movement of a pair of electrons from a filled orbital to an empty one
- A full arrowhead represents an electron pair
- A half arrowhead represents a single electron

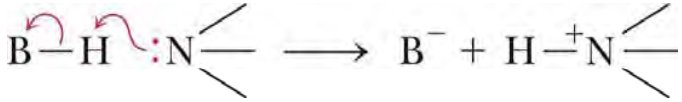
How to read and write mechanisms Step 2

For a bond-breaking event, the arrow begins in the middle of the bond, and the arrow points to the atom that will accept the electrons.

For a bond-making event, the arrow begins at the source of the electrons (for example, a nonbonded pair), and the arrowhead points to the atom where the new bond will be formed.

How to read and write mechanisms Step 3

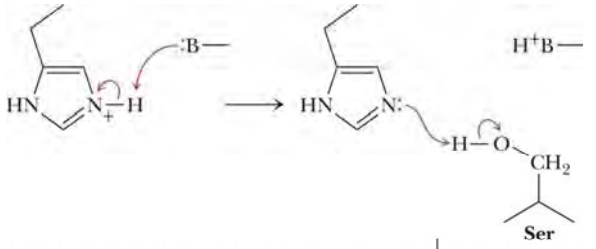
- It has been estimated that 75% of the steps in enzyme reaction mechanisms are proton (H^+) transfers.
- If the proton is donated or accepted by a group on the enzyme, it is often convenient (and traditional) to represent the group as "B", for "base", even if B is protonated and behaving as an acid:



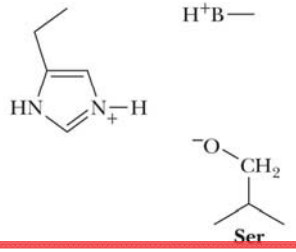
How to read and write mechanisms Step 4

- It is important to appreciate that a proton transfer can change a nucleophile into an electrophile, and vice versa.
- Thus, it is necessary to consider:
  - The protonation states of substrate and active-site residues
  - How pK_a values can change in the environment of the active site

How to read and write mechanisms

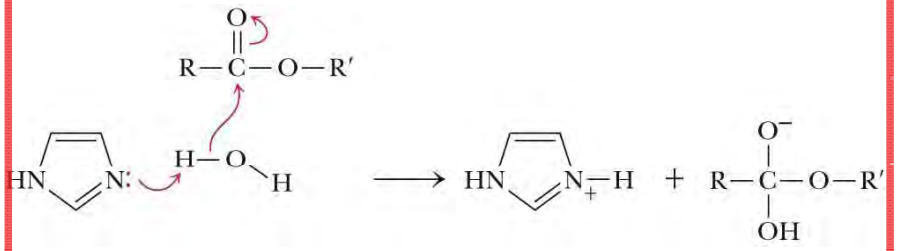


An active-site histidine, which might normally be protonated, can be deprotonated by another group and then act as a base, accepting a proton from the substrate.



How to read and write mechanisms

Water can often act as an acid or base at the active site through proton transfer with an assisting active-site residue:



This type of chemistry is the basis for general acid-base catalysis (discussed on pages 430-431).

Mechanism 1: Near-attack complexes

- X-ray crystal structure studies have shown that the reacting atoms and catalytic groups are precisely positioned for their roles.
- The preorganization
  - selects substrate conformations
  - the reacting atoms are in van der Waals contact
  - at an angle resembling the bond to be formed in the transition state
- Thomas Bruice has termed such arrangements **near-attack conformations (NACs)**
- NACs are precursors to reaction transition states

Enzymes facilitate formation of near-attack complexes

- In the absence of an enzyme, potential reactant molecules adopt a NAC only about 0.0001% of the time
- On the other hand, NACs have been shown to form in enzyme active sites from 1% to 70% of the time

Requirements of the formation of near-attack complexes

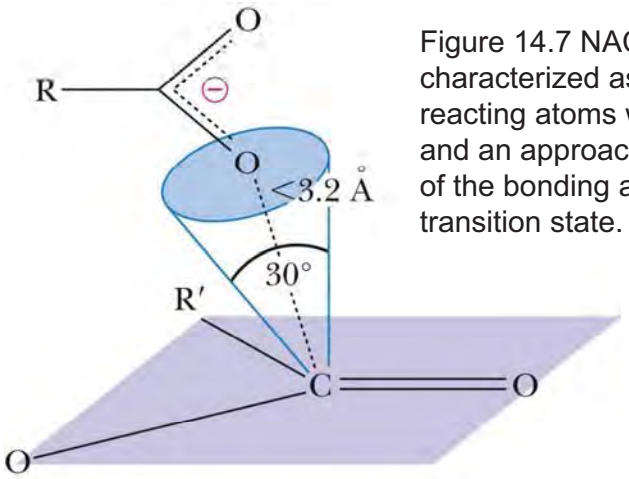
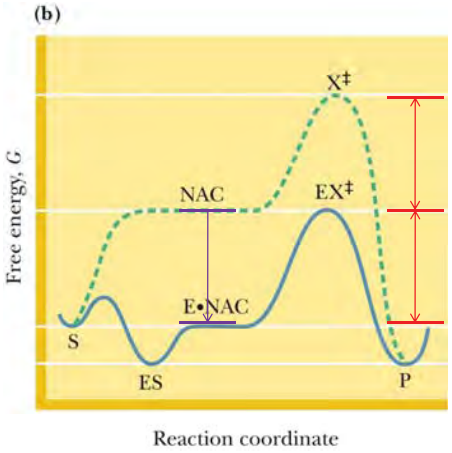


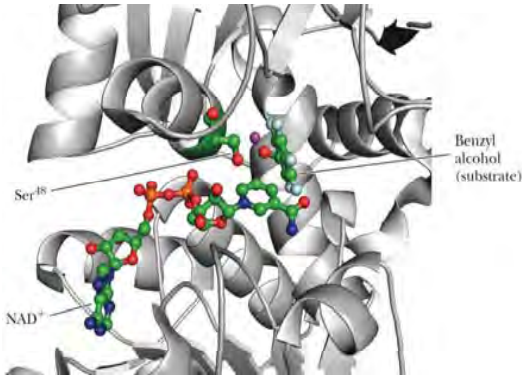
Figure 14.7 NACs are characterized as having reacting atoms within 3.2 Å and an approach angle of ±15° of the bonding angle in the transition state.

Enzymes facilitate formation of near-attack complexes

- The energy separation between the NAC and the transition state is approximately the same in the presence and absence of the enzyme.
- In an enzyme active site, the NAC forms more readily than in the uncatalyzed reaction.



The active site of liver alcohol dehydrogenase – a near-attack complex.



- An ordered, single-displacement Rx.
- Intermediate exists as a NAC 60% of the time

Mechanism 2: Covalent Catalysis

- Some enzyme reactions derive much of their rate acceleration from the formation of **covalent bonds** between enzyme and substrate

$$BX + Y \rightarrow BY + X$$

$$BX + Enz \rightarrow E : B + X + Y \rightarrow Enz + BY$$

covalent intermediate

Mechanism of Covalent catalysis

- The side chains of amino acids in proteins (Enzyme) offer a variety of nucleophilic centers for catalysis, including amines, carboxylates, aryl and alkyl hydroxyls, imidazoles, and thiol groups.
- These groups readily attack electrophilic centers of substrates, forming covalently bonded enzyme-substrate intermediates.
- The covalent intermediate can be attacked in a second step by water or by a second substrate, forming the desired product

Nucleophilic groups in enzymes

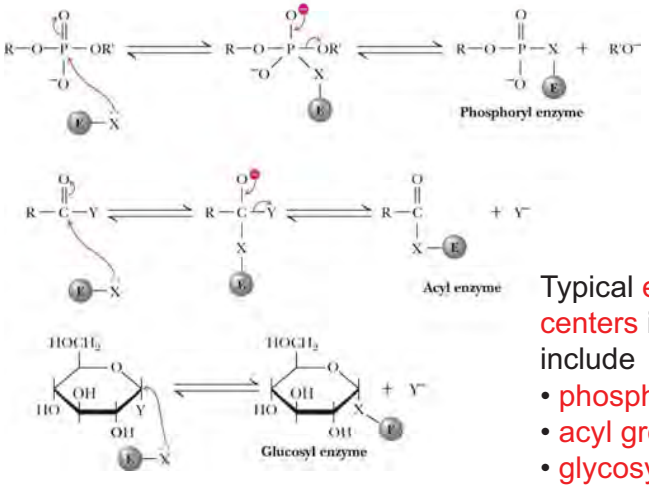
Nucleophilic group	Amino Acid
OH	Serine
SH	Cysteine
COO-	Aspartic acid
NH2	Lysine
imidazole	Histidine

Enzymes that form covalent intermediates

TABLE 14.2 Enzymes That Form Covalent Intermediates

Enzyme	Reacting Group	Covalent Intermediate
Trypsin Chymotrypsin (pages 434–439)	Serine	Acyl-Ser
Glyceraldehyde-3-P dehydrogenase (page 547)	Cysteine	Acyl-Cys
Phosphoglucomutase (page 447)	Serine	Phospho-Ser
Phosphoglycerate mutase (page 548) Succinyl-CoA synthetase (page 576)	Histidine	Phospho-His
Aldolase (page 545) Pyridoxal phosphate enzymes (pages 408, 782, and 807)	Lysine and other amino groups	Schiff base

Example of Covalent Catalysis



Typical electrophilic centers in substrates include
- phosphoryl group
- acyl group
- glycosyl group

Mechanism 3: Acid - Base Catalysis

- There are two types of acid-base catalysis:
  (1) specific acid-base catalysis, in which H^+ or OH^- accelerates the reaction,
  (2) general acid-base catalysis, in which an acid or base other than H^+ or OH^- accelerates the reaction.

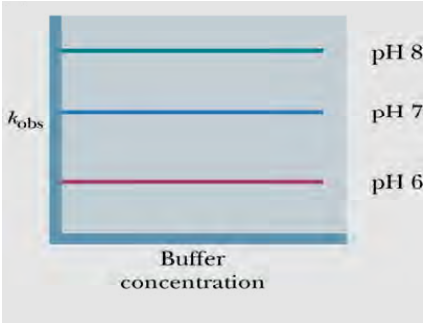
What are "acid and base" ?

	Acid	Base
• **Arrhenius**	H^+ forming	OH^- forming
• **Bronsted-Lowry**	H^+ donor	H^+ acceptor
• **Lewis**	e^- acceptor	e^- donor

See General chemistry chap 15

Specific acid or base catalysis

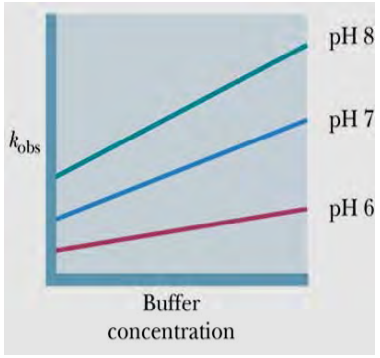
In specific acid or base catalysis, the buffer concentration has no effect.



In specific acid-base catalysis, H^+ or OH^- concentration affects the reaction rate, k_{obs} is pH-dependent, but buffers concentration (which accept or donate H^+/OH^-) have no effect.

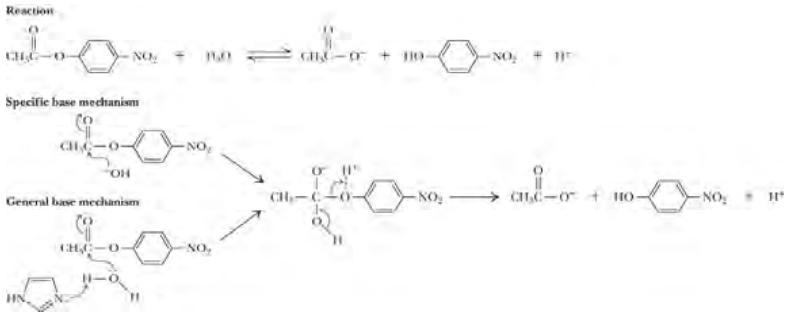
General acid-base catalysis

- *general acid-base catalysis is catalysis in which a **proton** is transferred in the **transition state.***



In general acid - base catalysis, in which an ionizable buffer may donate or accept a proton in the transition state, k_{obs} is dependent on buffer concentration.

Catalysis of p-nitrophenylacetate hydrolysis can occur either by specific acid hydrolysis or by general base catalysis



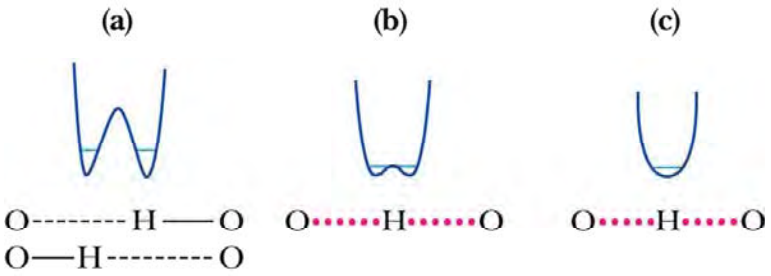
Histidine is most effective general acid or base because its pKa is near 7!

Mechanism 4: Low-Barrier Hydrogen Bonds (LBHBs)

- In normal hydrogen bonds (O:•••H–O), the O•••O separation is 2.8 Å
- In LBHBs, the O•••O separation decreases until the H atom becomes centered, leaving the H atom to freely exchange between the two O atoms
- pK_a values of the two electronegative atoms must be similar
- LBHB are very strong, transient intermediates that help to accelerate enzyme-catalyzed reactions
  - LBHB may be to redistribute electron density in the intermediate

Ex: Serine Proteases, Asp Protease

Low-Barrier Hydrogen Bonds (LBHBs)



O-H-O hydrogen bond of length 0.28 nm, with the hydrogen bond order for the stronger O-H interaction is approximately 1.0, and the weaker O-H interaction is 0.07.

the O-O distances is 0.25 nm

the O-O distance is 0.23 to 0.24 nm, and the bond order of each O-H interaction is 0.5.

Mechanism 5: Metal-Ion Catalysis

- Many enzymes require metal ions for maximal activity.
- metalloenzyme : enzyme binds the metal very tightly or requires the metal ion to maintain its stable, native state. (metal is coenzyme)
  - *e.g.* Fe, Cu, Zn, Mn, Co.
- metal-activated enzymes :Enzymes that bind metal ions more weakly, perhaps only during the catalytic cycle.
  - *e.g* Na, K, Mg, Ca

Functions of Metal-Ion

- One role for metals in metal-activated enzymes and metalloenzymes is to act as electrophilic catalysts, stabilizing the increased electron density or negative charge that can develop during reactions.
- Another potential function: coordination and increase the acidity of a nucleophile

Example of Metal Ion Catalysis

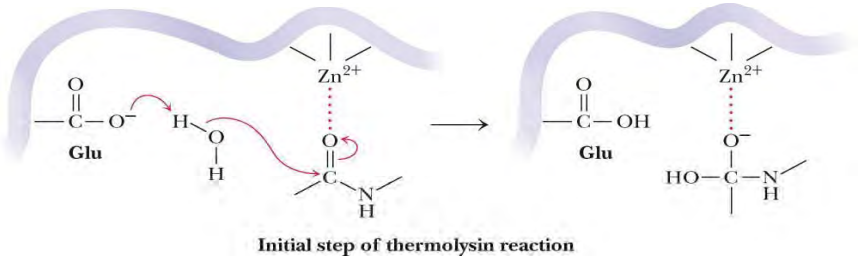


Figure 14.14 Thermolysin is an endoprotease with a catalytic Zn^{2+} ion in the active site. The Zn^{2+} ion stabilizes the buildup of negative charge on the peptide carbonyl oxygen, as a glutamate residue deprotonates water, promoting hydroxide attack on the carbonyl carbon.

How Do Active-Site Residues Interact to Support Catalysis?

- About half of the amino acids engage directly in catalytic effects in enzyme active sites
- Other residues may function in secondary roles in the active site:
  - Raising or lowering catalytic residue pK_a values
  - Orientation of catalytic residues
  - Charge stabilization
  - Proton transfers via hydrogen tunneling

End of Part 2

- Ask yourself…
  - What are general mechanism of enzyme catalysis?
  - Do you know how to read the reaction mechanism?
  - What are general nucleophilic groups in amino acid side chains?
  - What's different between specific and general acid-base reaction?
  - What are the functions of metal ions in enzyme catalysis?

14.5 What Can Be Learned From Typical Enzyme Mechanisms?

- Three typical enzymes to explain the mechanisms:
  - The serine protease:
    - covalent catalysis, general acid-base catalysis, substrate selectivity, LBHB
  - The aspartic protease:
    - general acid-base catalysis, LBHB
  - Chorismate mutase:
    - NAC

Example 1: The Serine Proteases Family

- All involve a serine in catalysis - thus the name
- Serine proteases includes
  - Trypsin, chymotrypsin, elastase are digestive enzymes and are synthesized in the pancreas and secreted into the digestive tract as inactive proenzymes, or zymogens.
  - Thrombin is a crucial enzyme in the blood-clotting cascade
  - Subtilisin is a bacterial protease
  - Plasmin breaks down the fibrin polymers of blood clots.
  - Tissue plasminogen activator (TPA) specifically cleaves the proenzyme *plasminogen*, yielding plasmin

zymogen

- Proenzyme or zymogen: is an inactive enzyme precursor.
- A zymogen requires a biochemical change (such as a hydrolysis reaction revealing the active site, or changing the configuration to reveal the active site) for it to become an active enzyme.
- Feed forward reaction (positive feedback)!

Catalytic Triad

- Ser is part of a "catalytic triad" of Ser, His, Asp
- Serine proteases are homologous, but locations of the three crucial residues differ somewhat
- Enzymologists agree to number the triad always as His^{57}, Asp^{102}, Ser^{195}

Similarity of 3 Serine Proteases

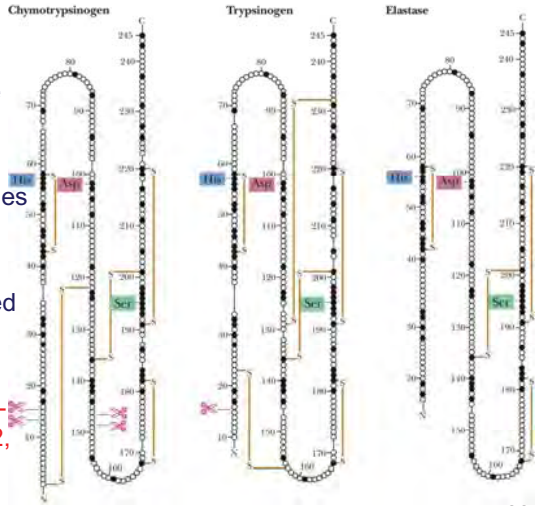
-Each circle represents one amino acid.

- Numbering is based on the sequence of chymotrypsinogen.

-Filled circles indicate residues that are identical in all three proteins.

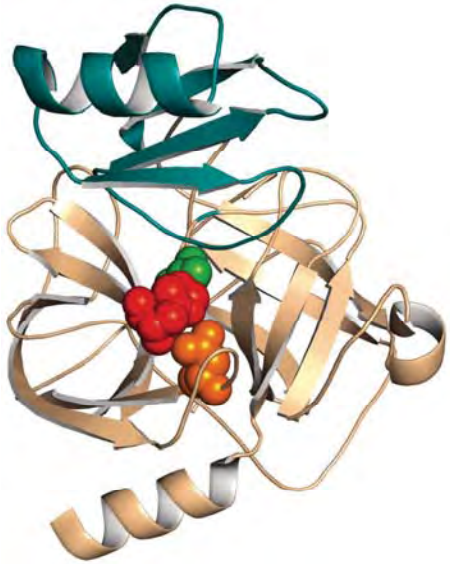
-Disulfide bonds are indicated in yellow.

-The positions of the three catalytically important active-site residues (His57, Asp102, and Ser195) are indicated.

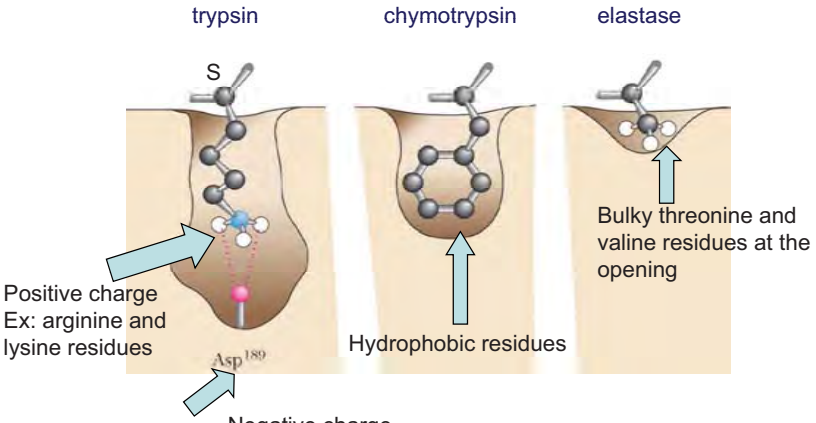


The Catalytic Triad of the Serine Proteases

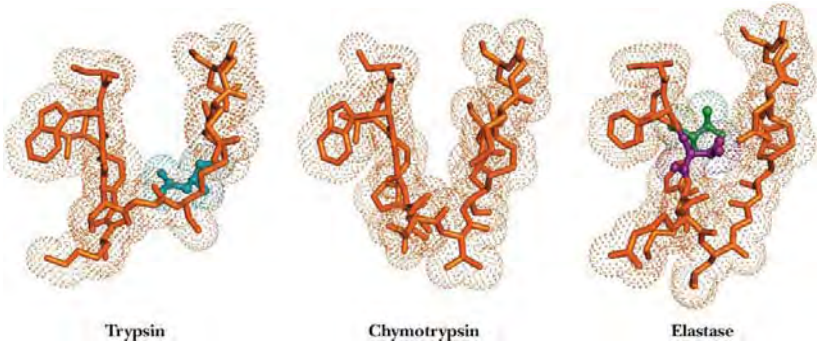
Figure 14.16 Structure of chymotrypsin (white) in a complex with eglin C (blue ribbon structure), a target substrate. His^{57} (red) is flanked by Asp^{102} (gold) and Ser^{195} (green). The catalytic site is filled by a peptide segment of eglin. Note how close Ser^{195} is to the peptide that would be cleaved in the reaction.



The substrate-binding pockets determine the substrate selectivity

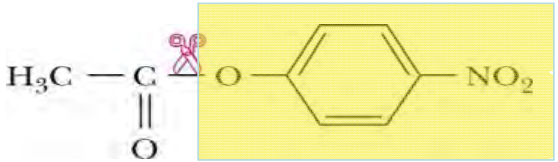


Serine Protease Binding Pockets are Adapted to Particular Substrates



How to assay the activity of protease?

- Serine Proteases Cleave Simple Organic Esters, such as p-Nitrophenylacetate

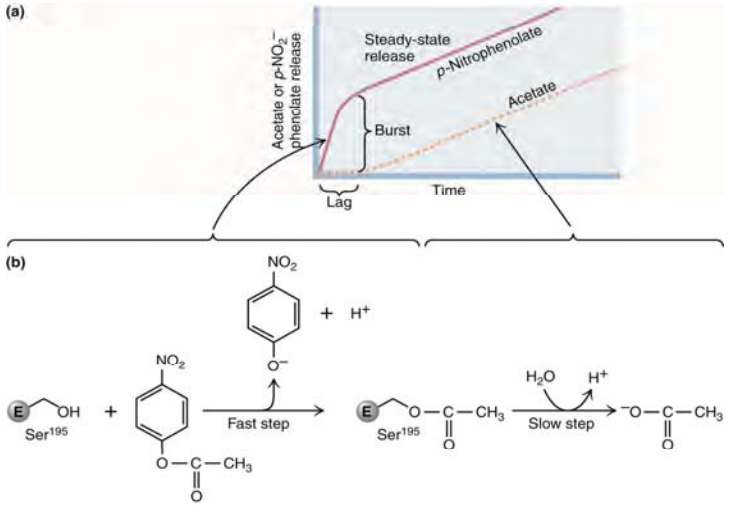


Release of this part give yellow color!

***p*-Nitrophenylacetate**

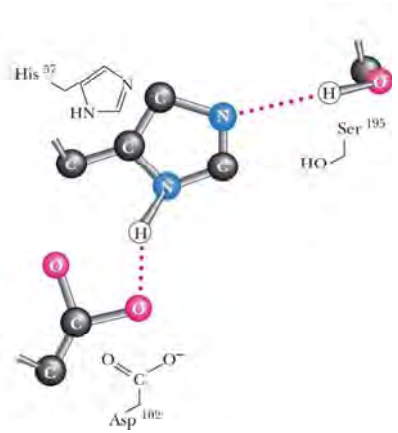
Which protease would cut it?

Burst kinetics tell the mechanism of serine protease

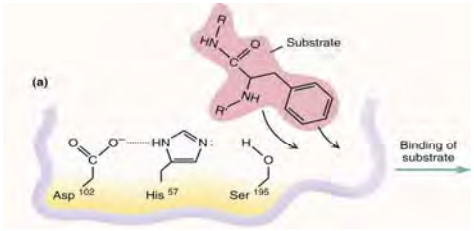


Serine Protease Mechanism

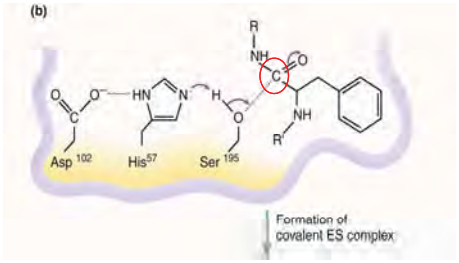
- *A mixture of covalent and general acid-base catalysis*
  - Asp[102] functions only to orient His[57]
  - His[57] acts as a general acid and base
  - Ser[195] forms a covalent bond with peptide to be cleaved



The Serine Protease Mechanism in Detail 1/5



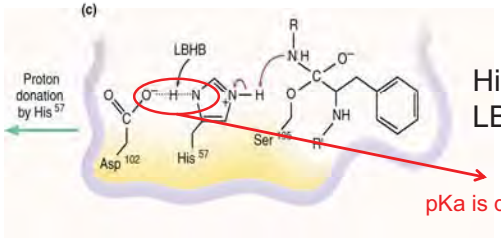
Binding of a model substrate.



Formation of the covalent ES complex involves general base catalysis by His[57]

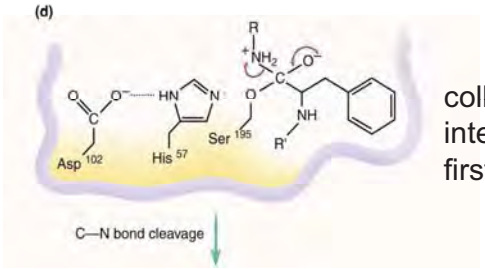
Covalent bond formation turns a trigonal C into a tetrahedral C

The Serine Protease Mechanism in Detail 2/5



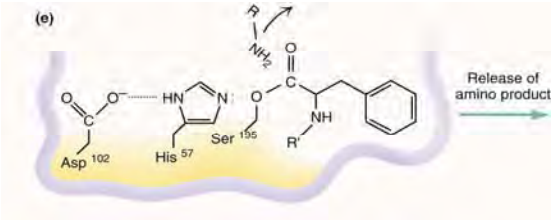
His[57] stabilized by a LBHB.

pKa is different, how LBHB formation?

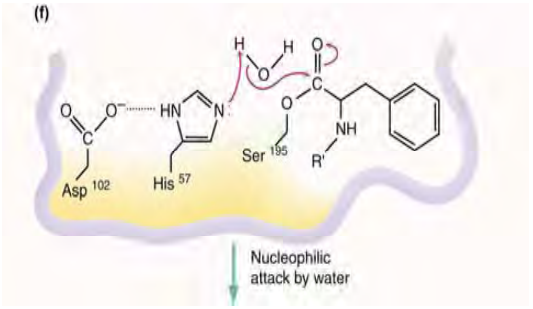


collapse of the tetrahedral intermediate releases the first product.

The Serine Protease Mechanism in Detail 3/5

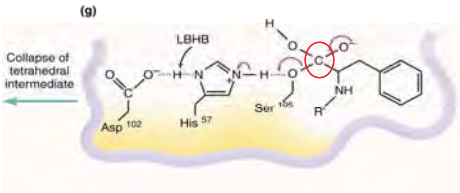


The amino product departs, making room for an entering water molecule.

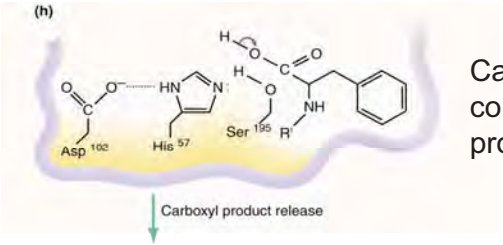


Nucleophilic attack by water is facilitated by His[57], acting as a general base.

The Serine Protease Mechanism in Detail 4/5



Collapse of the tetrahedral intermediate cleaves the covalent intermediate, releasing the second product.



Carboxyl product release completes the serine protease mechanism.

The Serine Protease Mechanism in Detail 5/5

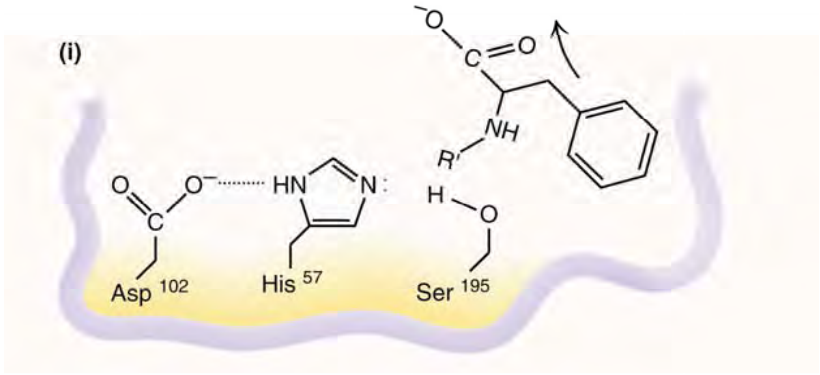
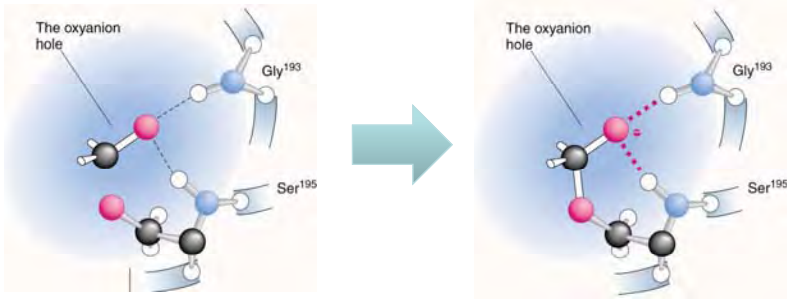


Figure 14.21 The chymotrypsin mechanism: At the completion of the reaction, the side chains of the catalytic triad are restored to their original states.

Transition-State Stabilization in the Serine Proteases

- The chymotrypsin mechanism involves two tetrahedral oxyanion intermediates
- These intermediates are stabilized by a pair of amide groups that is termed the "oxyanion hole"
- The amide N-H groups of Ser^{195} and Gly^{193} provide primary stabilization of the tetrahedral oxyanion

The "oxyanion hole"



The oxyanion hole of chymotrypsin stabilizes the tetrahedral oxyanion intermediate seen in the mechanism of Figure 14.21.

Example 2: The Aspartic Proteases

- All involve two Asp residues at the active site
- These two Asp residues work together as general acid-base catalysts
- Most aspartic proteases have a tertiary structure consisting of two lobes (N-terminal and C-terminal) with approximate two-fold symmetry

Aspartic proteases play many roles in humans

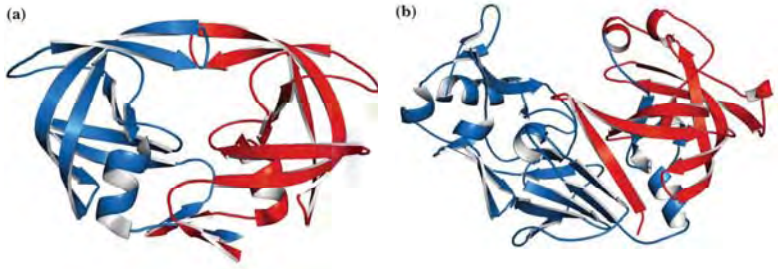
TABLE 14.3 Some Representative Aspartic Proteases

Name	Source	Function
Pepsin*	Stomach	Digestion of dietary protein
Chymosin†	Stomach	Digestion of dietary protein
Cathepsin D	Spleen, liver, and many other animal tissues	Lysosomal digestion of proteins
Renin‡	Kidney	Conversion of angiotensinogen to angiotensin I; regulation of blood pressure
HIV-protease§	AIDS virus	Processing of AIDS virus proteins

The Aspartic Proteases

Most aspartic proteases exhibit a two-lobed structure. Each lobe contributes one catalytic aspartate to the active site. HIV-1 protease is a homodimeric enzyme, with each subunit contributing a catalytic Asp residue.

Figure 14.22 Structures of (a) HIV-1 protease and (b) pepsin. Pepsin's N-terminal half is shown in red; the C-terminal half is shown in blue.



Aspartic Protease Mechanism

- Enzymologists said aspartic proteases is general acid-base catalysis
  - show one relatively low pK_a, and one relatively high pK_a
  - once thought to represent pK_a values of the two aspartate residues
- Structural Chemists said it is a LBHB catalysis
  - LBHB disperse electron density (electron tunnel)

A Mechanism for the Aspartic Proteases

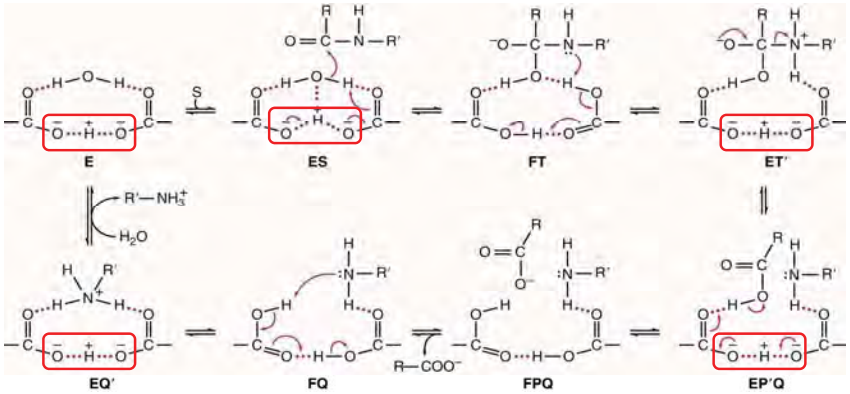


Figure 14.24 Mechanism for the aspartic proteases. LBHBs play a role in states E, ES, ET', EQ', and EP'Q.

HIV-1 Protease

- HIV-1 protease cleaves the polyprotein products of the HIV genome
- This is a remarkable imitation of mammalian aspartic proteases
- HIV-1 protease is a homodimer - more genetically economical for the virus
- Active site is two-fold symmetric

Proteolytic cleavage pattern for the HIV genome

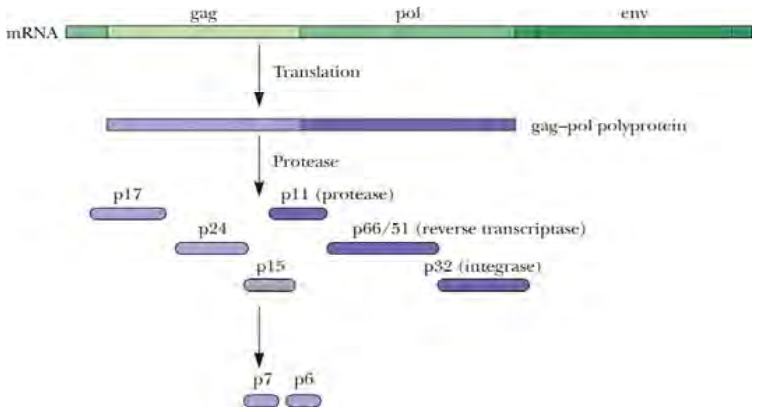


Figure 14.26 HIV mRNA provides the genetic information for synthesis of a polyprotein. Cleavage yields the active products.

Protease Inhibitors Block the Active Site of HIV-1 Protease

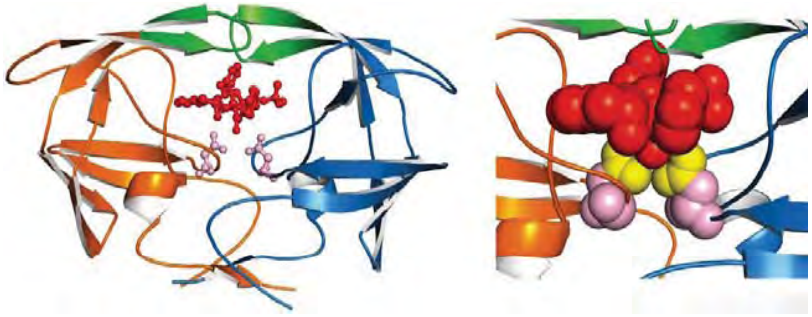


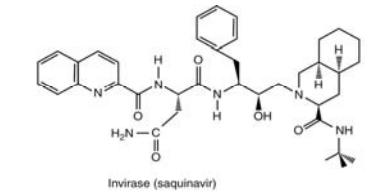
Figure 14.27 HIV-1 protease complexed with the inhibitor Crixivan (red) made by Merck. The "flaps" that cover the active site are green; the catalytic active site Asp residues are violet.

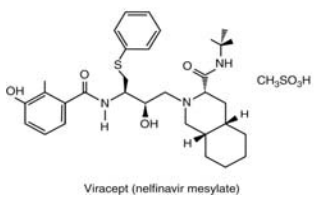
Protease Inhibitors Give Life to AIDS Patients

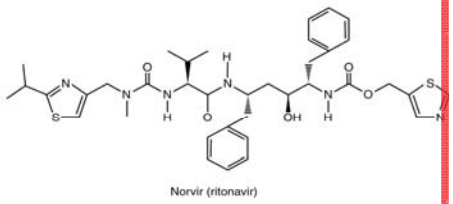
Protease inhibitors as AIDS drugs

- If the HIV-1 protease can be selectively inhibited, then new HIV particles cannot form
- Several novel protease inhibitors are currently marketed as AIDS drugs
- Many such inhibitors work in a culture dish
- However, a successful drug must be able to kill the virus in a human subject without blocking other essential proteases in the body

Protease Inhibitors Give Life to AIDS Patients







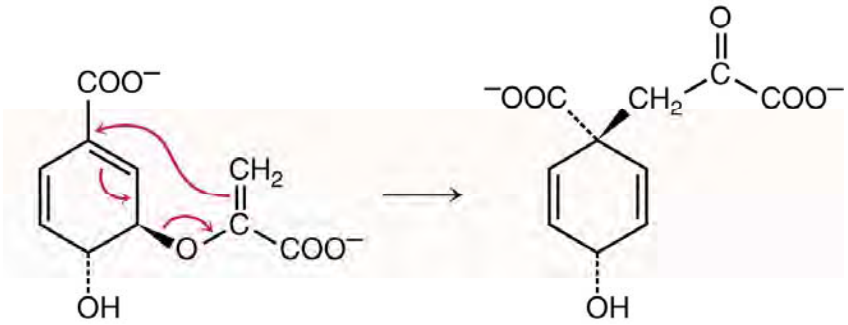
Protease inhibitor drugs used by AIDS Patients

Example 3: Chorismate Mutase

- Biosynthesis of Phe and Tyr in microbe and plant.
- Single substrate!
- Intramolecular rearrangement
- Good example of the catalytic power of enzyme
  - Uncatalyzed reaction use the same transition state!

Chorismate Mutase: A Model for Understanding Catalytic Power and Efficiency

(a) Chorismate mutase reaction



Chorismate — Intramolecular rearrangement — Prephenate

Chorismate Mutase: A Model for Understanding Catalytic Power and Efficiency

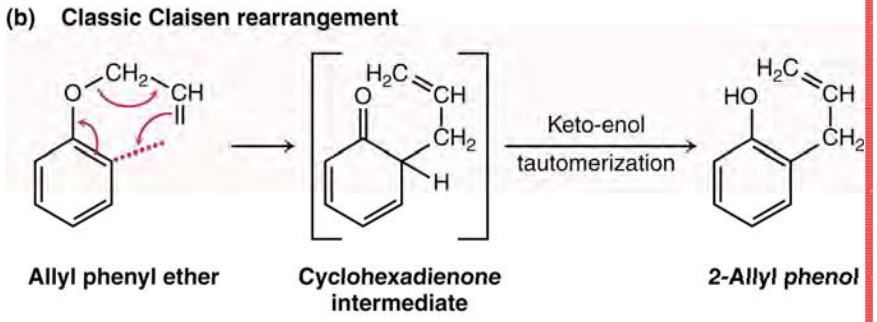


Figure 24.28 A classic Claisen rearrangement. Conversion of allyl phenyl ether to 2-allyl alcohol proceeds through a cyclohexadienone intermediate, which then undergoes a keto-enol tautomerization.

Two possible mechanisms of chorismate rearrangement reaction

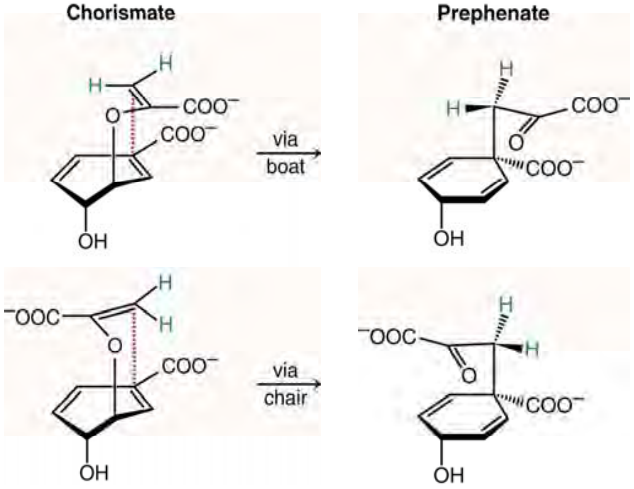
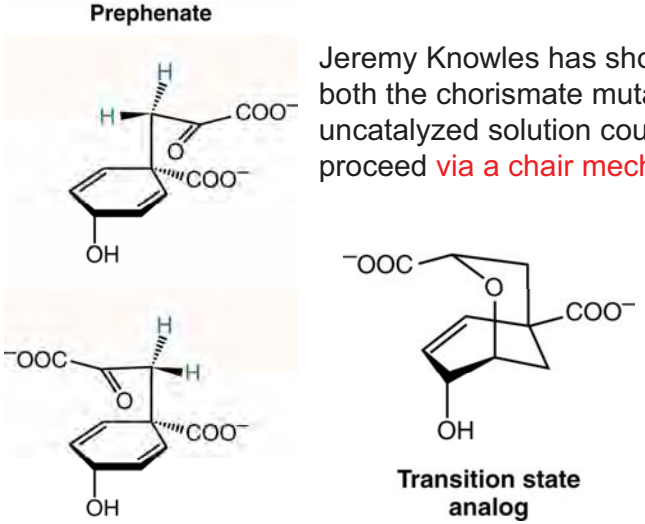


Figure 14.29 The critical H atoms are distinguished in this figure by blue and green colors.

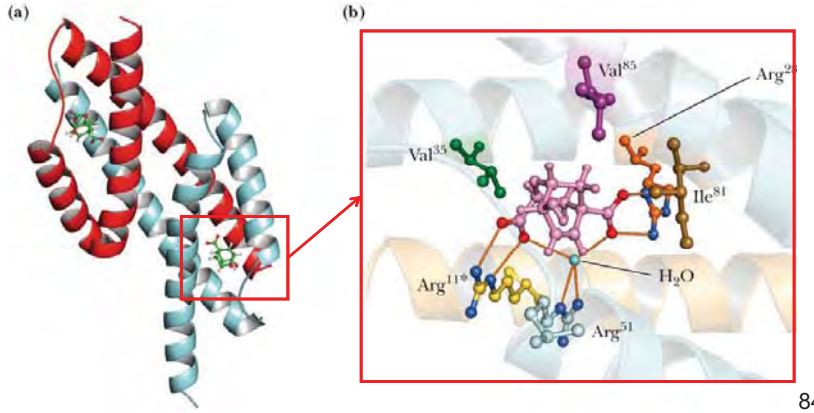
Use TSA to understand the chair mechanism of chorismate mutase



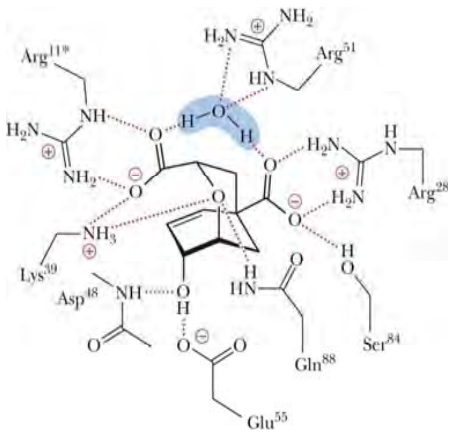
Jeremy Knowles has shown that both the chorismate mutase and its uncatalyzed solution counterpart proceed via a chair mechanism.

The structure of *E. coli* chorismate mutase

- Chorismate mutase is a homodimer.
- The active site is formed by each dimer!



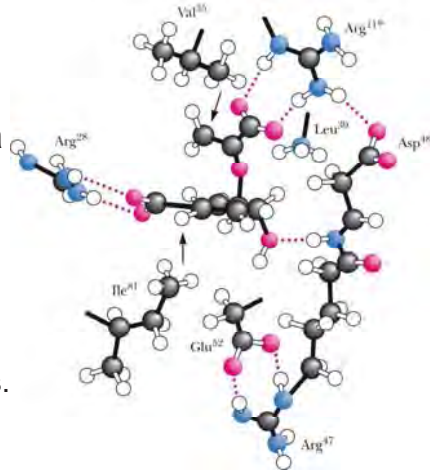
The Chorismate Mutase Active Site Favors a NAC



TSA stabilization by

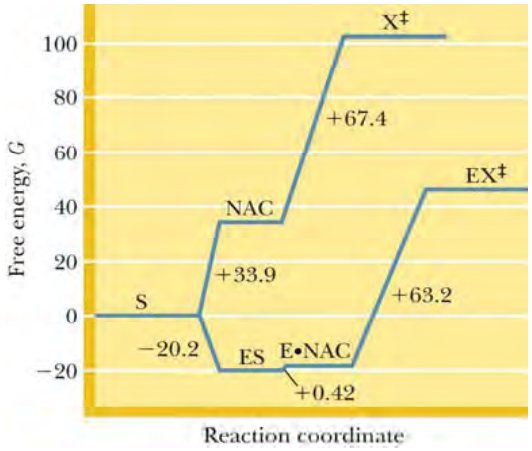
- Twelve electrostatic and hydrogen-bonding interactions

- Figure 14.33 Chorismate bound to the active site of chorismate mutase in a structure that resembles a NAC.
  - Arrows: hydrophobic interactions
  - Red dotted lines: electrostatic interactions.



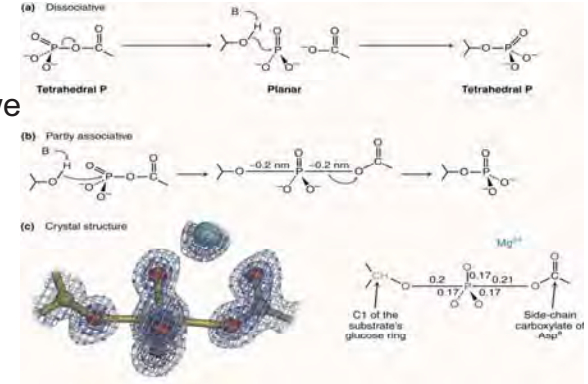
Formation of a NAC is facile in the chorismate mutase active site

Figure 14.34 Chorismate mutase facilitates NAC formation. The energy required to move from the NAC to the transition state is essentially equivalent in the catalyzed and uncatalyzed reactions.



To see is to believe!

- A High-Energy Intermediate in the Phosphoglucomutase Reaction was seen by X-ray diffraction!
- Transition state has very short lifetime? Why we could see it?



End of Part 3

- Ask yourself…
  - What is the catalytic mechanism of serine protease?
  - What is the catalytic mechanism of aspartic protease?
  - What is the catalytic mechanism of chorismate mutase?

End of this class

- You should know…
  - Why enzyme could accelerate a reaction?
    - 2 major reasons
  - How enzyme could accelerate a reaction?
    - 5 mechanisms
  - Examples of enzyme catalysis mechanism
    - Remember serine protease!