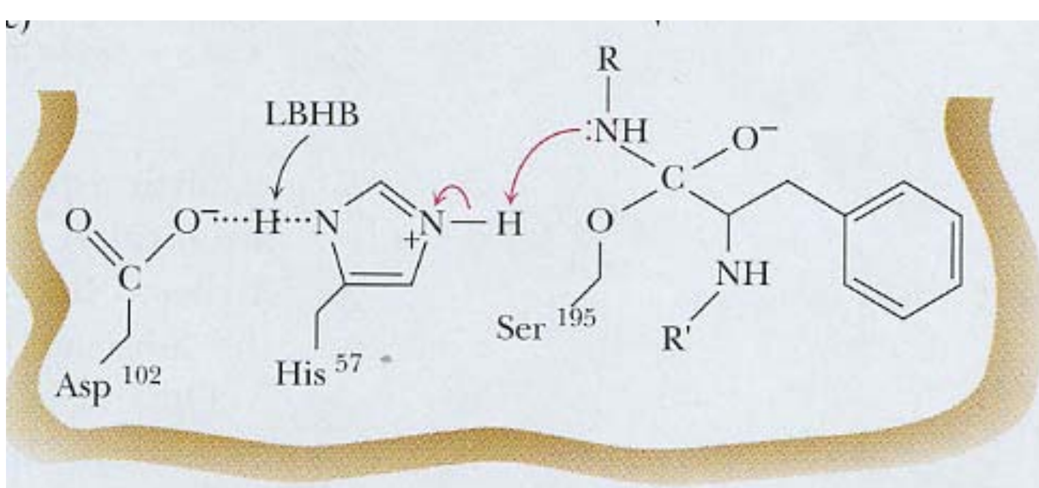
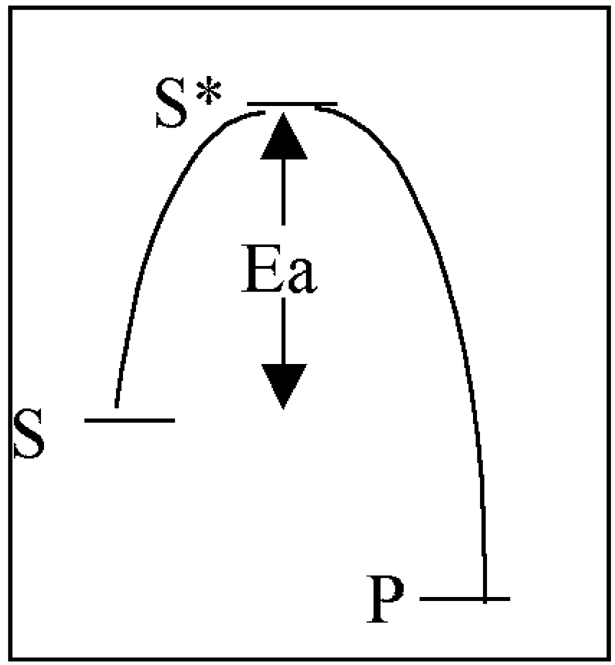
Mechanisms of Enzyme Action



Kinetics of an uncatalyzed chemical reaction:

$$S \rightleftharpoons S^* \rightleftharpoons P$$

Free energy, ΔG

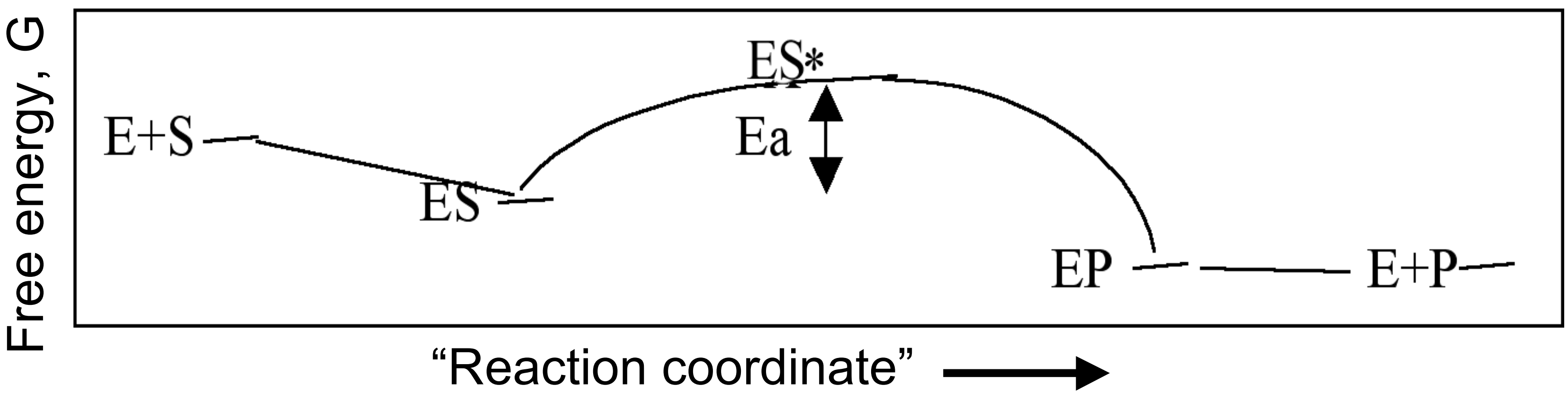


“Reaction coordinate”

Ea is “activation energy”

Kinetics of a catalyzed chemical reaction:

$$S + E \rightleftharpoons ES \rightleftharpoons ES^* \rightleftharpoons EP \rightleftharpoons E + P$$

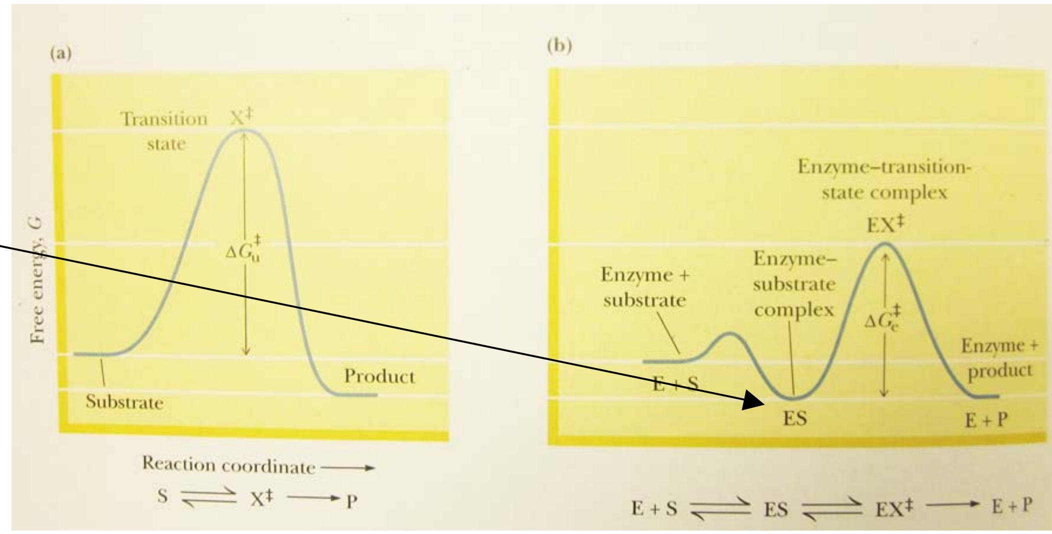


1. Enzyme does not affect ΔG or ΔG° between S and P (i.e., equilibrium)
2. Enzyme reduces Ea: Ea (catalyzed) < Ea (uncatalyzed)

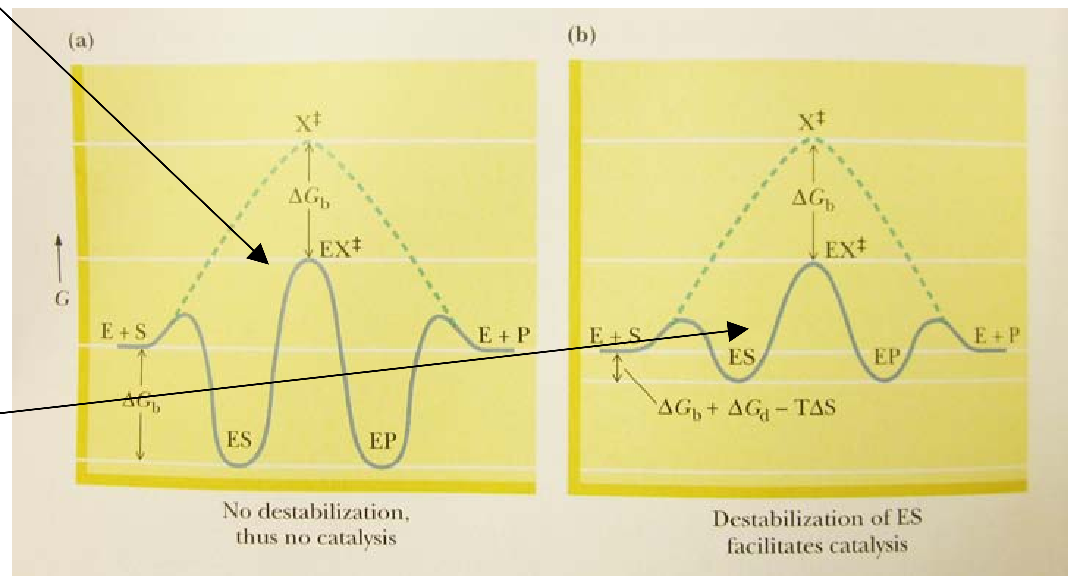
A more complete way of showing the effects of enzymes:

Enzymes bind to substrates, so G(ES) < G(E+S).

However, if all they did was to bind, then Ea =ΔG(ES*) for the reaction would not be reduced.



So when they bind the substrate, they stress It in some way, raising G(ES) for part of the substrate and reducing ΔG(ES*)(=Ea).



Quantitatively, what is the effect of reducing Ea?

For reaction A → B, V = k[A]

$k = (\kappa T/h)\exp(-Ea/RT)$

κ = Boltzman's constant; h = Plank's constant,

So k and thus V are inversely and exponentially related to Ea and directly related to T:

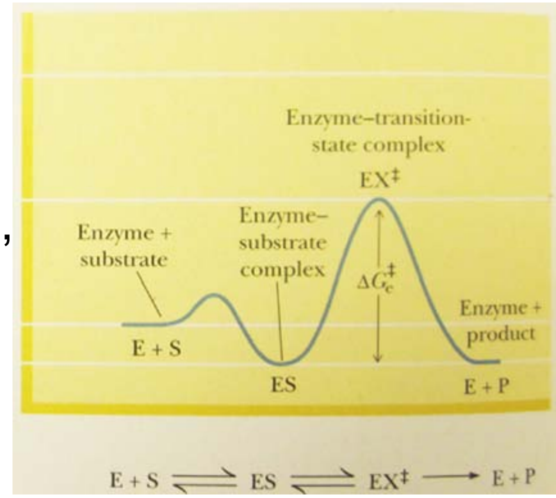
A 6 kJ/mol reduction in Ea gives ca 10x increase in k and V

$\Delta k \sim \exp(+6000/8.3*300) \sim 11$

(reduction in Ea is an increase from –Ea)

V(catalyzed)/V(uncatalyzed) for various enzymes varies from 10^4 to 10^{21}, meaning Ea is reduced by ca 23 to 126 kJ/mol

How do enzymes reduce Ea?



These effects raise G(ES): cage effect, orientation, steric straining of bonds (stress from H-, Vanderwaal's, ionic bonds), dislocation of bonding electrons through +/- charges

These effects reduce G(ES*): covalent bonds, acid-base catalysis, low-barrier hydrogen bonds, and metal ion catalysis

Different classes of enzymes may use different mechanisms:

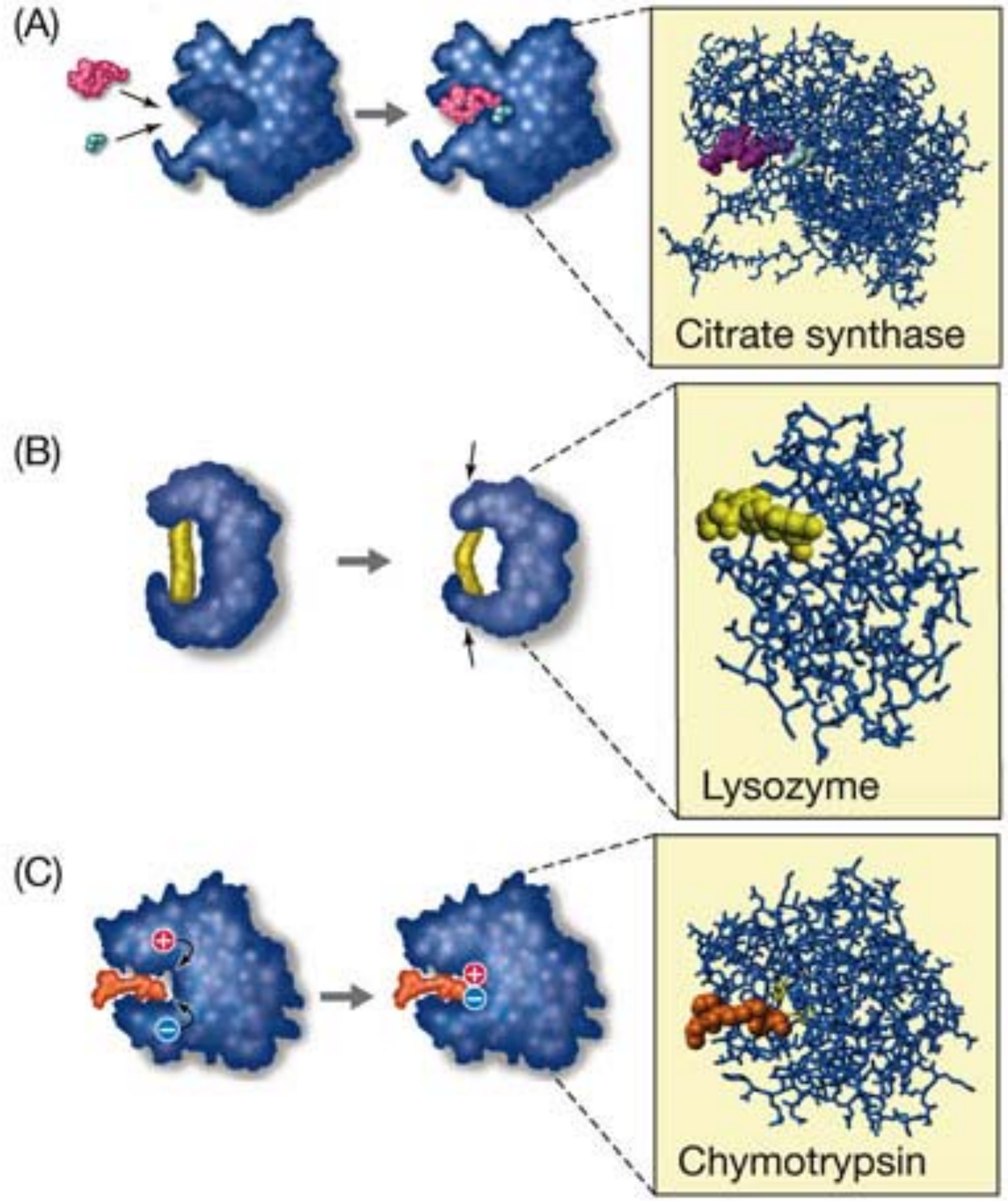
1. Oxidoreductases (oxidation-reduction reactions)
2. Transferases (transfer of functional groups)
3. Hydrolases (hydrolysis reactions)
4. Lyases (addition to double bonds)
5. Isomerases (isomerization reactions)
6. Ligases (formation of bonds with ATP cleavage)

Examples:

Orientation,
Cage effect

Strain

Charge effects,
Covalent bonds,
Acid-base catalysis

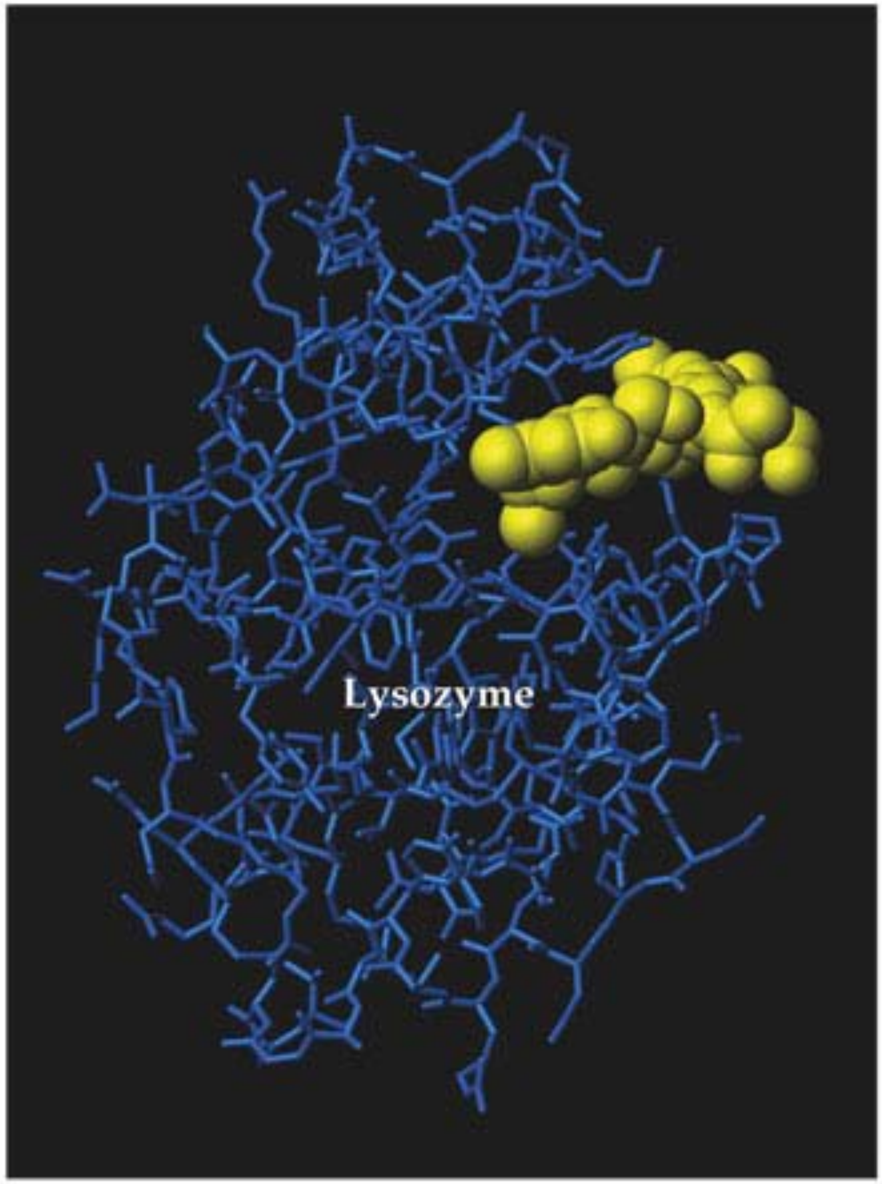


LIFE 8e, Figure 6.11

An example
of an enzyme
that sterically
strains the
substrate:

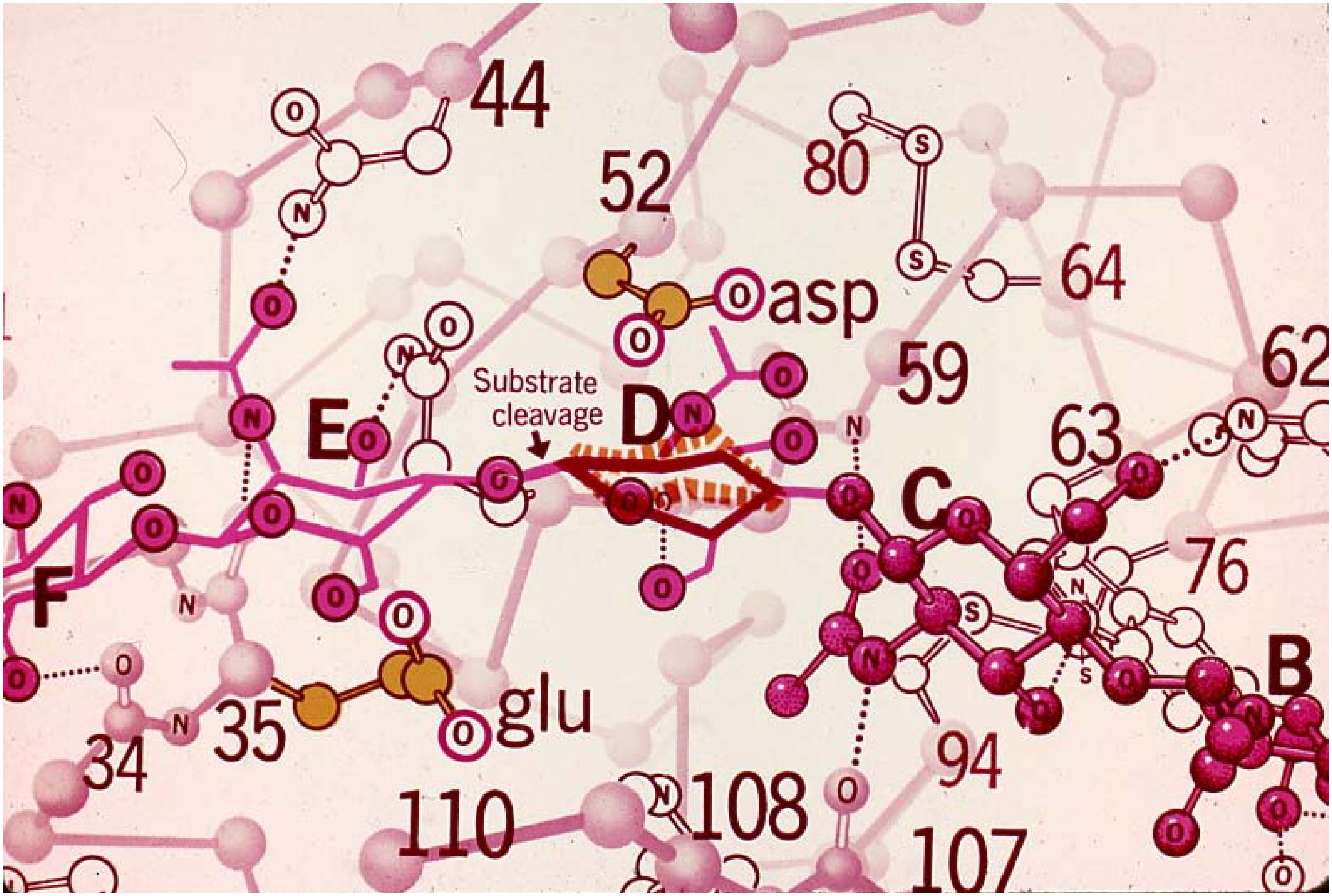
Lysozyme
distorts the bonds
of one of the
sugars in the
polysaccharide
of a bacterial
cell wall

It also places a
partial charge on
the substrate,
making it react
more easily
with water
(hydrolysis).



Hydrolysis
breaks the
polysaccharide
chain and
weakens
the wall so
that the cell
lyses.

LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 6.13 Tertiary Structure of Lysozyme

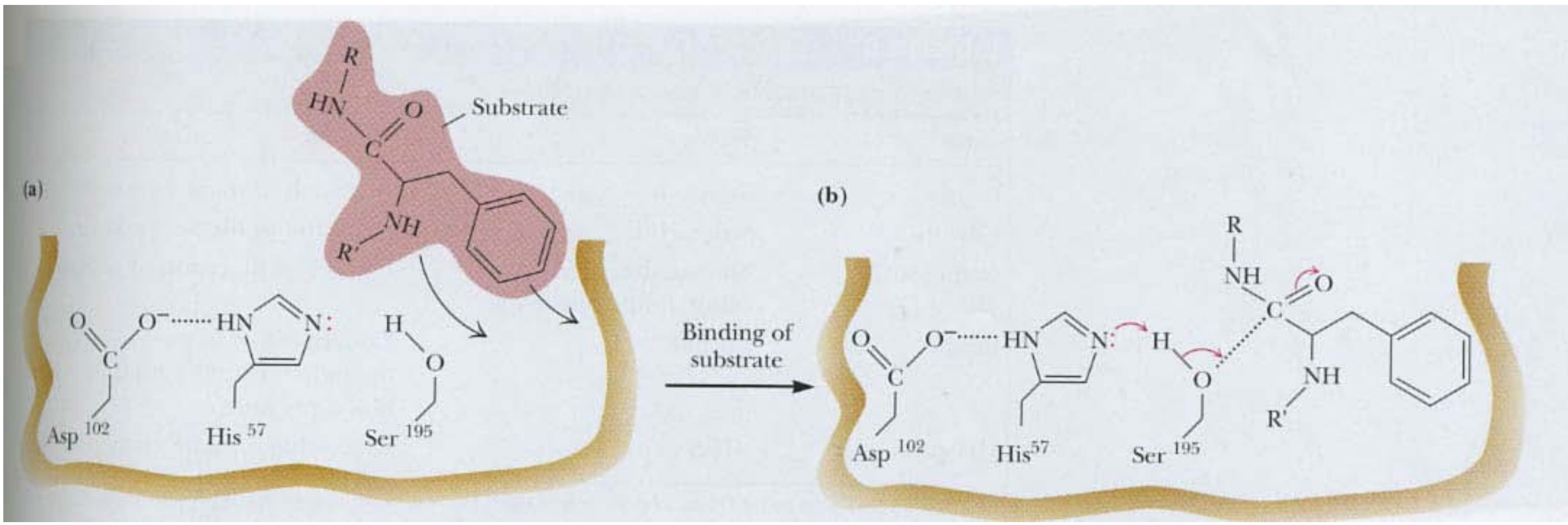


44
52
80
64
asp
59
62
63
Substrate
cleavage
D
E
C
F
76
B
glu
35
34
94
108
110
107

Example of an enzyme mechanism using covalent bonds, acid-base catalysis, low-barrier hydrogen bonds

Serine protease (e.g., trypsin, chymotrypsin, acetylcholinesterase): hydrolyzes peptide bond of proteins (or acetylcholine),

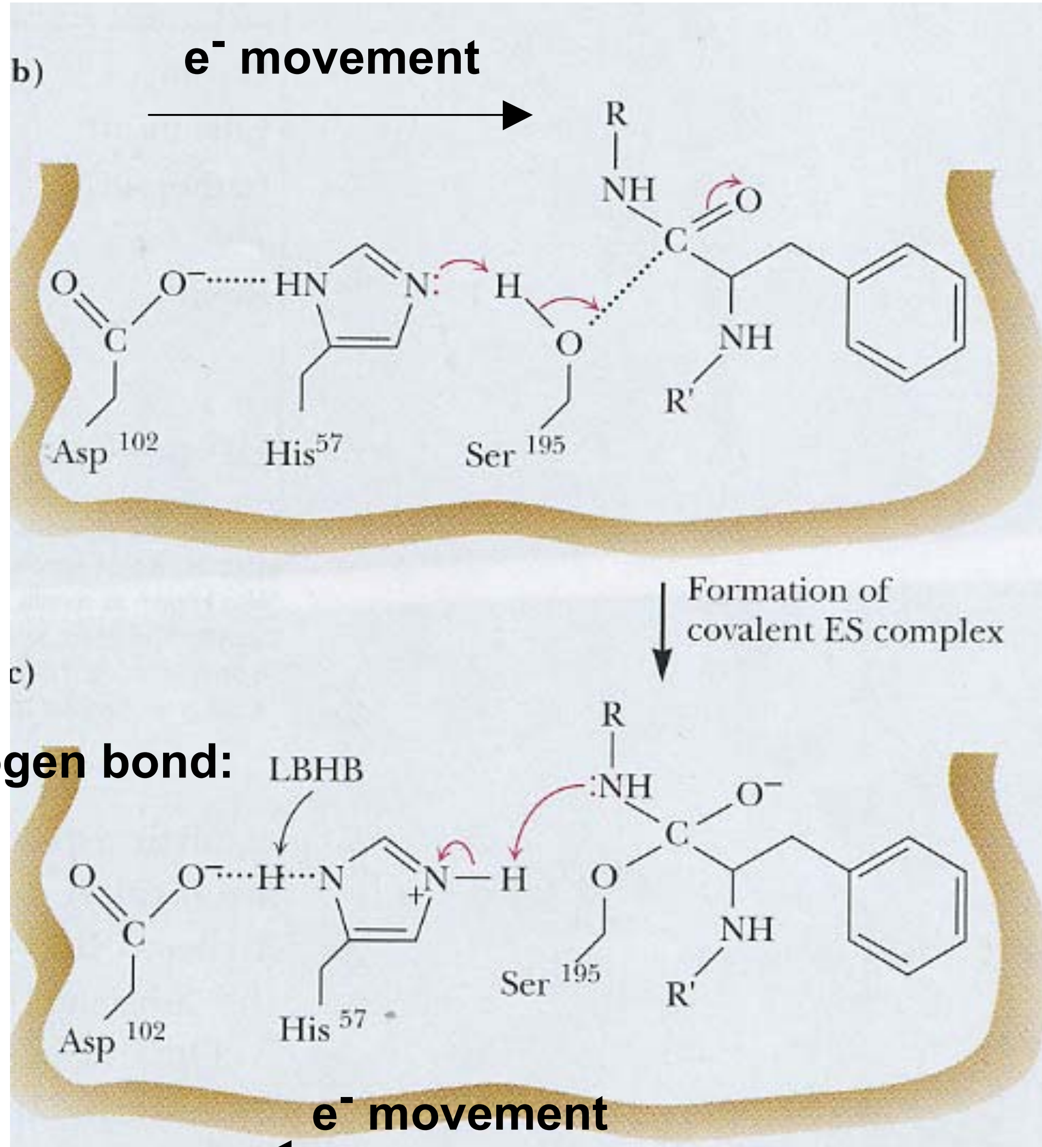
substrate (A-CO-NH-B) + H_2O → A-COOH + H_2N-B



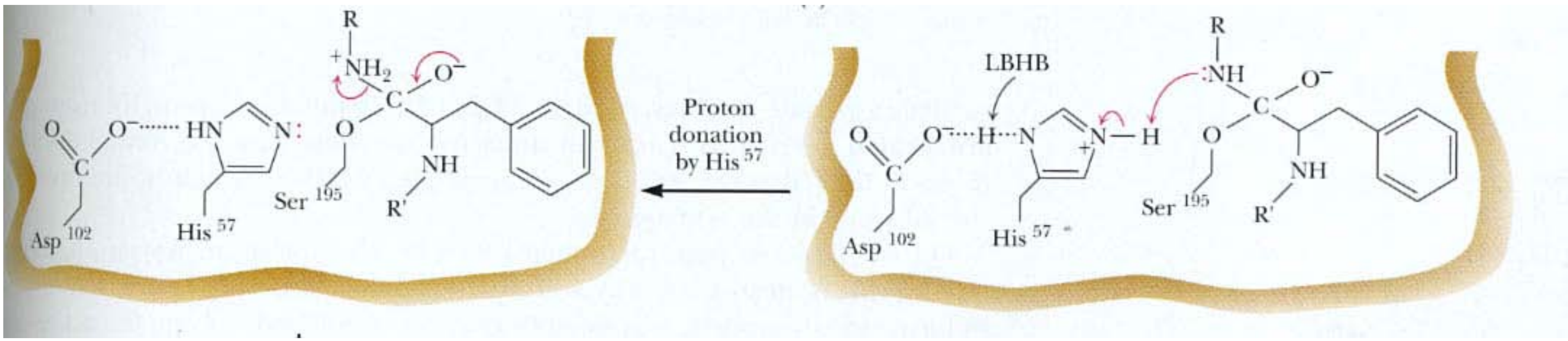
Asp-His-Ser = DHS

e^- movement

Low-barrier hydrogen bond:

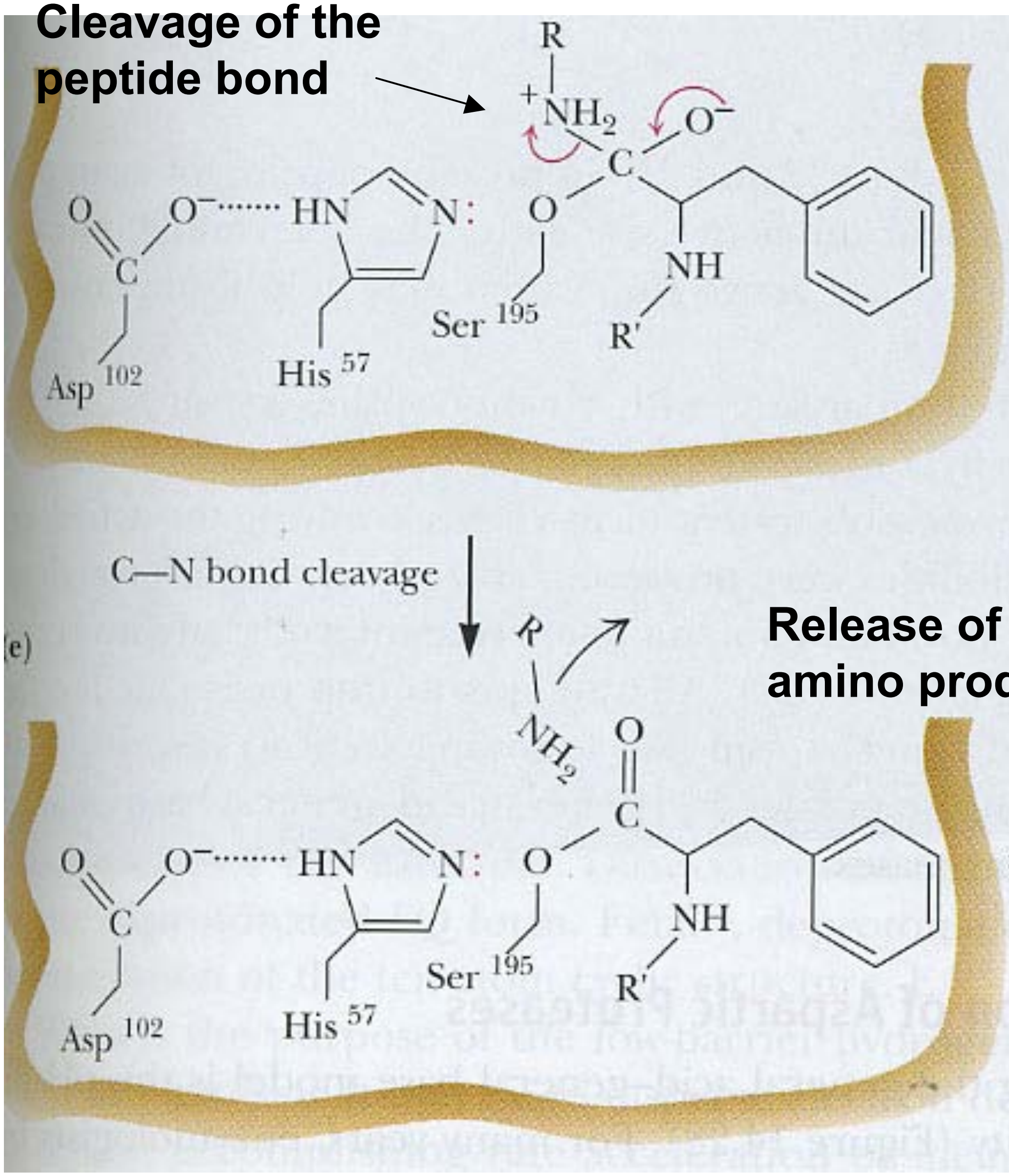


e^- movement

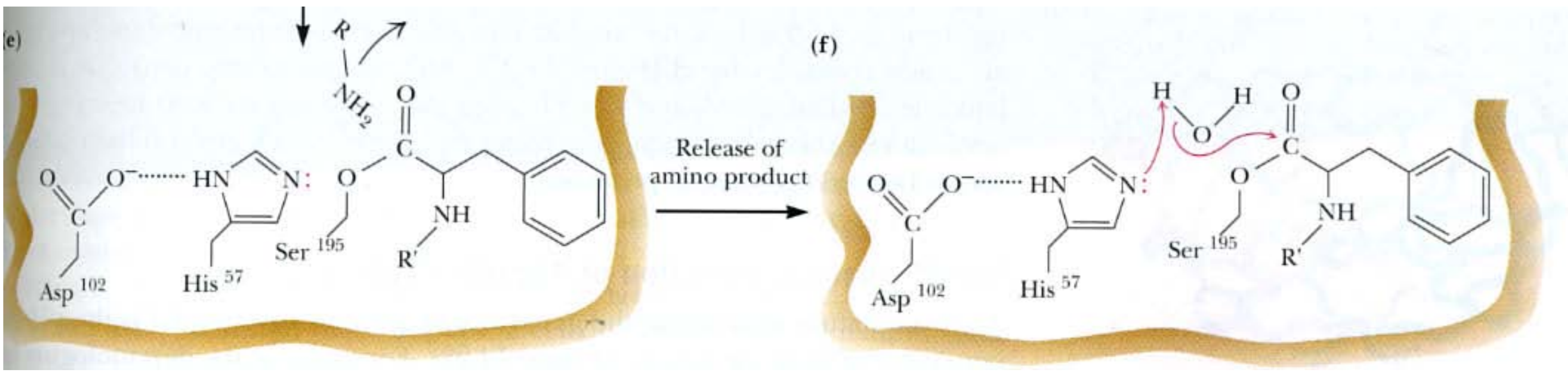


(same picture as previous)

Cleavage of the peptide bond



Release of the amino product



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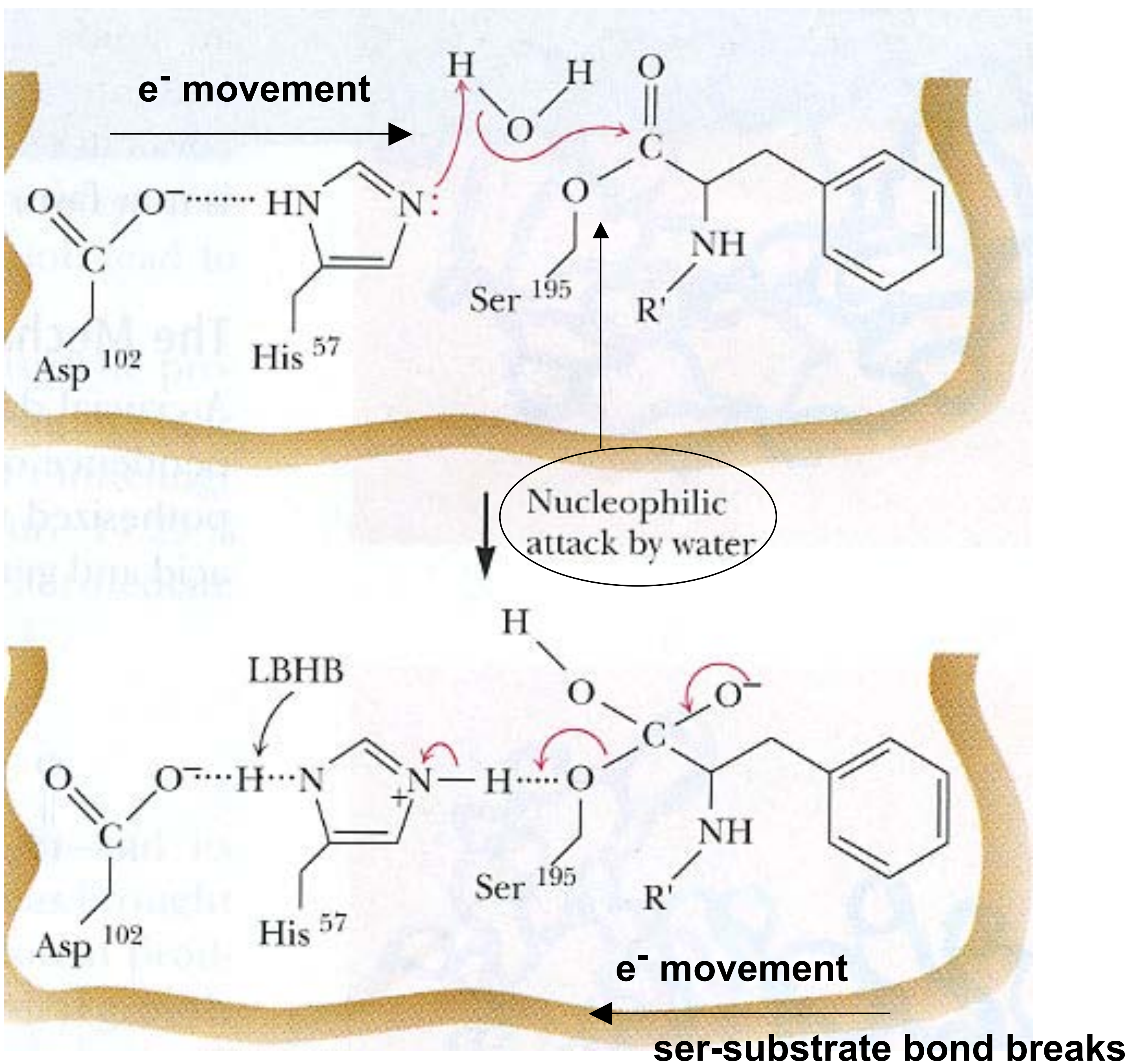
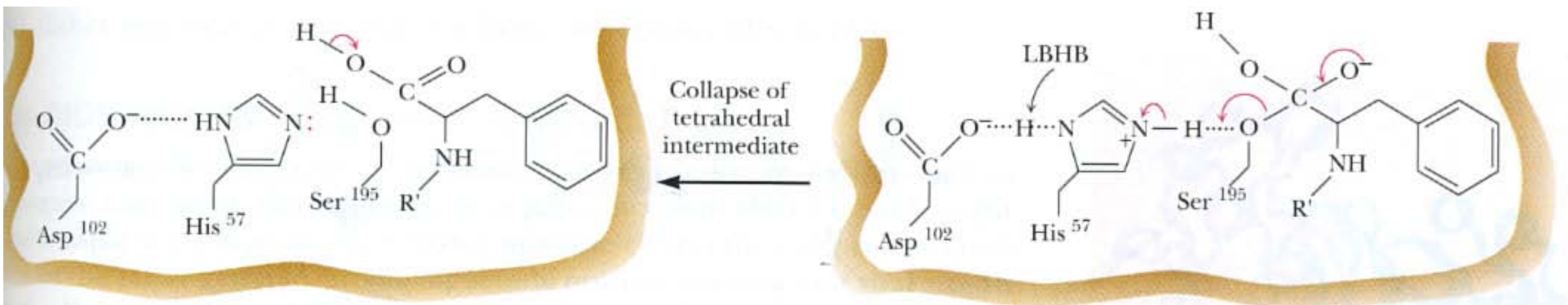

e- movement
H
H
O
O
C
O
O⁻
HN
N:
C
Ser 195
NH
R'
Asp 102
His 57
Nucleophilic attack by water
H
LBHB
O
O⁻
C
O
O⁻····H···N
N
+
N— H····· O
C
NH
Ser 195
R'
Asp 102
His 57
e- movement
ser-substrate bond breaks



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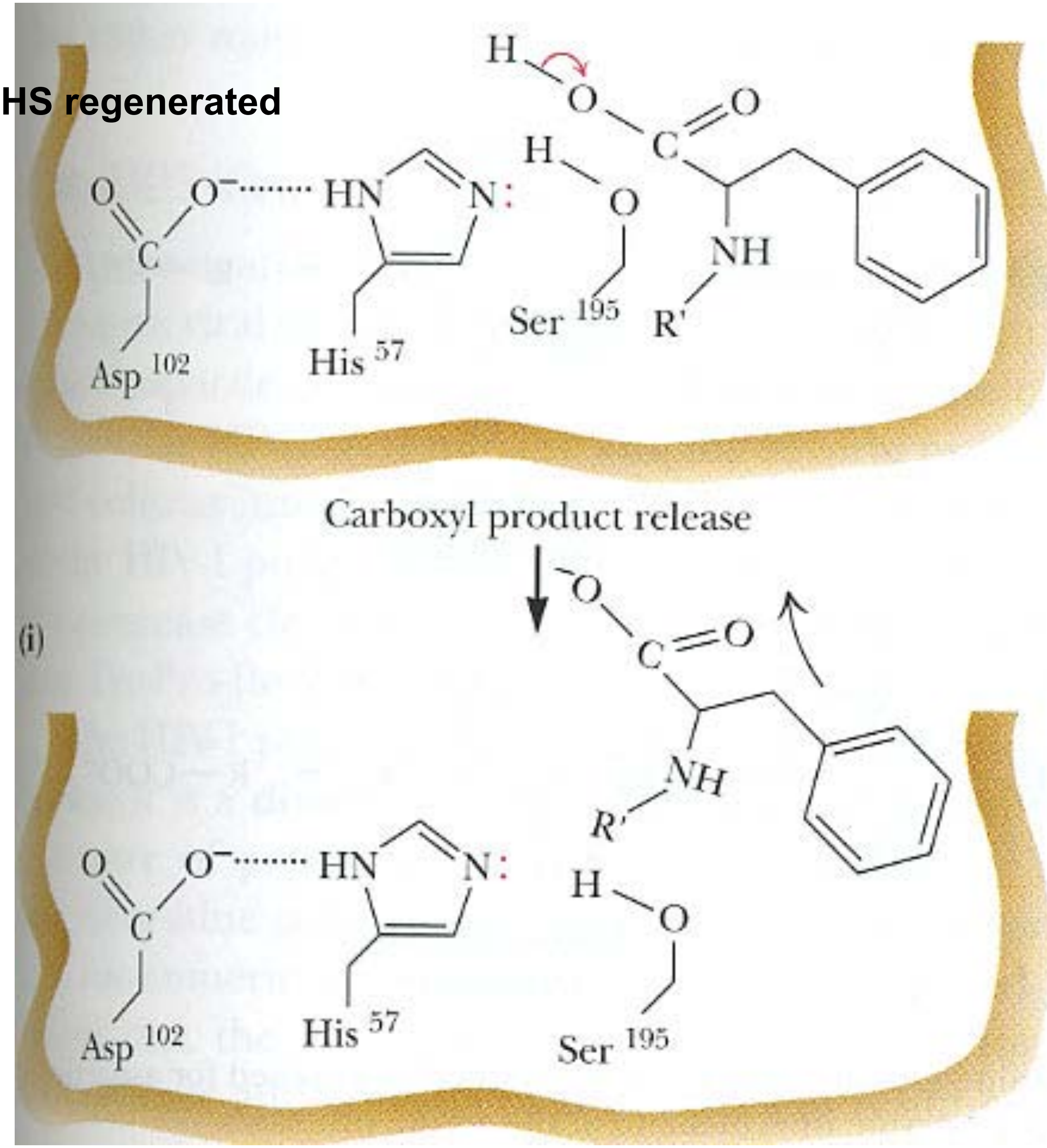
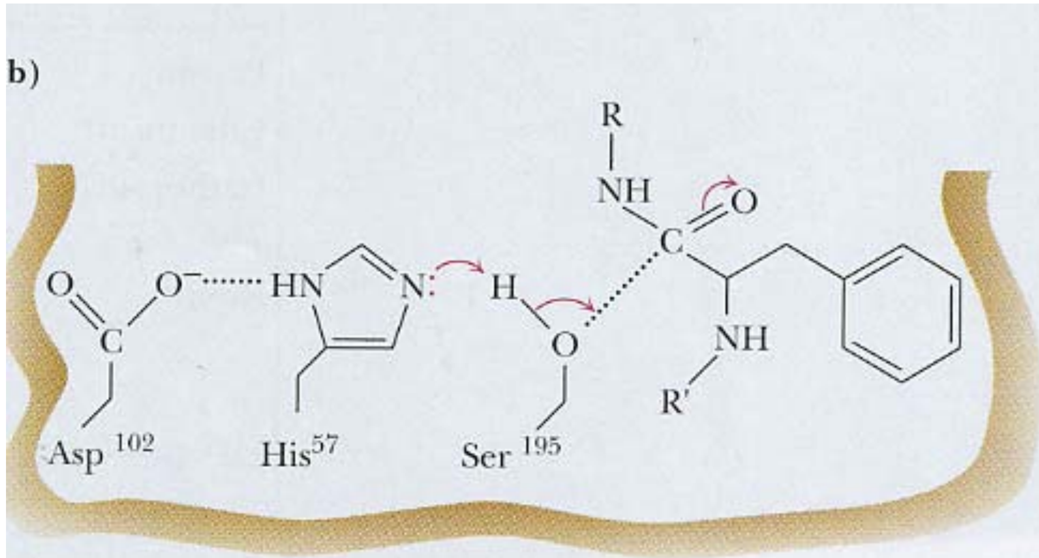
DHS regenerated





Specificity of reaction: depends on DHS in active site
Specificity of substrate: geometry of the activity site

Note the pH dependence: >6 needed for his^{o}

Summary

Enzymes speed reactions by reducing Ea

Enzyme reduce Ea by stressing substrate (raising G(ES))
and by reducing G(ES*)

Lysozyme and chymotrypsin give examples of enzyme
pathways for hydrolysis