L7 Enzyme functional nature: Mechanism

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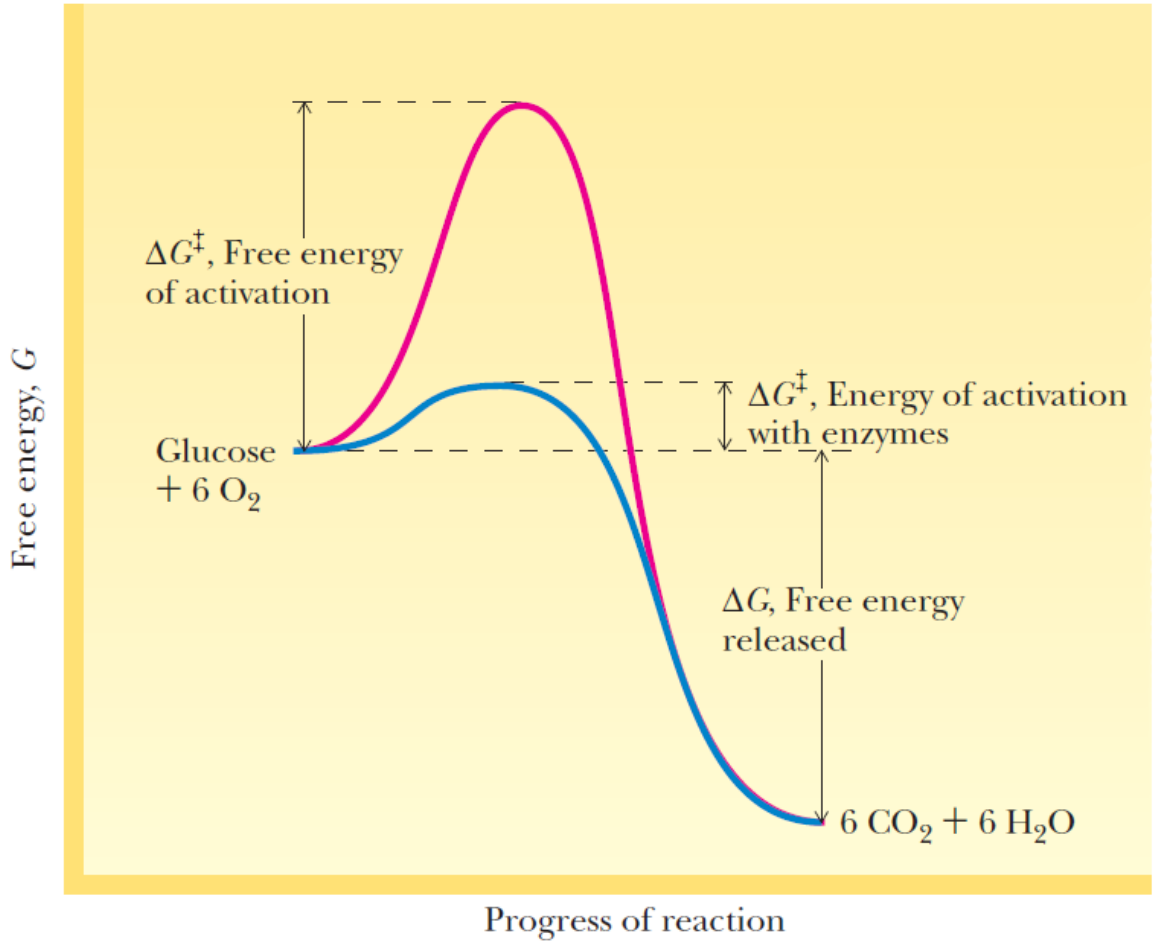
Enzyme; Functional nature and mechanism

Enzymes are catalyst,
That accelerate chemical reactions

Mostly enzymes are proteins,
some RNA enzymes also exist



Gibbs free energy (*G*)

- Thermodynamics: changes in free energy, entropy, …

$$\Delta G = \Delta H - T \cdot \Delta S$$
$$\Delta G = (\Delta U + P \cdot \Delta V) - T \cdot \Delta S$$

- For nearly all biochemical reactions ΔV is small and ΔH is almost equal to ΔU

- Hence, we can write:

$$\Delta G = \Delta U - T \cdot \Delta S$$

If ΔG is negative

Energy was released, products are simpler, greater entropy (2nd Law of Thermodynamics)

Exergonic / exothermic reaction (spontaneous)

If ΔG is positive

Energy input, product more complex, energy needed to go against 2nd Law

Endergonic / endothermic (non-spontaneous)

The Enthalpic term

- Changes in bonding
  - van der Waals
  - Hydrogen bonding
  - Charge interactions

The Entropic term

- Changes the arrangement of the solvent or counterions
- Reflects the degrees of freedom
- Rotational & Translational changes

Protein folding

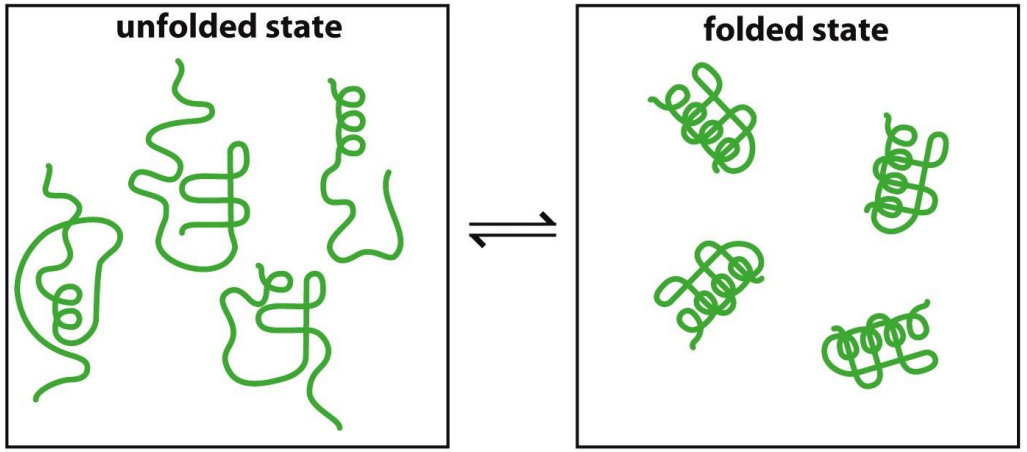


Figure 10.13 The Molecules of Life (© Garland Science 2013)

Protein folding is a spontaneous process?

$\Delta G < 0$???

Protein folding

(U) Unfolded $\rightleftharpoons$ Folded (F)

$K_{folding} = \frac{(F)}{(U)} = \frac{1}{K_{unfolding}}$

$\Delta G^{\circ}_{unfolding} = \Delta H^{\circ} - T\,\Delta S^{\circ}$

Enthalpy change

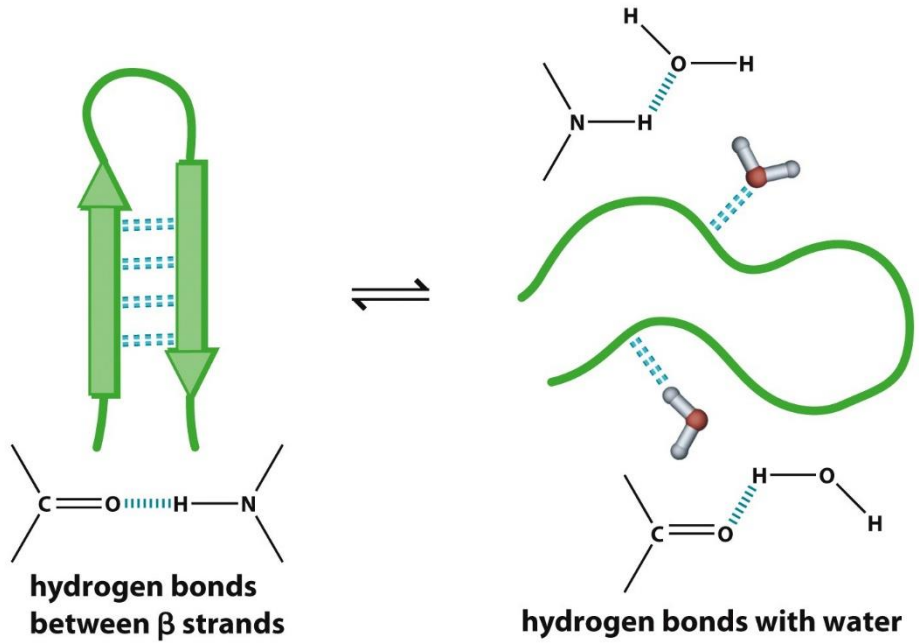


Figure 10.14 The Molecules of Life (© Garland Science 2013)

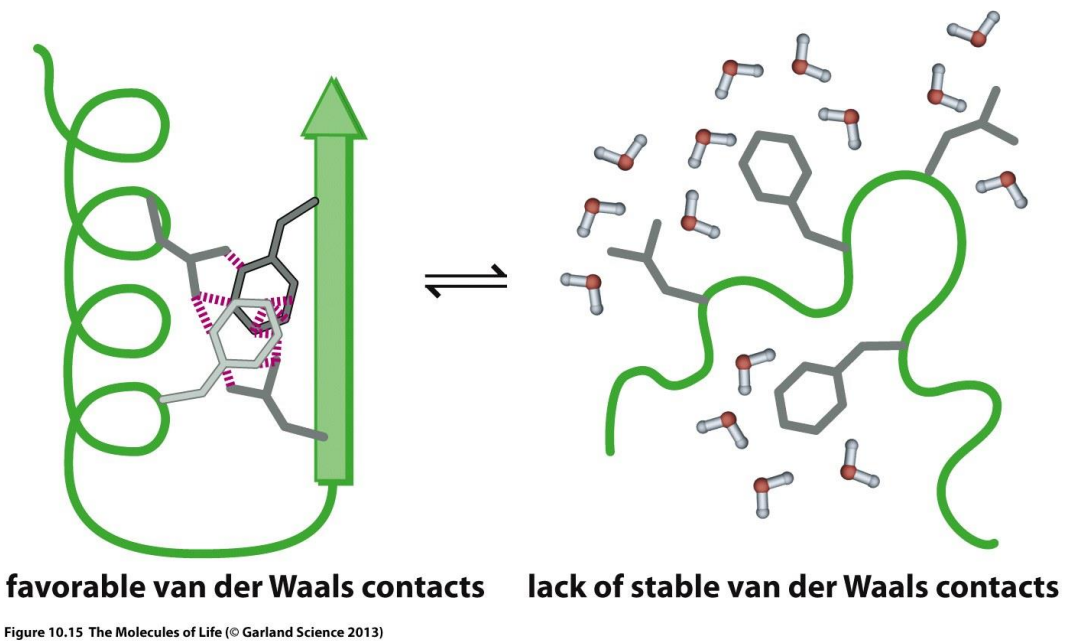


Figure 10.15 The Molecules of Life (© Garland Science 2013)

Entrophy contribution

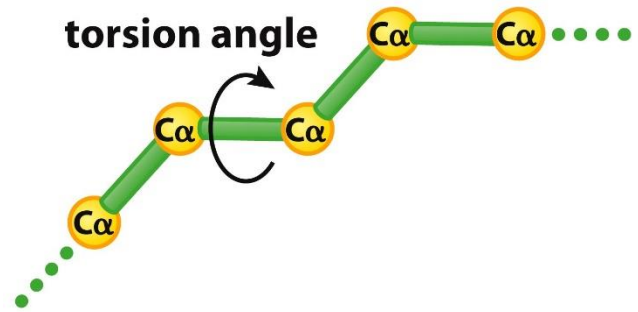


Figure 10.18 The Molecules of Life (© Garland Science 2013)

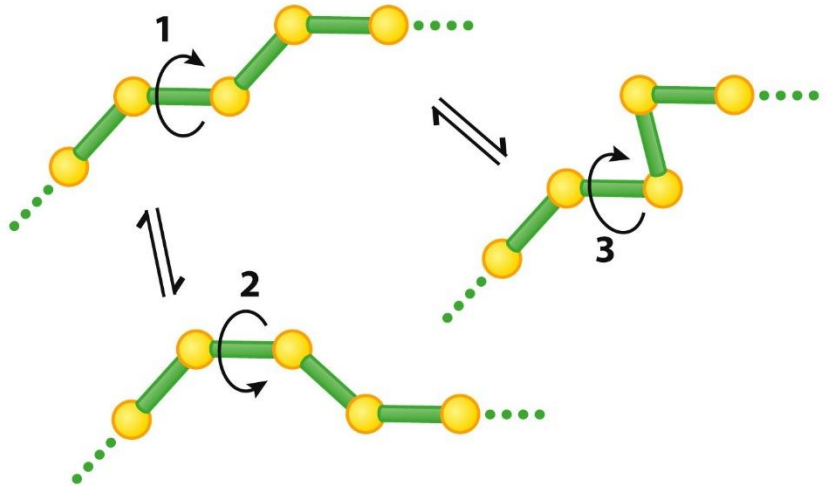


Figure 10.19 The Molecules of Life (© Garland Science 2013)

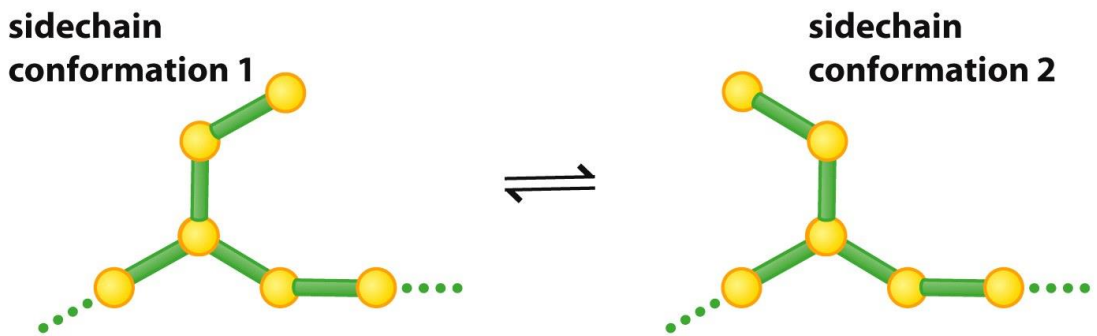


Figure 10.20 The Molecules of Life (© Garland Science 2013)

Entrophy contribution from water

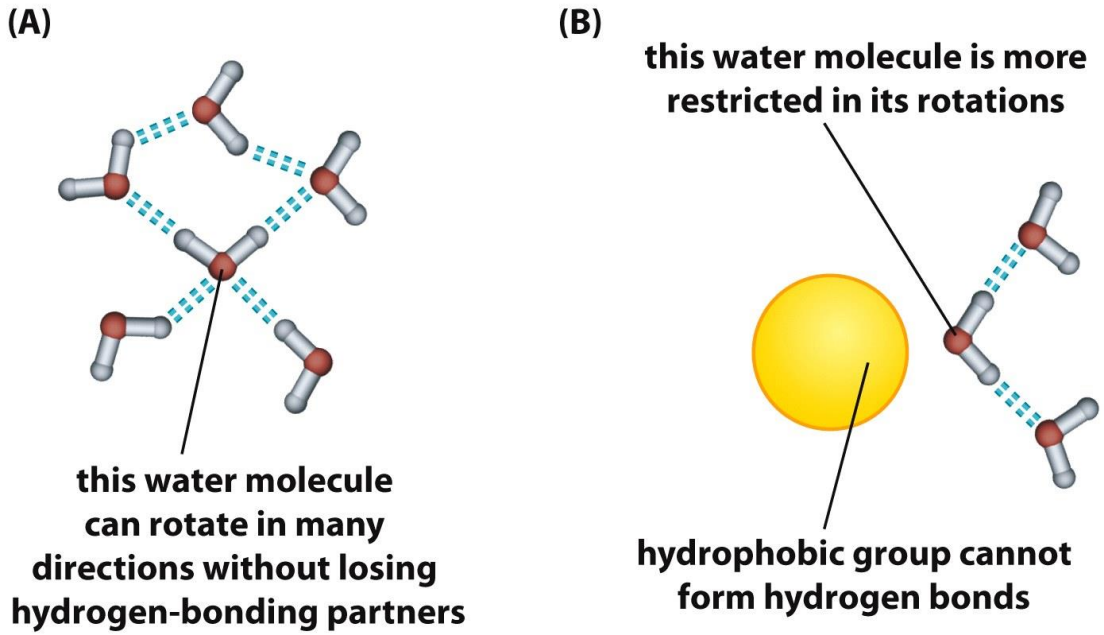


Figure 10.21 The Molecules of Life (© Garland Science 2013)

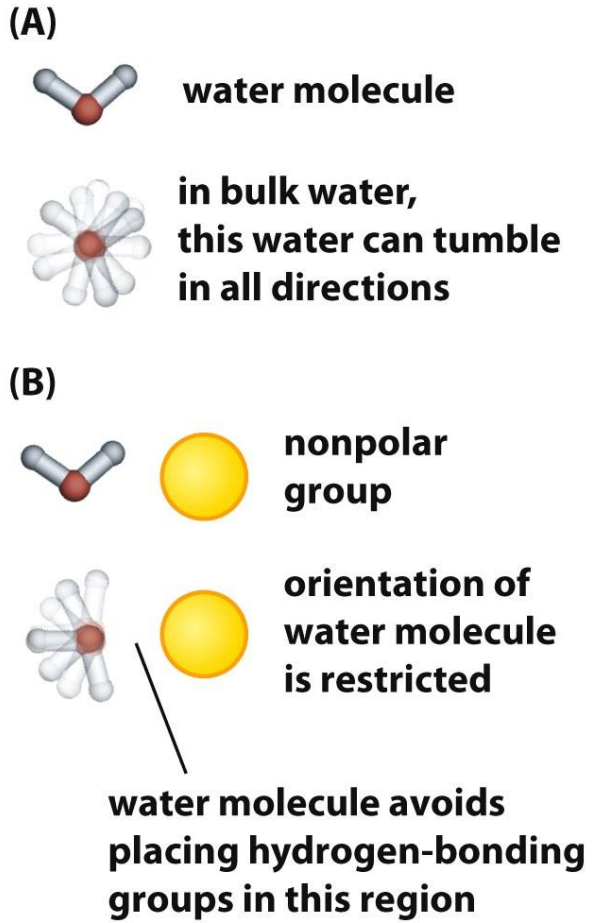


Figure 10.22 The Molecules of Life (© Garland Science 2013)

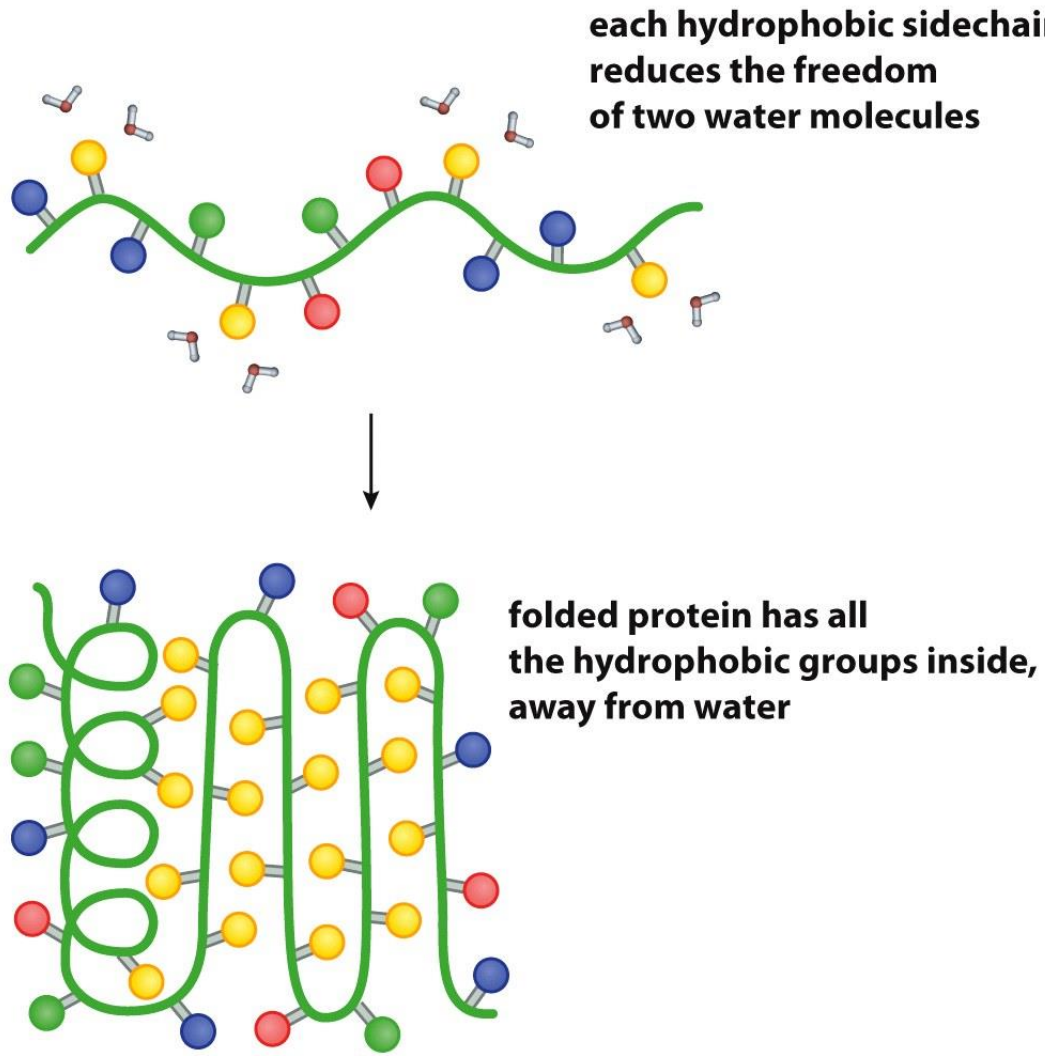
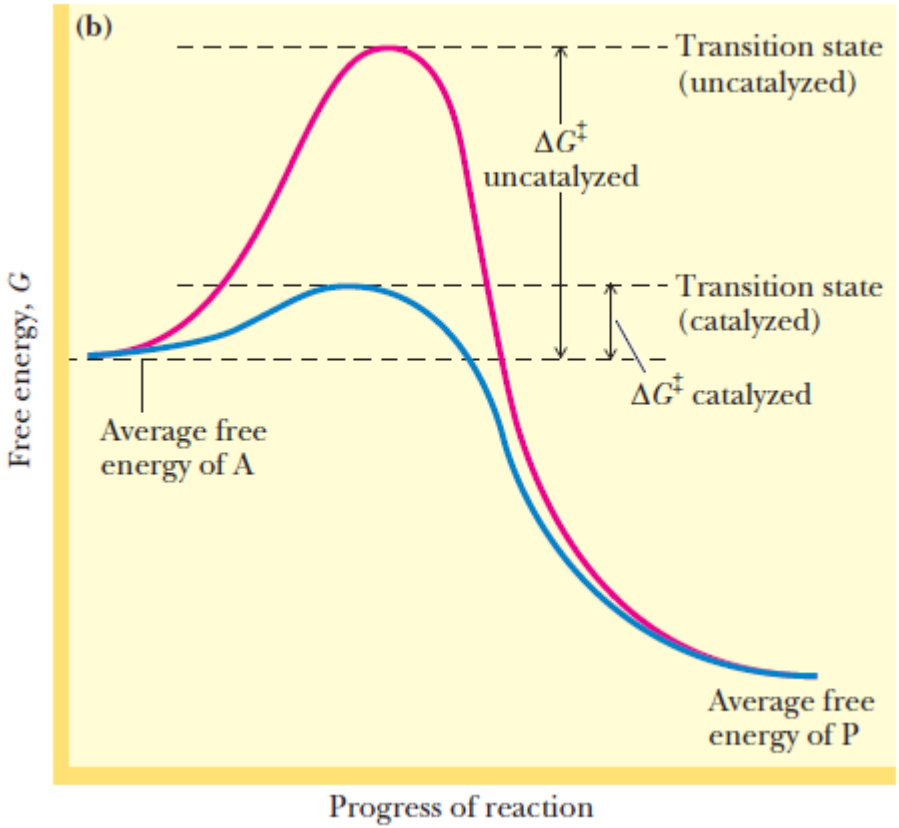
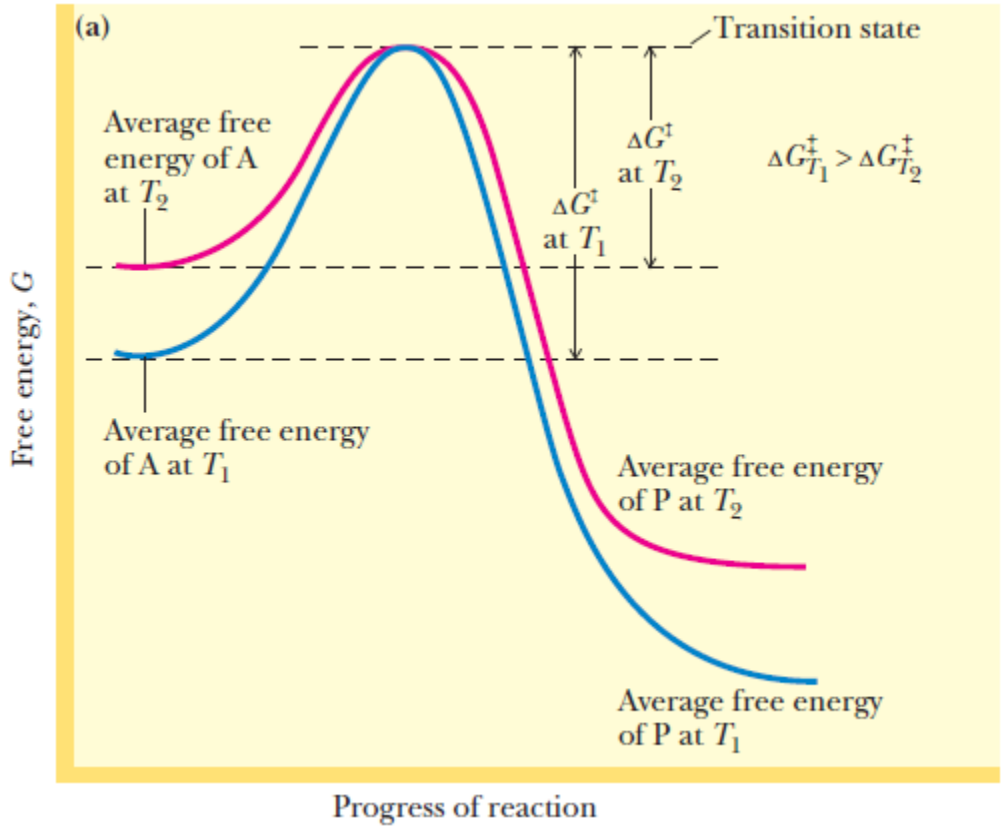


Figure 10.23 The Molecules of Life (© Garland Science 2013)

Mechanism :Catalysts Lower the Free Energy of Activation for a Reaction

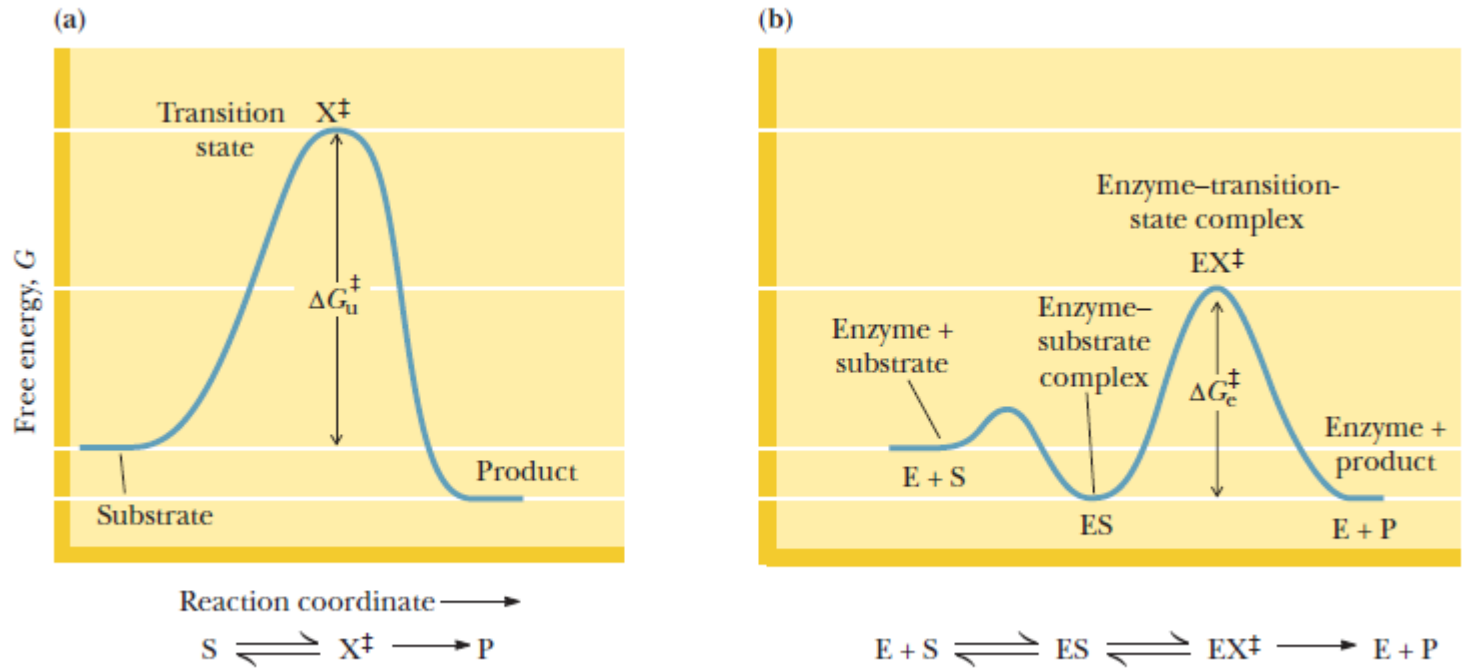




Transition intermediate in chemical reactions!

In all chemical reactions, the reacting atoms or molecules pass through a state that is intermediate in structure between the reactant(s) and the product(s). Consider the transfer of a proton from a water molecule to a chloride anion:

$$\underset{\text{Reactants}}{H{-}O{-}H + Cl^-} \rightleftharpoons \underset{\text{Transition state}}{H{-}O^{\delta-}\cdots H\cdots Cl^{\delta-}} \rightleftharpoons \underset{\text{Products}}{HO^- + H{-}Cl}$$



Without enzyme

With enzyme

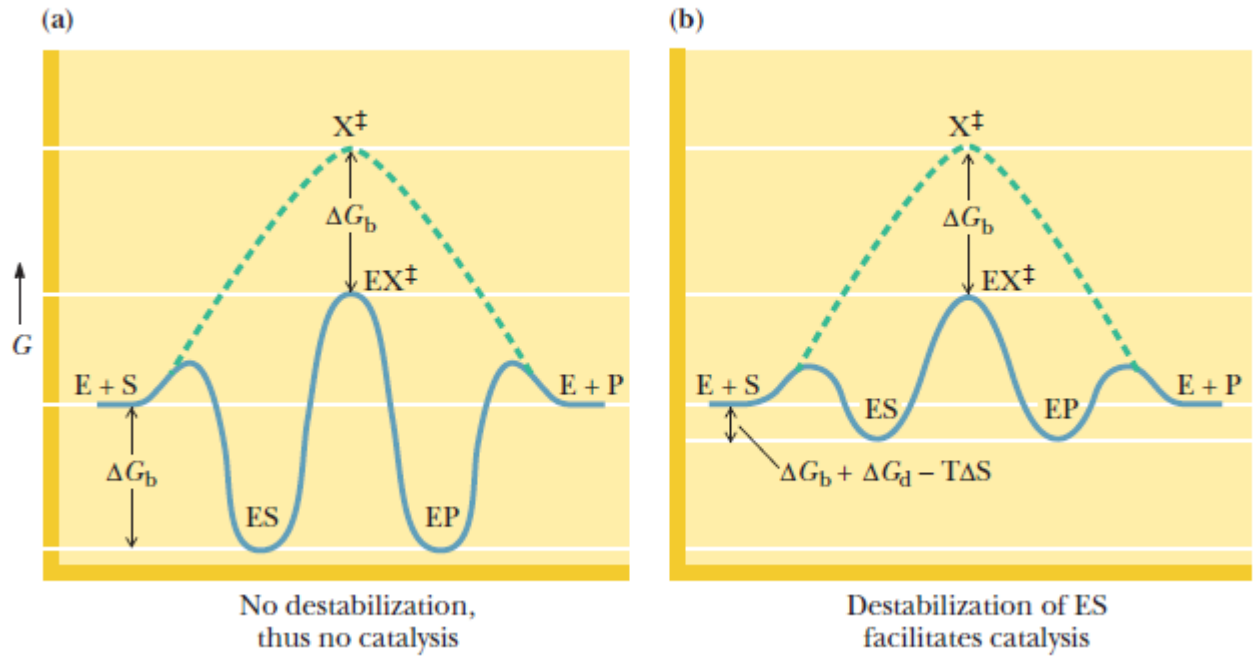
Enzyme substrate complex is partially destabilized!

$$E + S \rightleftharpoons ES \rightleftharpoons EX^{\ddagger} \longrightarrow E + P$$

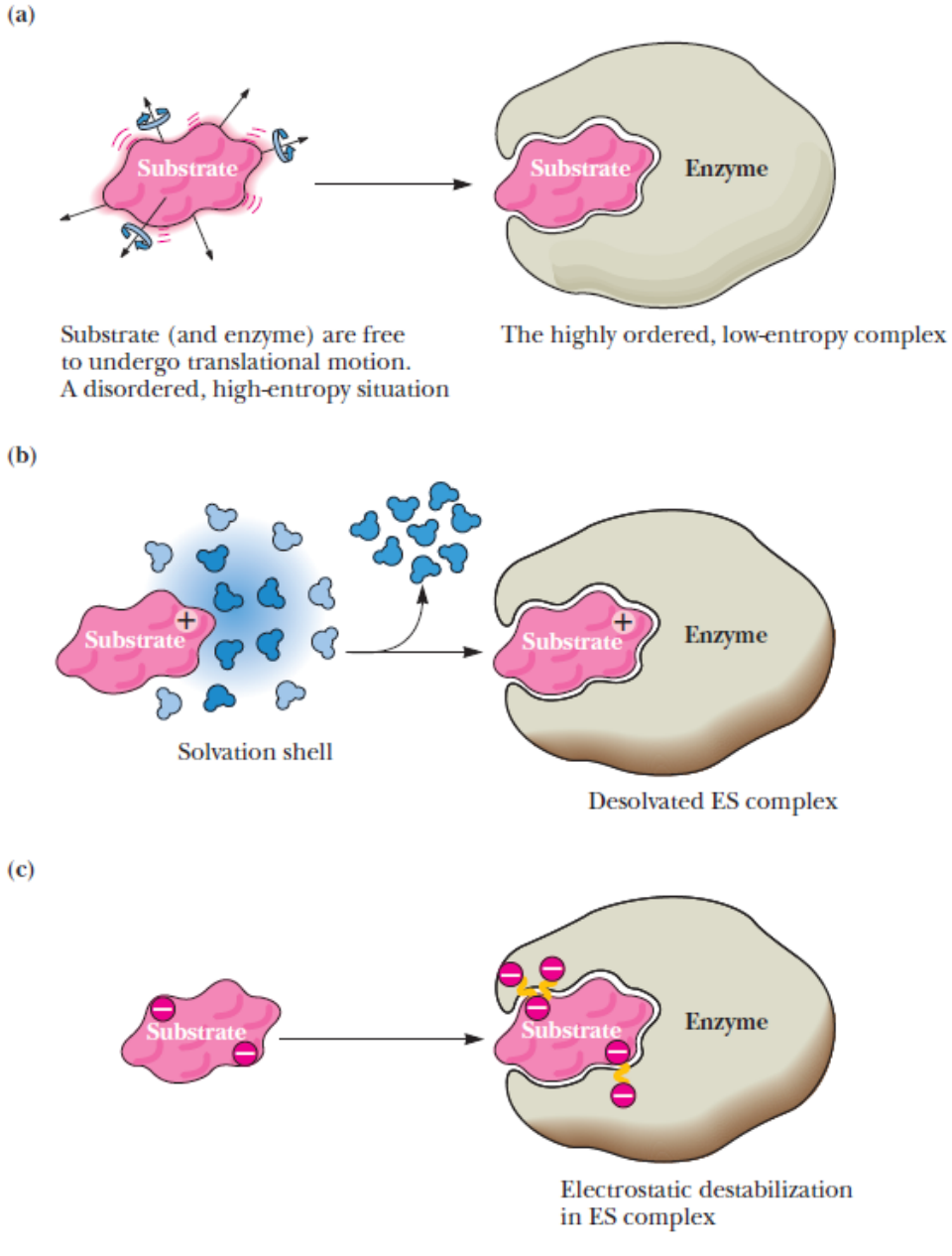
$$K_S = \frac{[E][S]}{[ES]}$$

$$K_T = \frac{[E][X^{\ddagger}]}{[EX^{\ddagger}]}$$

$$k_e/k_u \approx K_S/K_T$$



Partially de-stabilize
ES complex

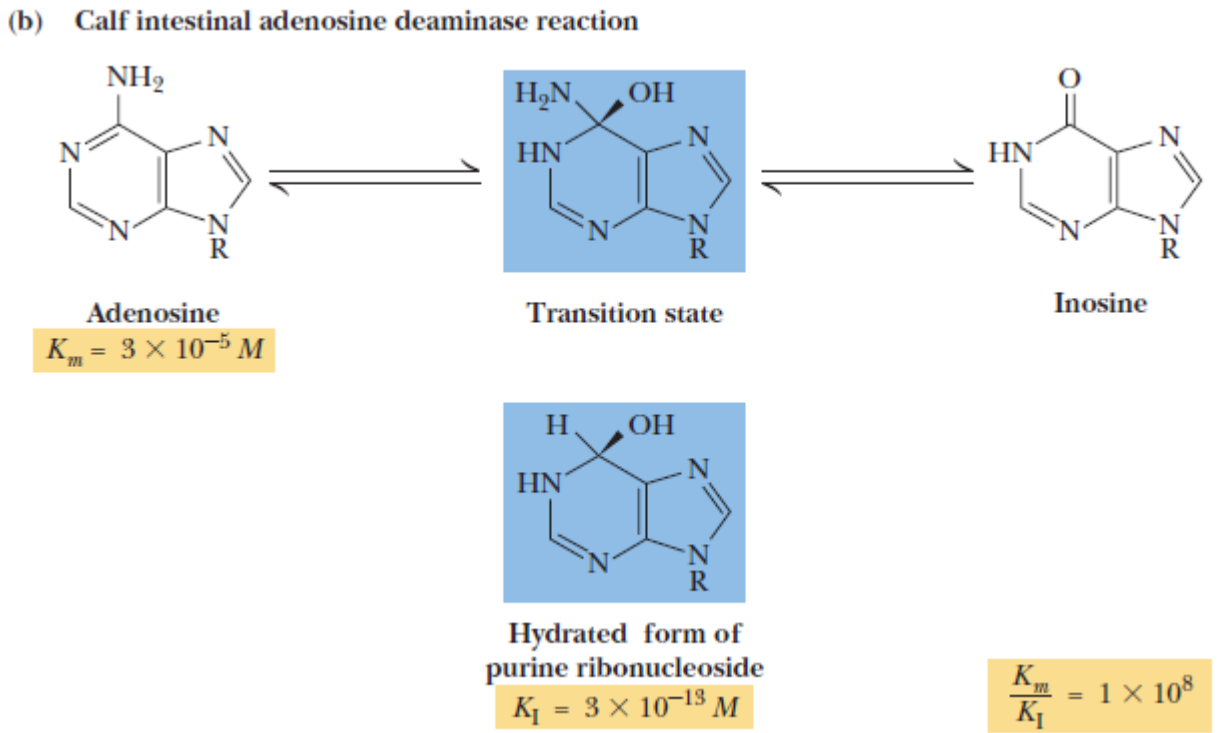


How analogs of transition state exploited?

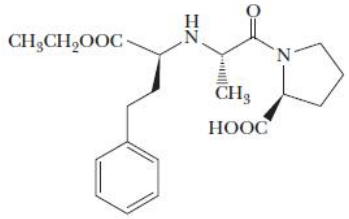
The value of K_T for fructose-1,6-bisphosphatase is an astounding 7 x 10^{-26} M!

This low value for binding constant means very tight binding between enzyme and transition complex

If you can mimic the transition state with similar compound, it will bind the enzyme complex very tightly. This idea is exploited very well and many know drug today have designed on this principle

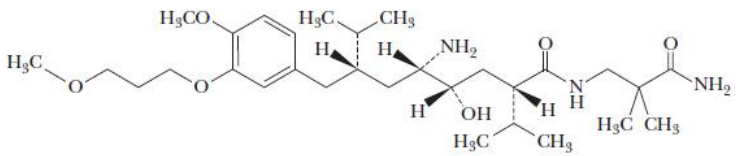


Angiotensin Concerting Enzyme (ACE)



Enalapril

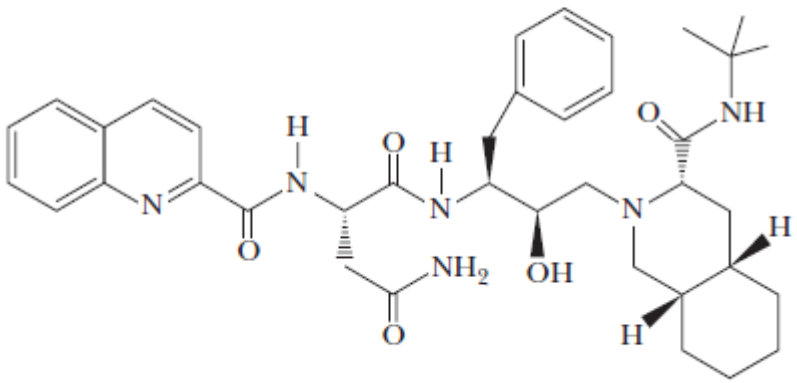
Courtesy of James Gathany/CDC



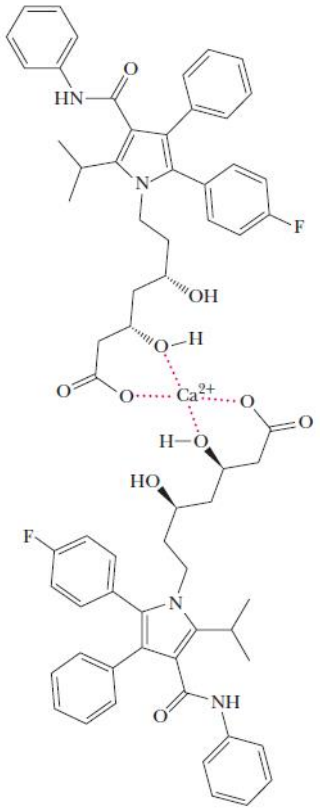
Aliskiren

Protease Inhibitors Are AIDS Drugs

Crixivan (indinavir) by Merck, Invirase (saquinavir) by Roche, and similar "protease inhibitor" drugs are transition-state analogs for the HIV-1 protease, discussed on pages 470–471.



Saquinavir



Atorvastatin (Lipitor)

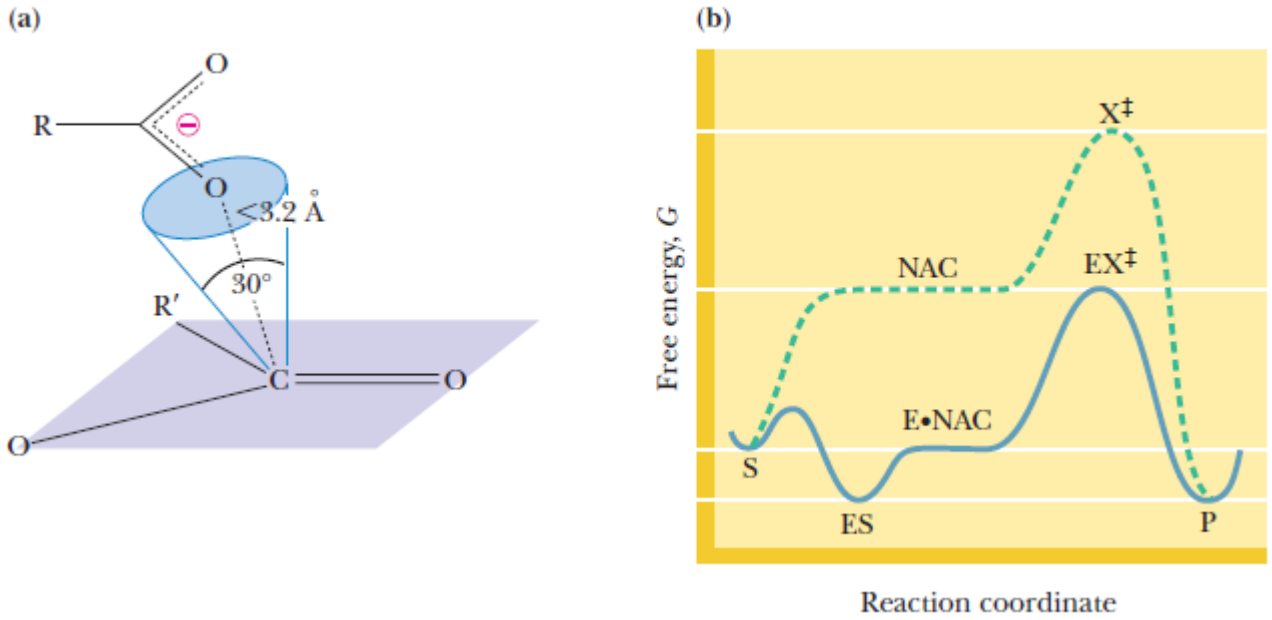
© AP Photo/Amy Sancetta, File

HMG-CoA reductase

Enzymes Facilitate Formation of Near-Attack Conformations

Near attack conformations

Reacting atoms are in *Van der waals* contact and at an angle resembling the bond to be formed



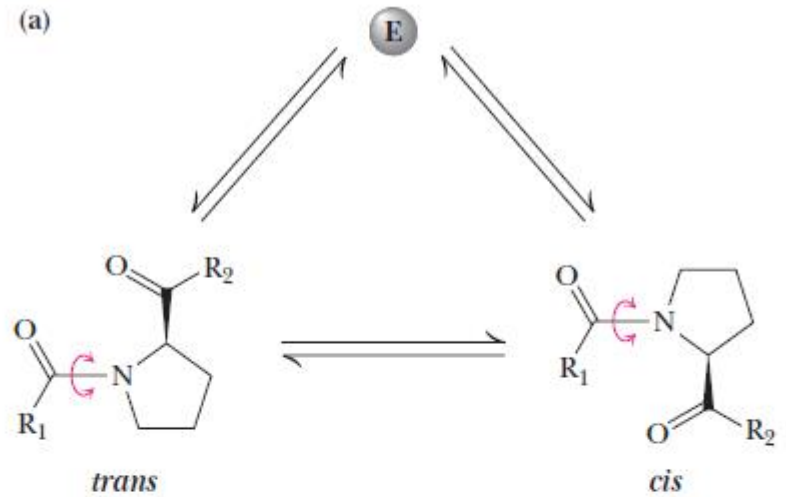
For reactions involving bonding between O, N, C & S atoms: NAC are characterized as Distance within 3.2 Å and ± 15 angle

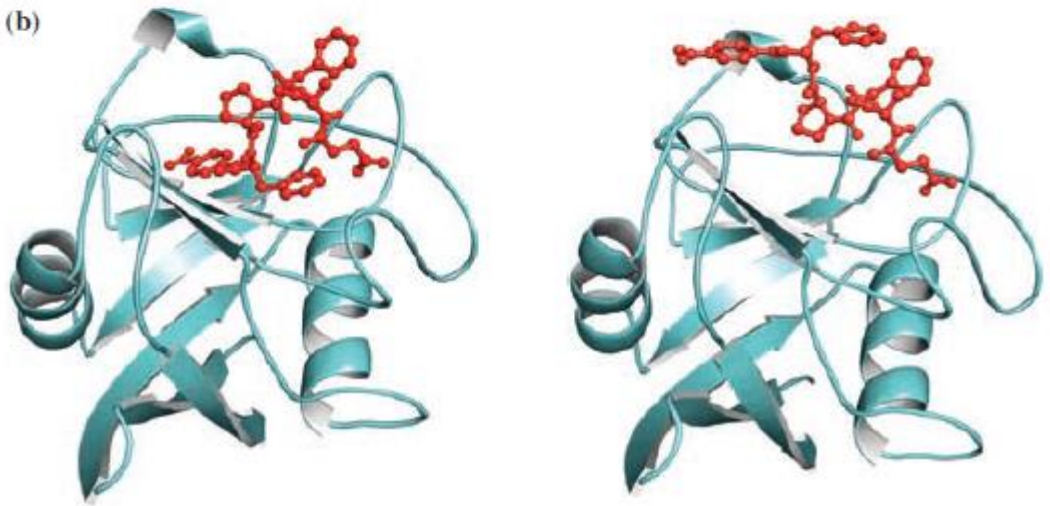
What exactly happens at protein active site??

Different amino-acids in protein active site are in constant motions and they assist in

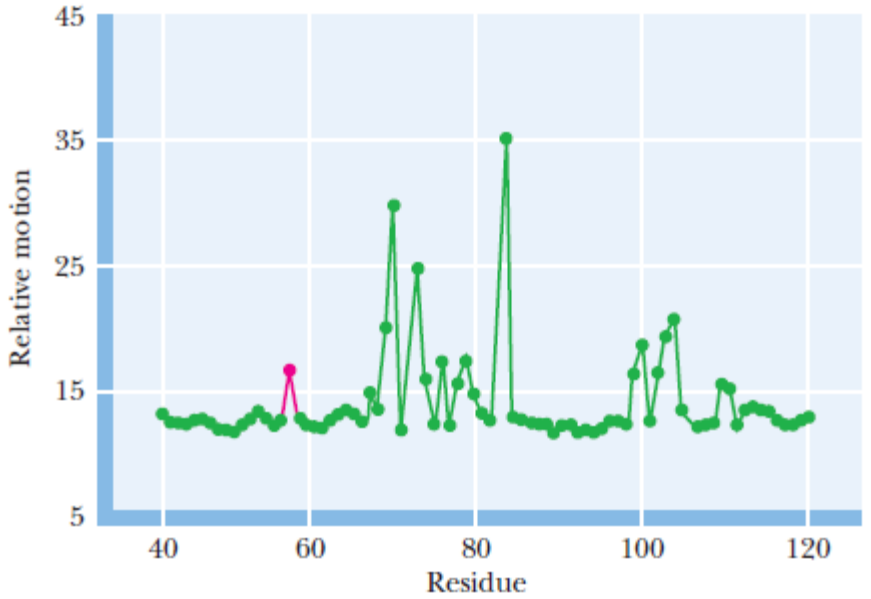
1. Substrate binding
2. Bring catalytic group into position around a substrate
3. Induce formation of NAC
4. Assist in bond making and bond breaking
5. Facilitate conversion of substrate to product

example





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

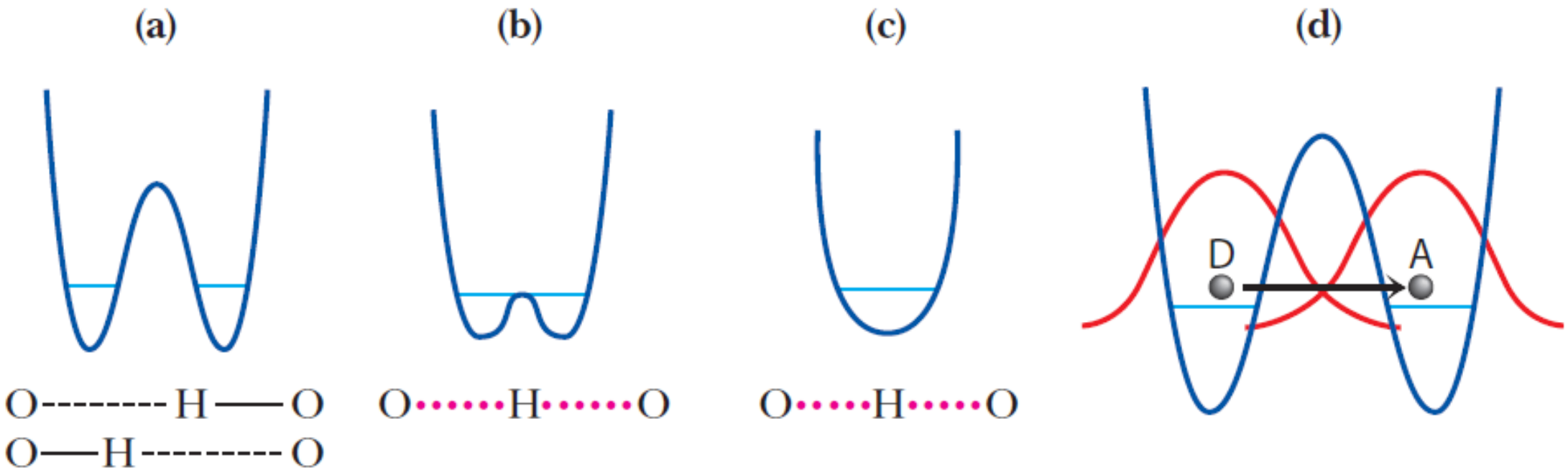


This network extends from the active site to the surface of the protein, and the motions in this network span time scales of femtoseconds to milliseconds. Such extensive networks of motion make it likely that the entire folded structure of the protein may be involved in catalysis at the active site.

Enzymes catalyze reactions by utilizing the same general reactions as studied in organic chemistry:

- Acid-base catalysis
- Covalent catalysis
- Metal ion catalysis
- Catalysis by alignment (approximation)

Low barrier hydrogen bonds & Proton tunneling



Weak bond

Strong bond

Covalent Catalysis

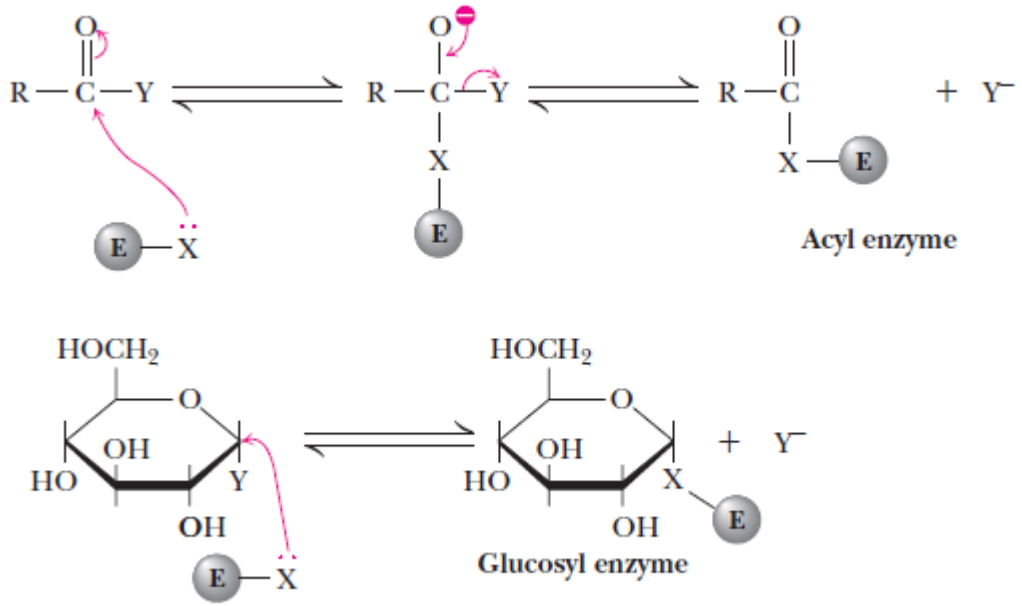
Some enzyme reactions derive much of their rate acceleration from the formation of **covalent bonds** between enzyme and substrate. Consider the reaction:

$$BX + Y \longrightarrow BY + X$$

and an enzymatic version of this reaction involving formation of a **covalent intermediate:**

$$BX + Enz \longrightarrow E{:}B + X + Y \longrightarrow Enz + BY$$

examples



Digestive serine protease

Ex., trypsin, chymotrypsin, thrombin, tissue plasminogen activator

Catalytic mechanism based on active site serine

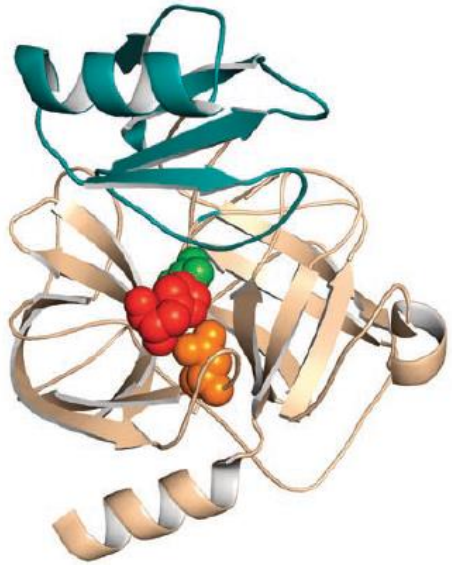


FIGURE 14.16 Structure of chymotrypsin (white) in a complex with eglin C (blue ribbon structure), a target protein. The residues of the catalytic triad (His^{57}, Asp^{102}, and Ser^{195}) are highlighted. His^{57} (red) is flanked by Asp^{102} (gold) and by Ser^{195} (green). The catalytic site is filled by a peptide segment of eglin. Note how close Ser^{195} is to the peptide that would be cleaved in the chymotrypsin reaction (pdb id = 1ACB).

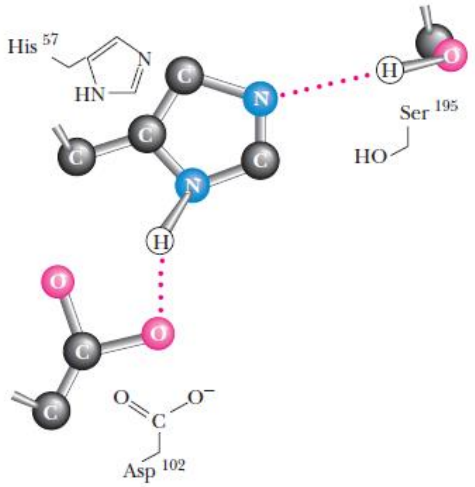
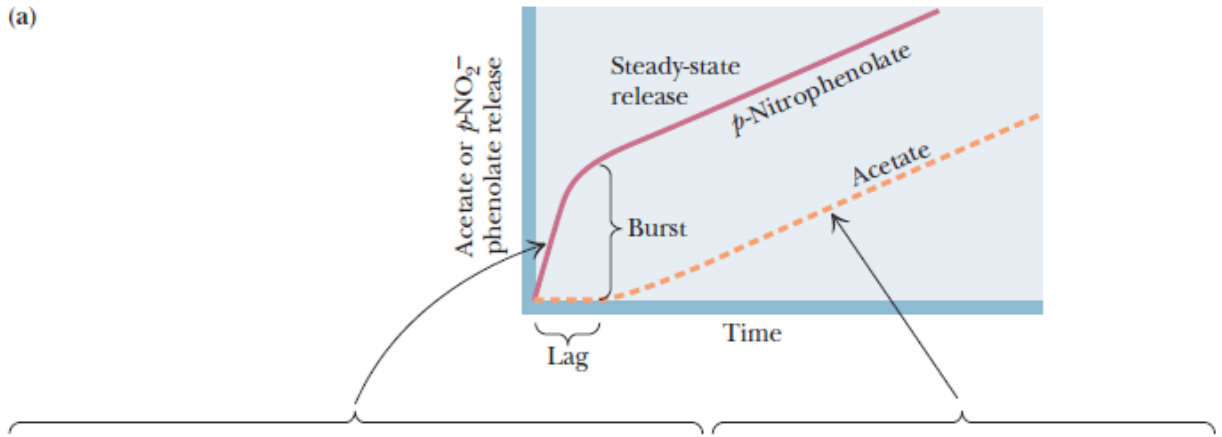
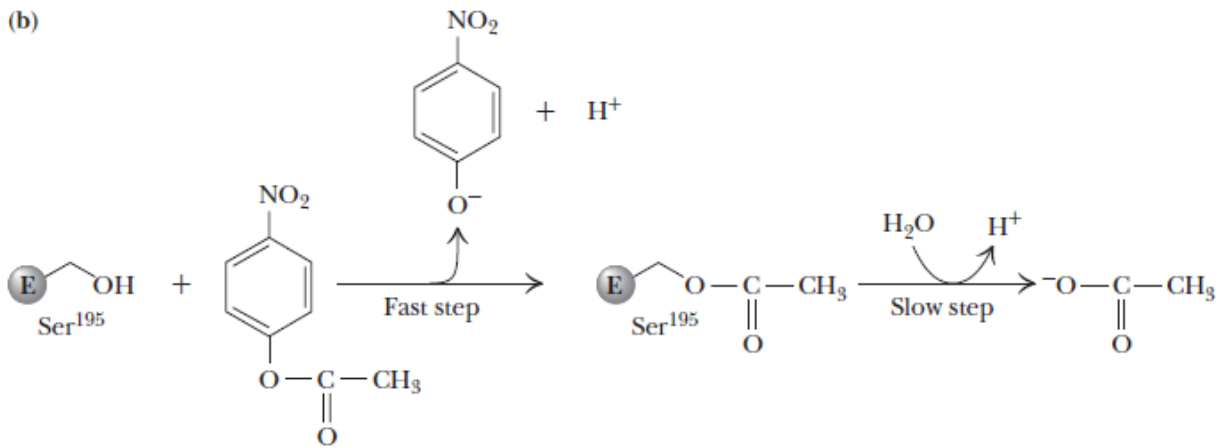
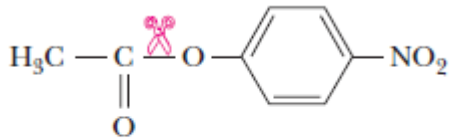


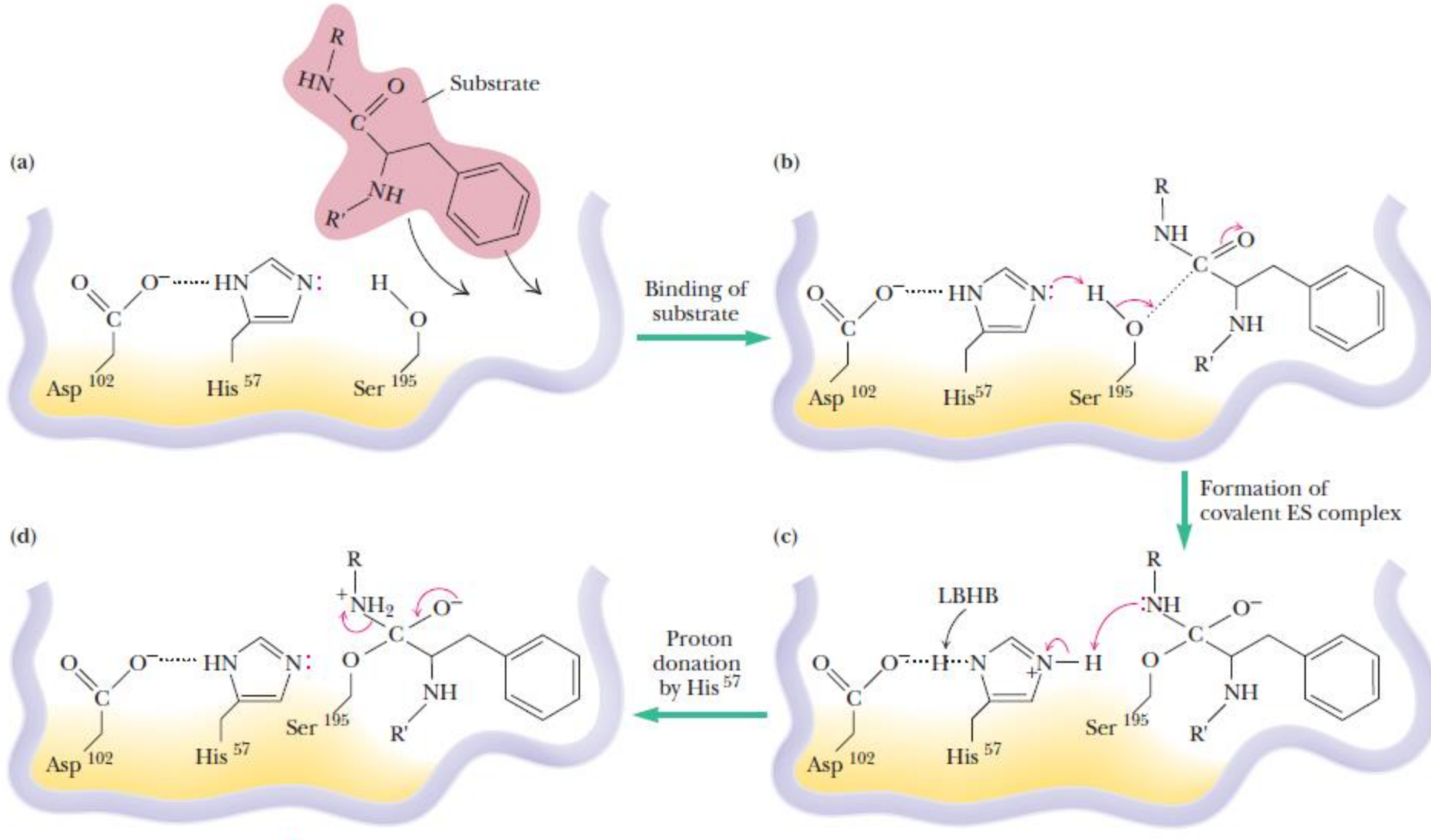
FIGURE 14.17 The catalytic triad of chymotrypsin.

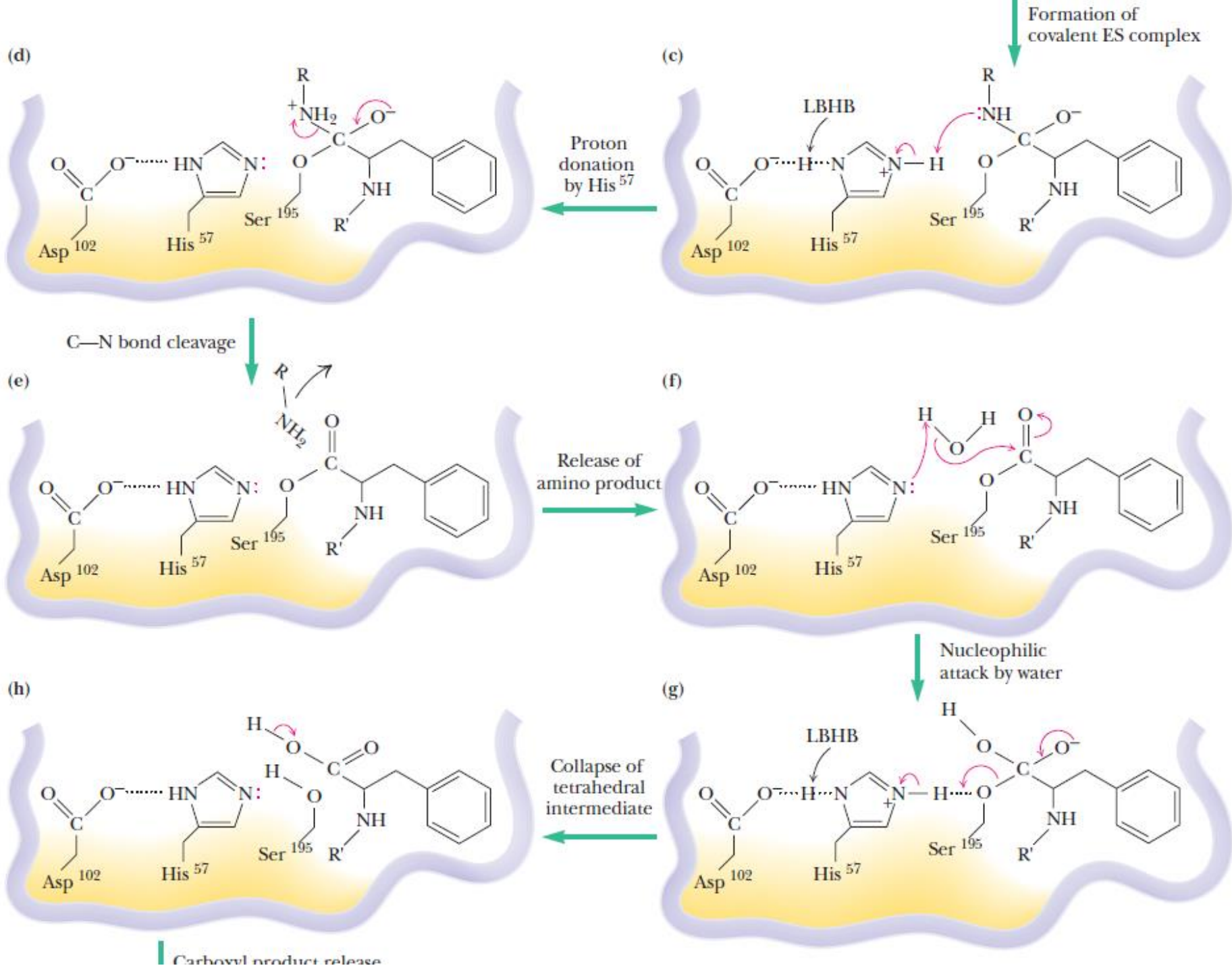






p-Nitrophenylacetate


(a)
Substrate
Asp 102
His 57
Ser 195
Binding of substrate
(b)
Asp 102
His57
Ser 195
Formation of covalent ES complex
(c)
LBHB
Asp 102
His 57
Ser 195
Proton donation by His 57
(d)
Asp 102
His 57
Ser 195


(d)
(c)
Formation of covalent ES complex
Proton donation by His 57
LBHB
C—N bond cleavage
(e)
(f)
Release of amino product
Nucleophilic attack by water
(h)
(g)
Collapse of tetrahedral intermediate
Asp 102
His 57
Ser 195
R'
NH
NH2

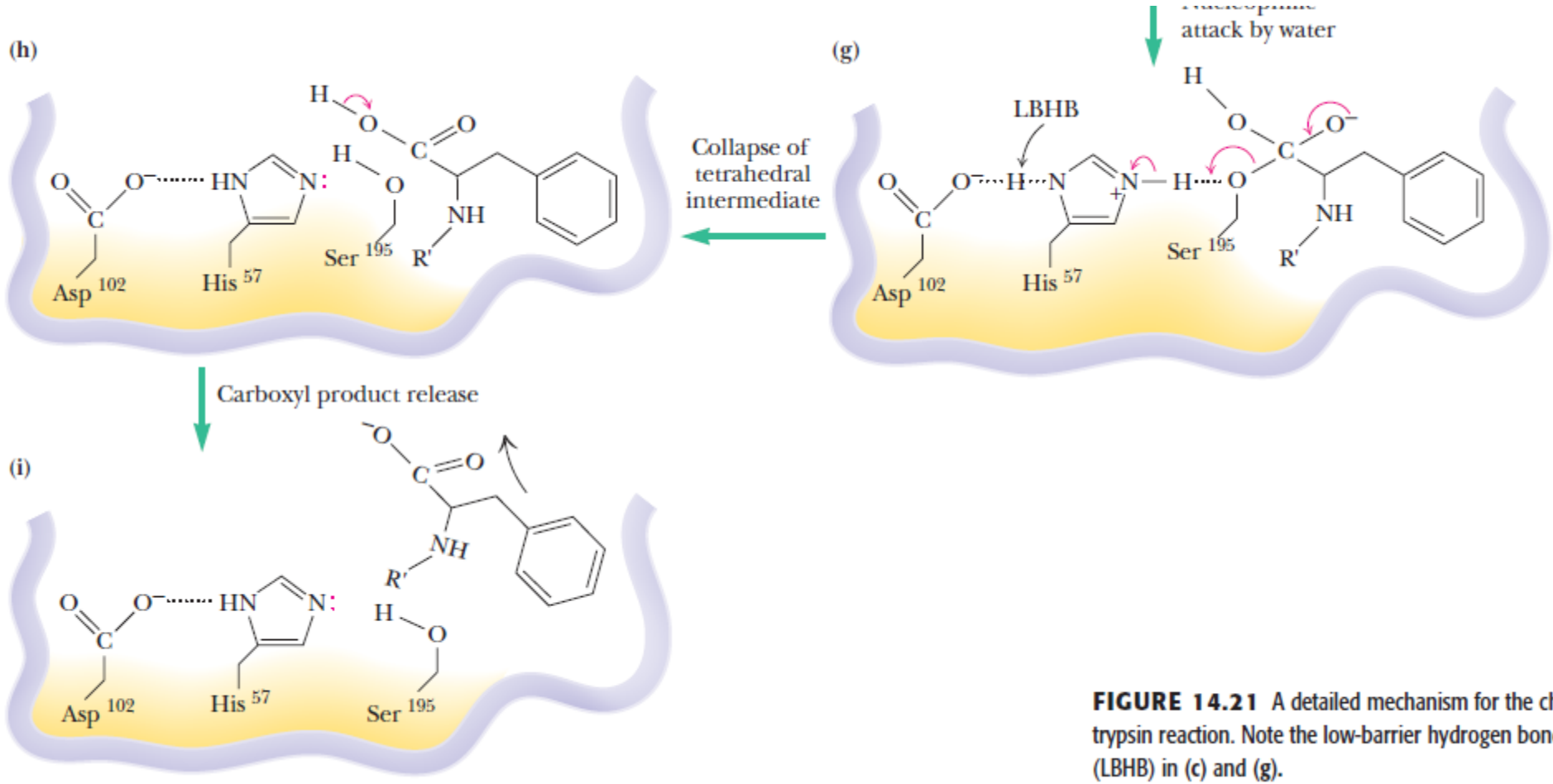


FIGURE 14.21 A detailed mechanism for the chymotrypsin reaction. Note the low-barrier hydrogen bond (LBHB) in (c) and (g).