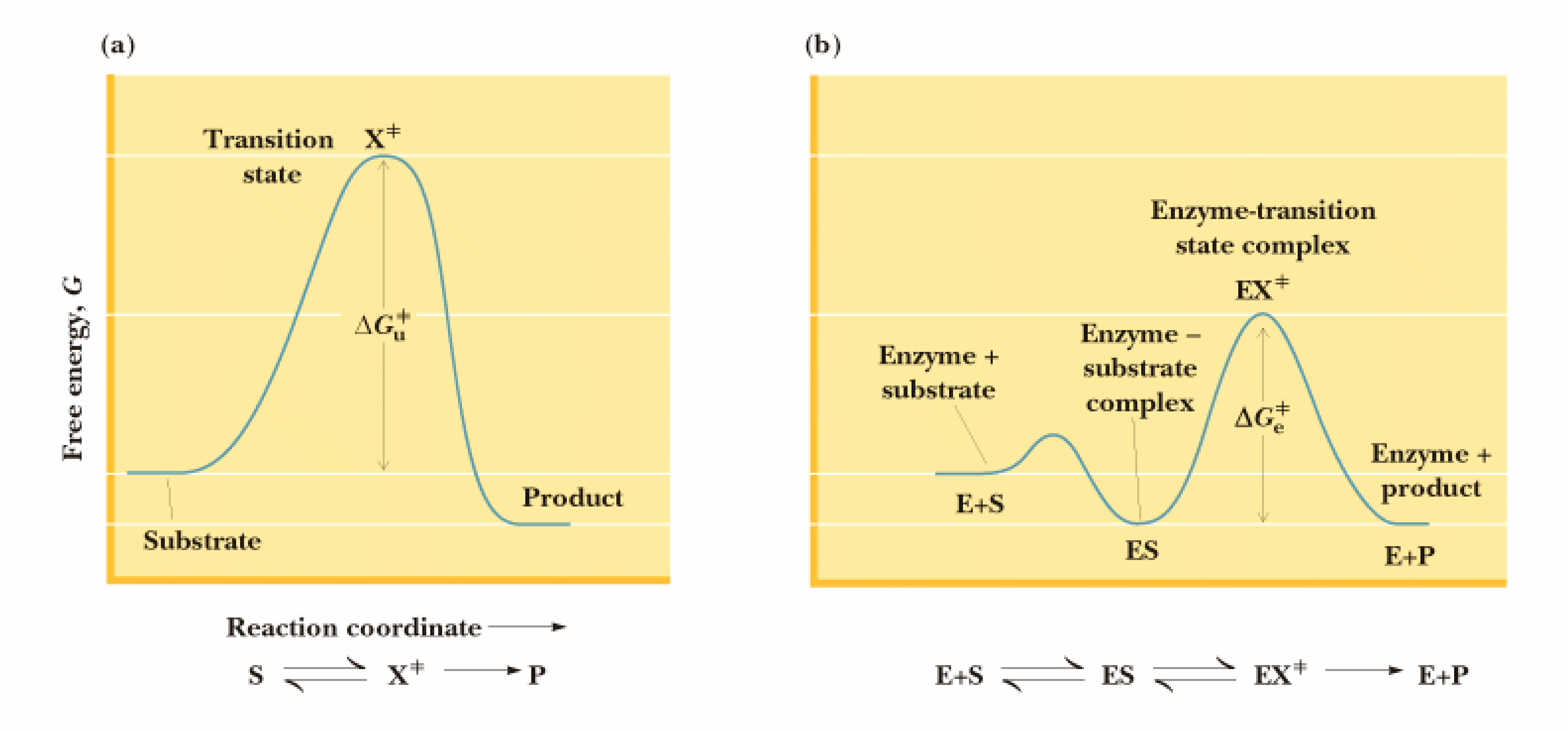
What is catalysis?

Stabilizing the Transition State

- Rate acceleration by an enzyme means that the energy barrier between ES and EX‡ must be smaller than the barrier between S and X‡
- This means that the enzyme must stabilize the EX‡ transition state more than it stabilizes ES



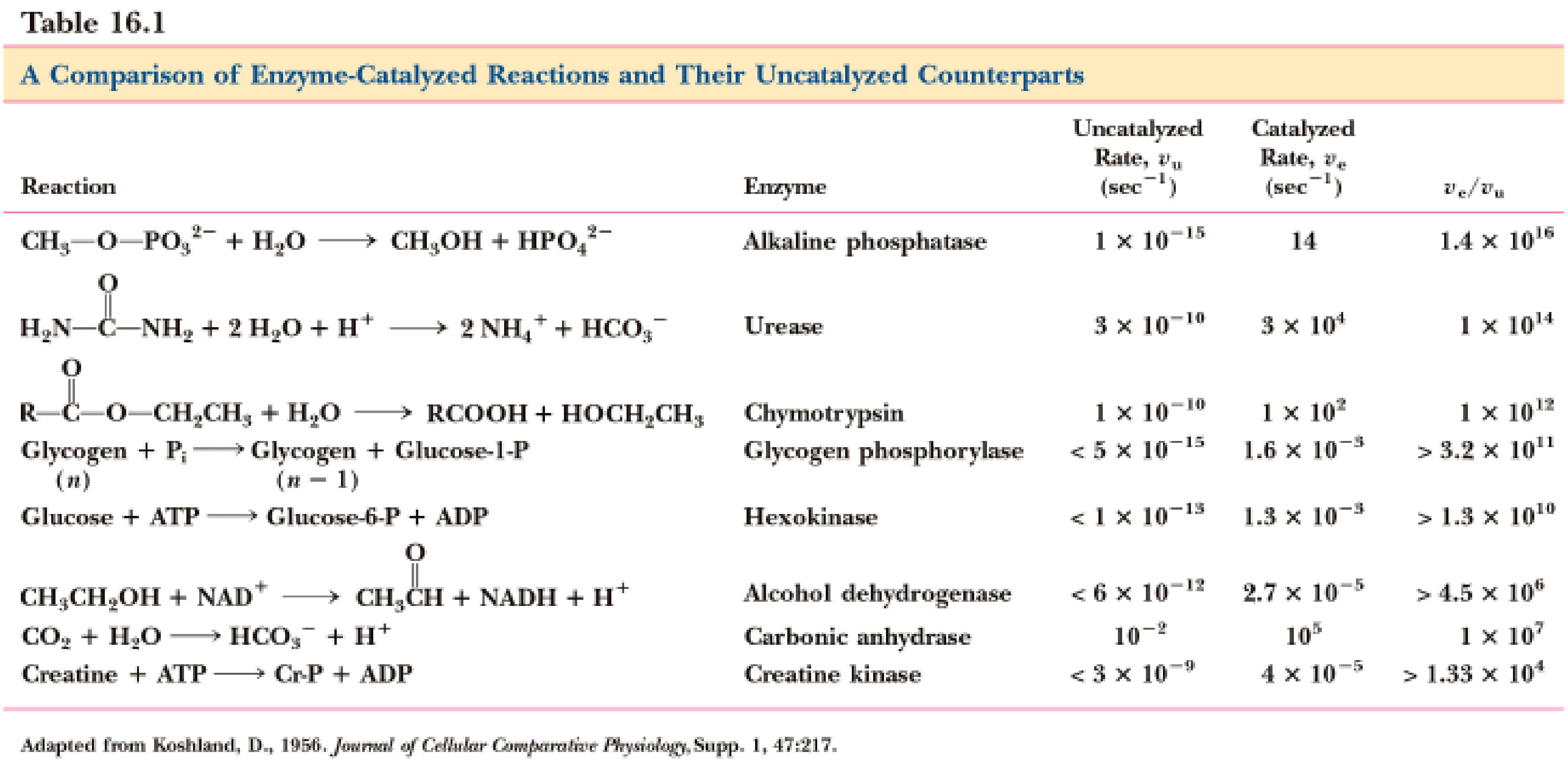


Table 16.1

A Comparison of Enzyme-Catalyzed Reactions and Their Uncatalyzed Counterparts

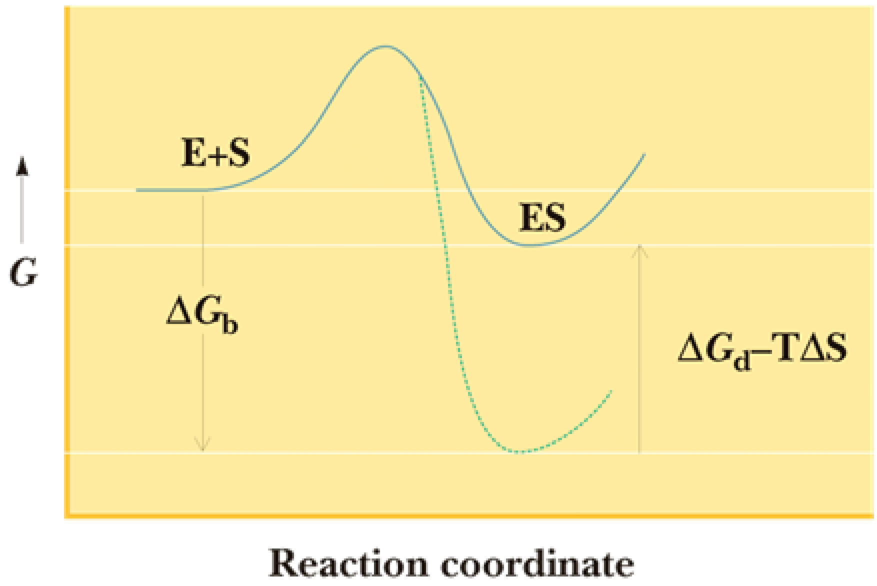
Reaction	Enzyme	Uncatalyzed Rate, v_u (sec^{-1})	Catalyzed Rate, v_e (sec^{-1})	v_e/v_u
$CH_3{-}O{-}PO_3^{2-} + H_2O \longrightarrow CH_3OH + HPO_4^{2-}$	Alkaline phosphatase	1×10^{-15}	14	1.4×10^{16}
$H_2N{-}\overset{\overset{\displaystyle O}{\Vert}}{C}{-}NH_2 + 2\,H_2O + H^+ \longrightarrow 2\,NH_4^+ + HCO_3^-$	Urease	3×10^{-10}	3×10^4	1×10^{14}
$R{-}\overset{\overset{\displaystyle O}{\Vert}}{C}{-}O{-}CH_2CH_3 + H_2O \longrightarrow RCOOH + HOCH_2CH_3$	Chymotrypsin	1×10^{-10}	1×10^2	1×10^{12}
$\underset{(n)}{\text{Glycogen}} + P_i \longrightarrow \underset{(n-1)}{\text{Glycogen}} + \text{Glucose-1-P}$	Glycogen phosphorylase	$< 5 \times 10^{-15}$	1.6×10^{-3}	$> 3.2 \times 10^{11}$
Glucose + ATP $\longrightarrow$ Glucose-6-P + ADP	Hexokinase	$< 1 \times 10^{-13}$	1.3×10^{-3}	$> 1.3 \times 10^{10}$
$CH_3CH_2OH + NAD^+ \longrightarrow CH_3\overset{\overset{\displaystyle O}{\Vert}}{C}H + NADH + H^+$	Alcohol dehydrogenase	$< 6 \times 10^{-12}$	2.7×10^{-5}	$> 4.5 \times 10^6$
$CO_2 + H_2O \longrightarrow HCO_3^- + H^+$	Carbonic anhydrase	10^{-2}	10^5	1×10^7
Creatine + ATP $\longrightarrow$ Cr-P + ADP	Creatine kinase	$< 3 \times 10^{-9}$	4×10^{-5}	$> 1.33 \times 10^4$

Adapted from Koshland, D., 1956. *Journal of Cellular Comparative Physiology,* Supp. 1, 47:217.

Binding Energy of ES

Competing effects determine the position of ES on the energy scale

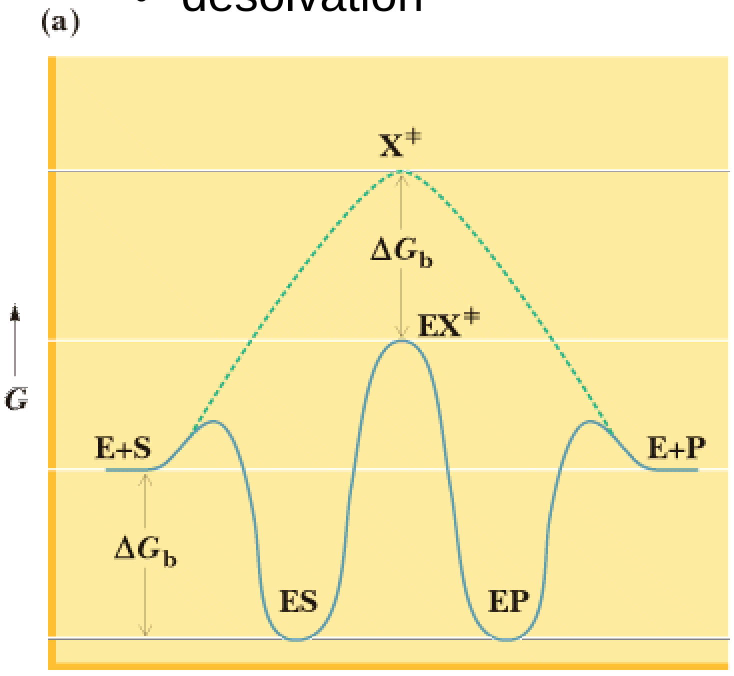
- Try to mentally decompose the binding effects at the active site into favorable and unfavorable
- The binding of S to E must be favorable
- But not too favorable!
- goal is to make the energy barrier between ES and $EX^{\ddagger}$ small



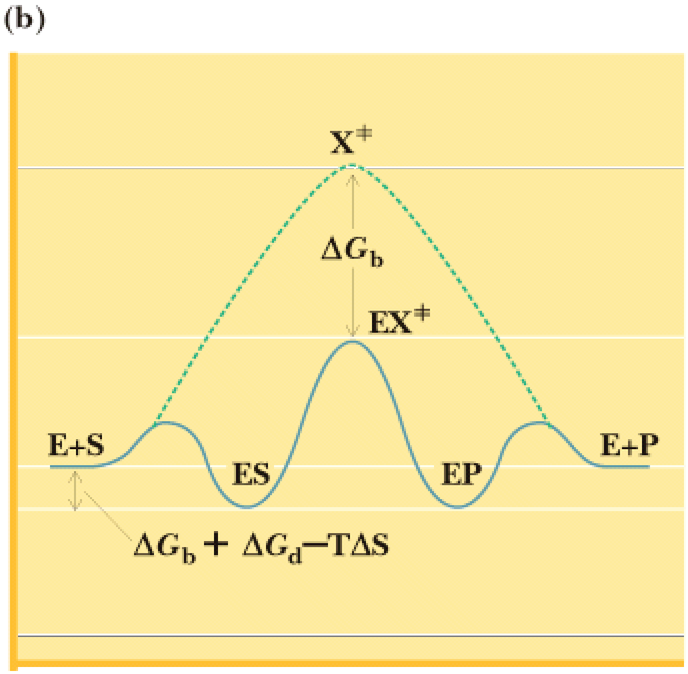
Entropy Loss and Destabilization of ES

Raising the energy of ES raises the rate

- For a given energy of $EX^{‡}$, raising the energy of ES will increase the catalyzed rate
- This is accomplished by
  - a) loss of entropy due to formation of ES
  - b) destabilization of ES by
    - strain
    - distortion
    - desolvation



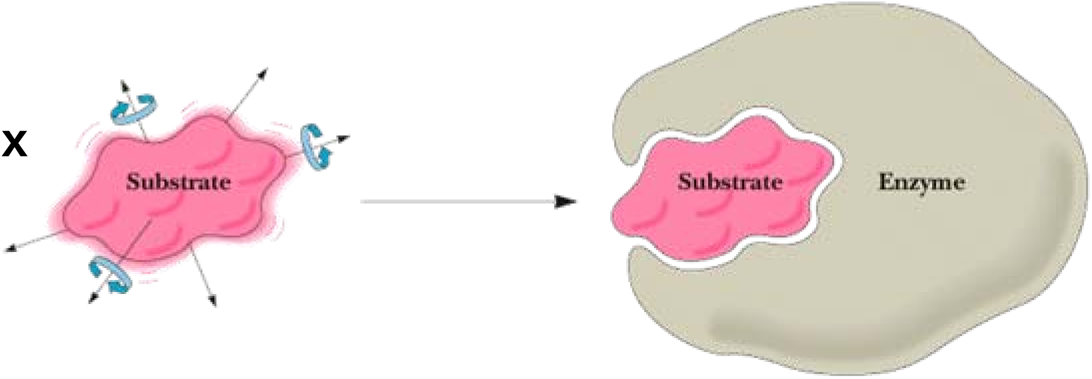
No destabilization, thus no catalysis



Destabilization of ES facilitates catalysis

Entropy Loss and Destabilization of ES

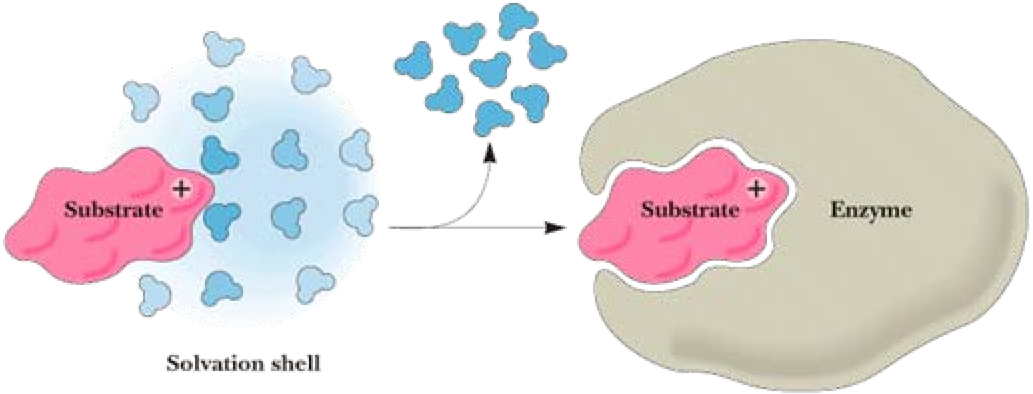
Formation of the ES complex results in a loss of entropy.



Substrate (and enzyme) are free
to undergo translational motion.
A disordered, high-entropy situation

The highly ordered, low-entropy complex

Substrates typically lose waters of hydration in the formation of the ES complex. Desolvation raises the energy of the ES complex



Desolvated ES complex

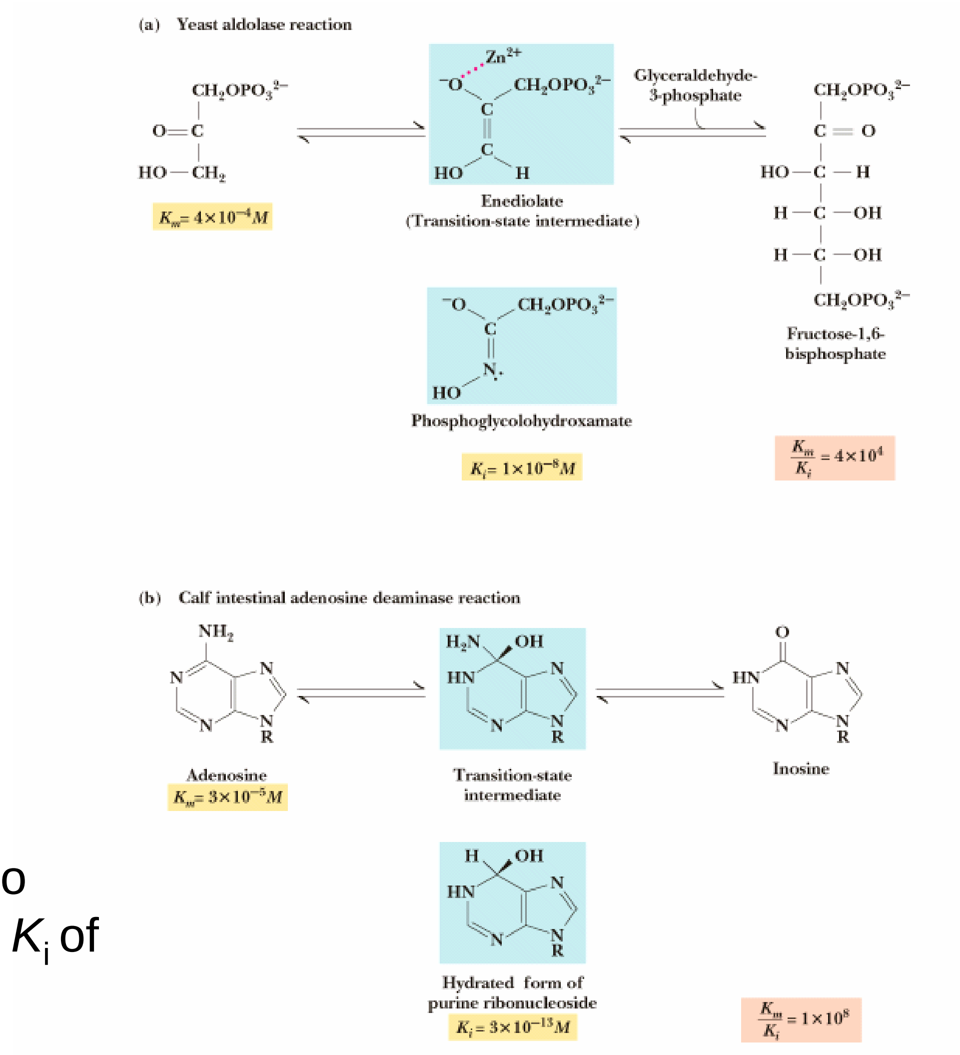
Affinity of Transition State must be higher than the affinity of the substrate

$K_T < K_S$

Insights of binding from transition state analogs

Phosphoglycolohydroxamate binds 40,000 times more tightly to yeast aldolase than the substrate dihydroxyacetone phosphate.

The 1, 6-hydrate of *purine ribonucleoside* has been estimated to bind to adenosine deaminase with a K_i of 3 x 10^{-13} M

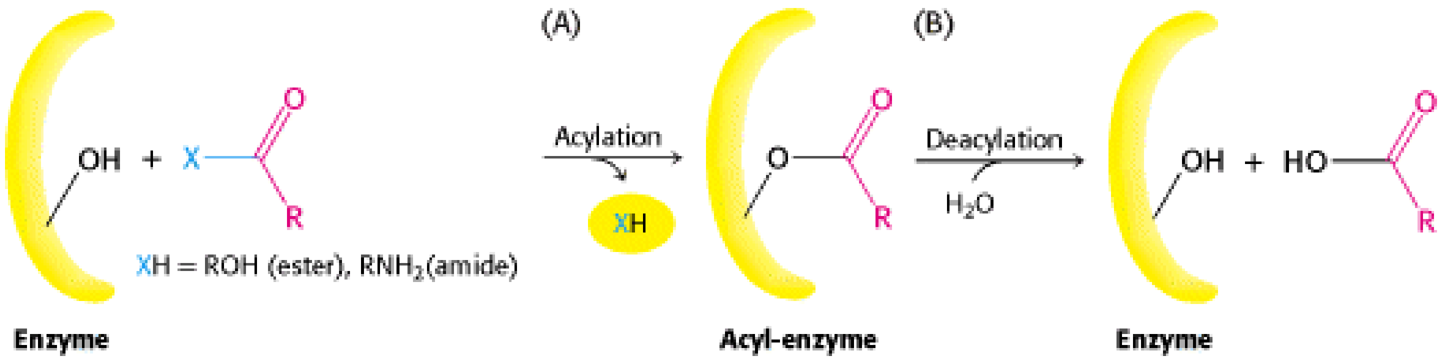


Mechanisms of catalysis

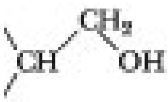
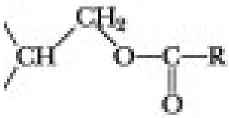
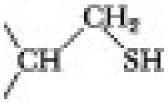
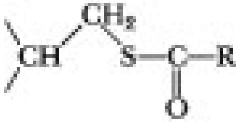
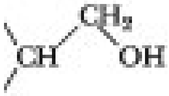
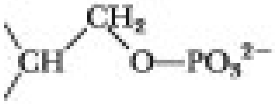
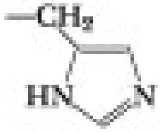
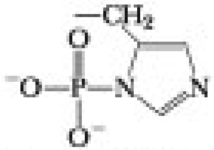
- **Covalent catalysis**
- **General acid/base catalysis**
- **Low-Barrier Hydrogen Bonds**
- **Metal ion catalysis**
- **Proximity and orientation**

Covalent Catalysis

- Enzyme and substrate become linked in a covalent bond at one or more points in the reaction pathway
- The formation of the covalent bond provides chemistry that speeds the reaction

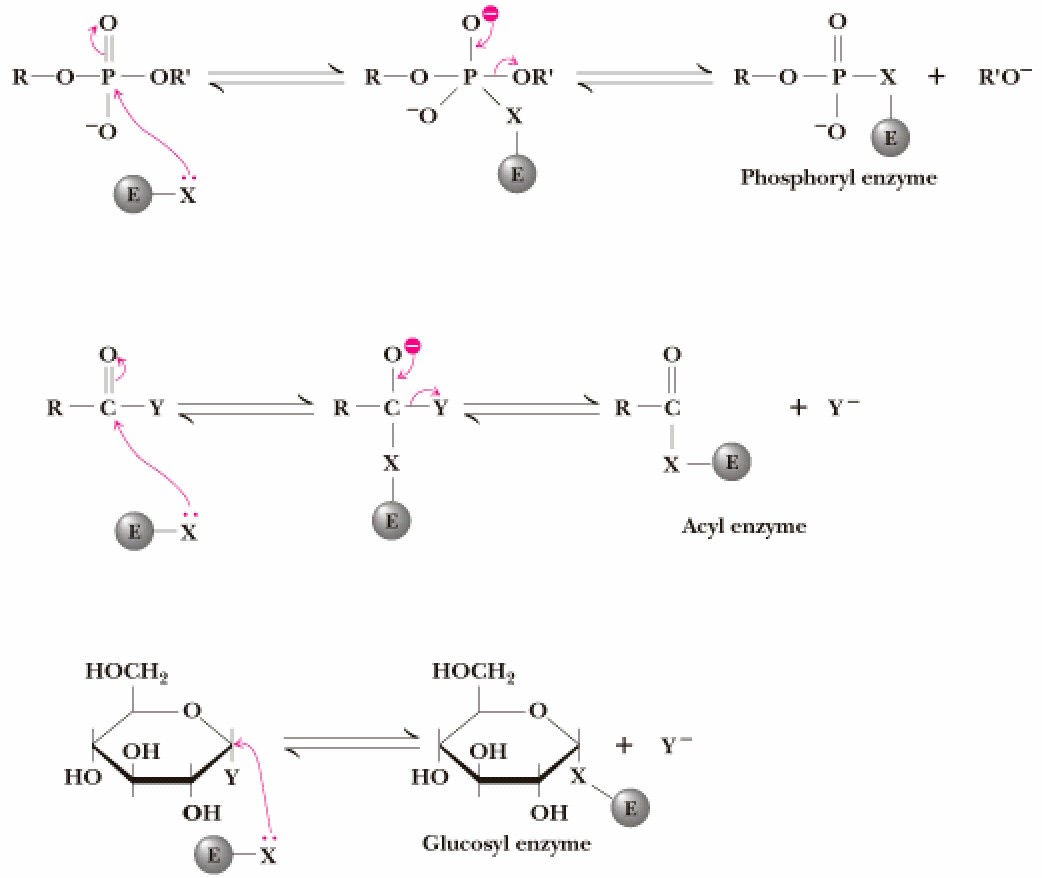


Examples of Enzymes that form Covalent Intermediates

Enzymes	Reacting Group	Covalent Intermediate
1. Chymotrypsin Elastase Esterases Subtilisin Thrombin Trypsin	 (Ser)	 (Acyl-Ser)
2. Glyceraldehyde-3-phosphate dehydrogenase Papain	 (Cys)	 (Acyl-Cys)
3. Alkaline phosphatase Phosphoglucomutase	 (Ser)	 (Phosphoserine)
4. Phosphoglycerate mutase Succinyl-CoA synthetase	 (His)	 (Phosphohistidine)
5. Aldolase Decarboxylases Pyridoxal phosphate-dependent enzymes	$R{-}NH_3^+$ (Amino)	 (Schiff base)

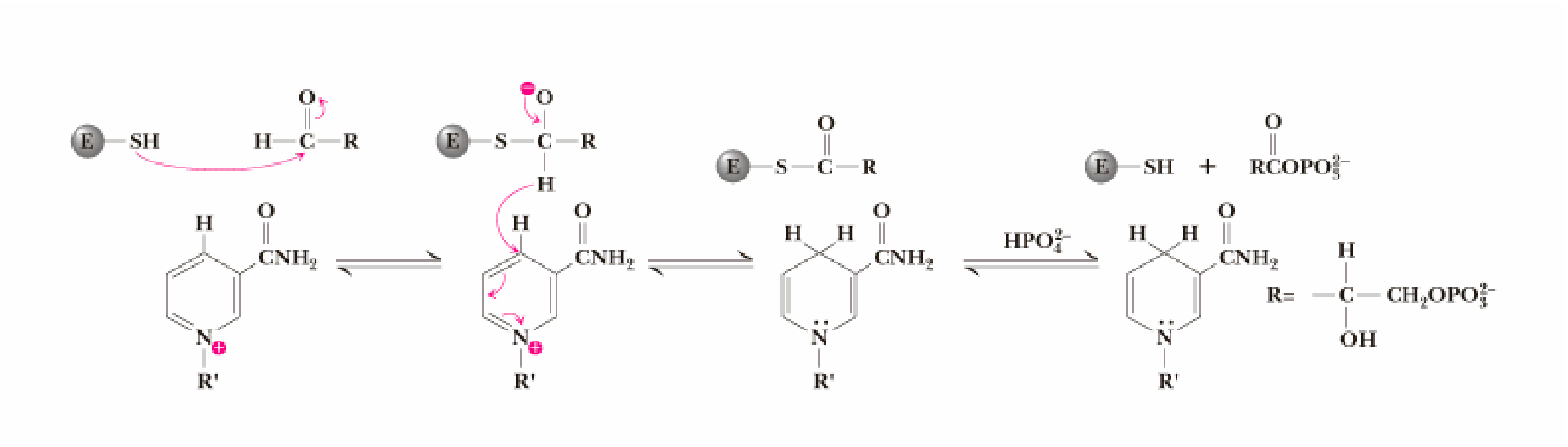
Examples of covalent bond formation between enzyme and substrate

In each case, a nucleophilic center (X:) on an enzyme attacks an electrophilic center on a substrate.



Formation of a covalent intermediate in the glyceraldehyde-3-phosphate dehydrogenase reaction

Glyceraldehyde-3-P + NAD$^+$ + P$_i$ → 1 , 3-Bisphosphoglycerate + NADH + H$^+$



General Acid-base Catalysis

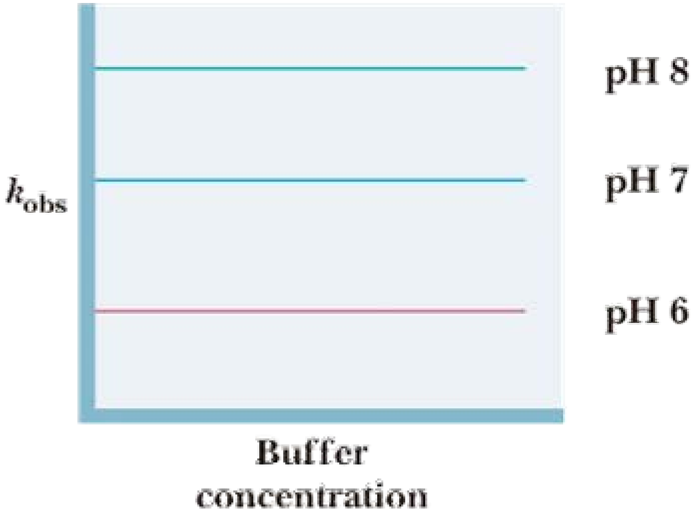
Catalysis in which a proton is transferred in the transition state

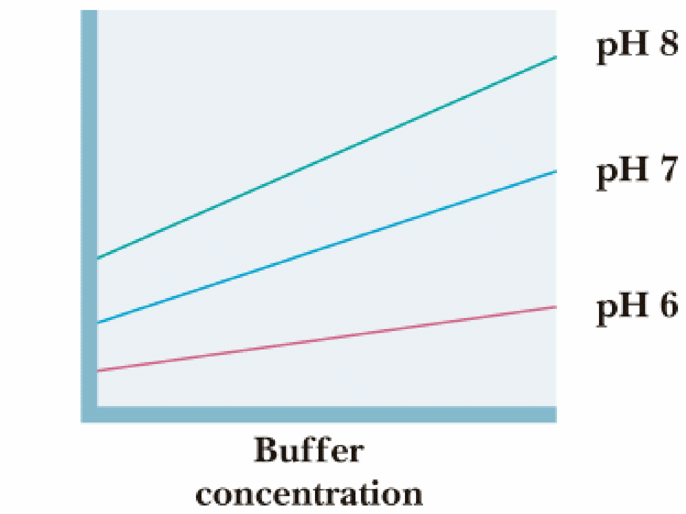
- "Specific" acid-base catalysis involves H^+ or OH^- from the solvent that diffuses into the catalytic center
- "General" acid-base catalysis involves acids and bases other than H^+ and OH^- , which facilitate transfer of H^+ in the transition state

Specific and general acid - base catalysis of simple reactions in solution may be distinguished by determining the dependence of observed reaction rate constants (*k*obs) on pH and buffer concentration.

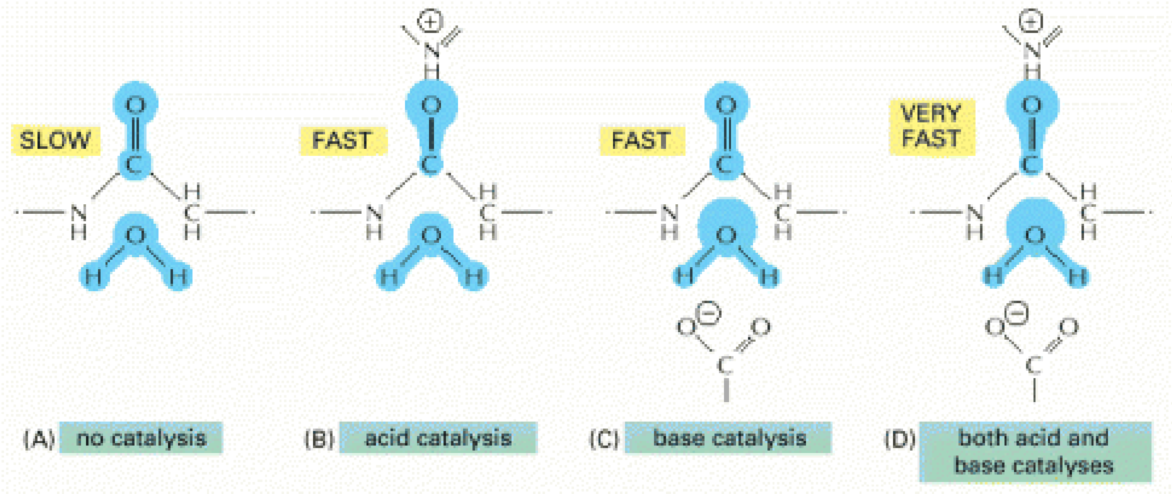
(a) In specific acid-base catalysis, H+ or OH- concentration affects the reaction rate, *k*obs is pH-dependent, but buffers concentration has no effect.

(b) 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 (dependent on all acids and bases in solution).





Example: Water attack of a carbonyl



(A) The start of the uncatalyzed reaction shown in is diagrammed, with *blue* indicating electron distribution in the water and carbonyl bonds.

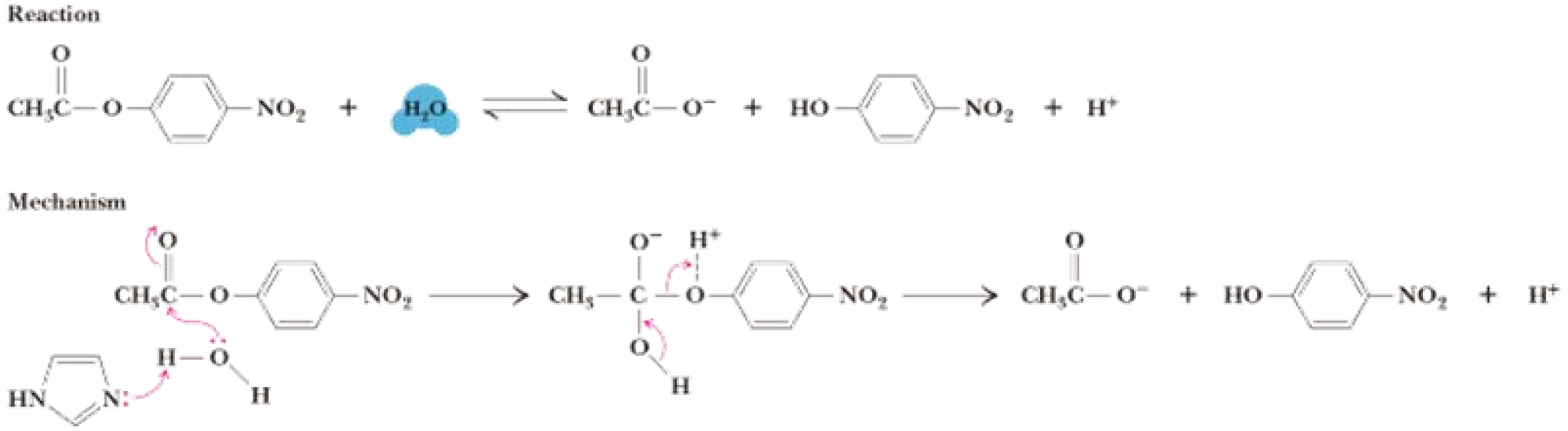
(B) An acid likes to donate a proton (H+) to other atoms. By pairing with the carbonyl oxygen, an acid causes electrons to move away from the carbonyl carbon, making this atom much more attractive to the electronegative oxygen of an attacking water molecule

(C) A base likes to take up H+. By pairing with a hydrogen of the attacking water molecule, a base causes electrons to move toward the water oxygen, making it a better attacking group for the carbonyl carbon.

(D) By having appropriately positioned atoms on its surface, an enzyme can perform both acid catalysis and base catalysis at the same time.

Catalysis of *p*-nitrophenylacetate hydrolysis by imidazole

an example of general base catalysis. Proton transfer to imidazole in the transition state facilitates hydroxyl attack on the substrate carbonyl carbon



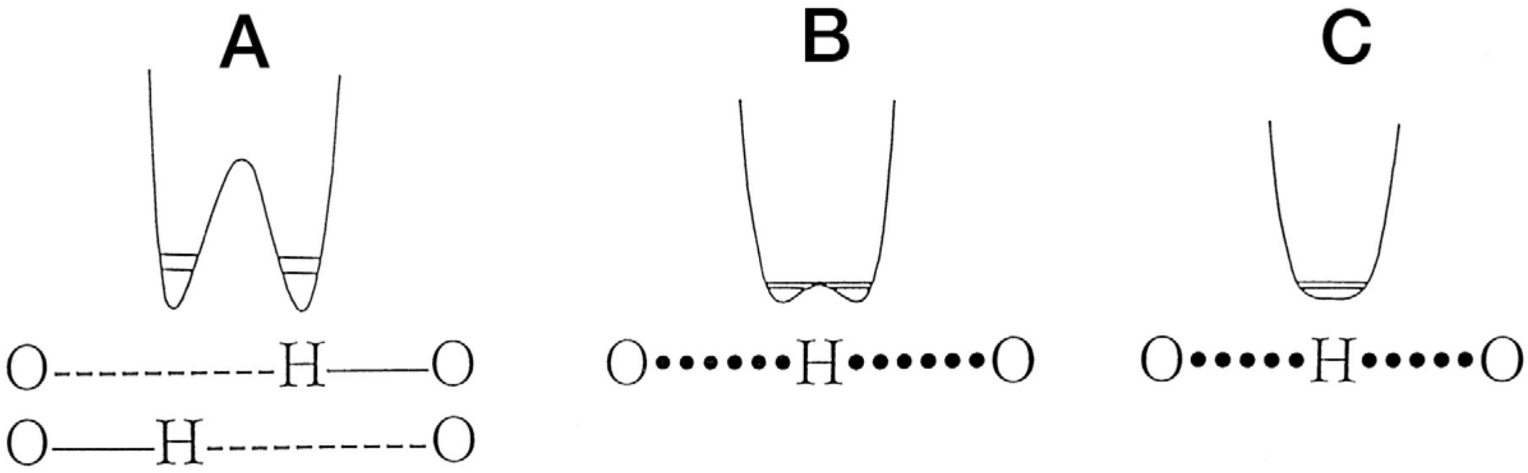
Low-Barrier Hydrogen Bond

Energy diagrams for hydrogen bonds between groups of equal p*K*.

A. weak hydrogen bond with O-O distance of **2.8 Å**; the two positions of the hydrogen are shown.

B. low barrier hydrogen bond of length 2.55 Å; the hydrogen is diffusely distributed, with average position in the center.

C. single-well hydrogen bond with length 2.29 Å. The *upper* and *lower horizontal lines* are zero point energy levels for hydrogen and deuterium, and the *curves* define the energetic barrier to changes in bond length. The distances are to scale



Metal Ion catalysis

Metalloenzyme – binds metal very tightly
Metal activated – binds metal weakly (perhaps only for catalysis)

metal ions that usually assist in catalysis
Fe2+ /Fe3+ Cu2+ Zn2+ Mn2+ Co2+
Metal ions that usually 'activate'
Na+ K+ Mg2+ Ca2+

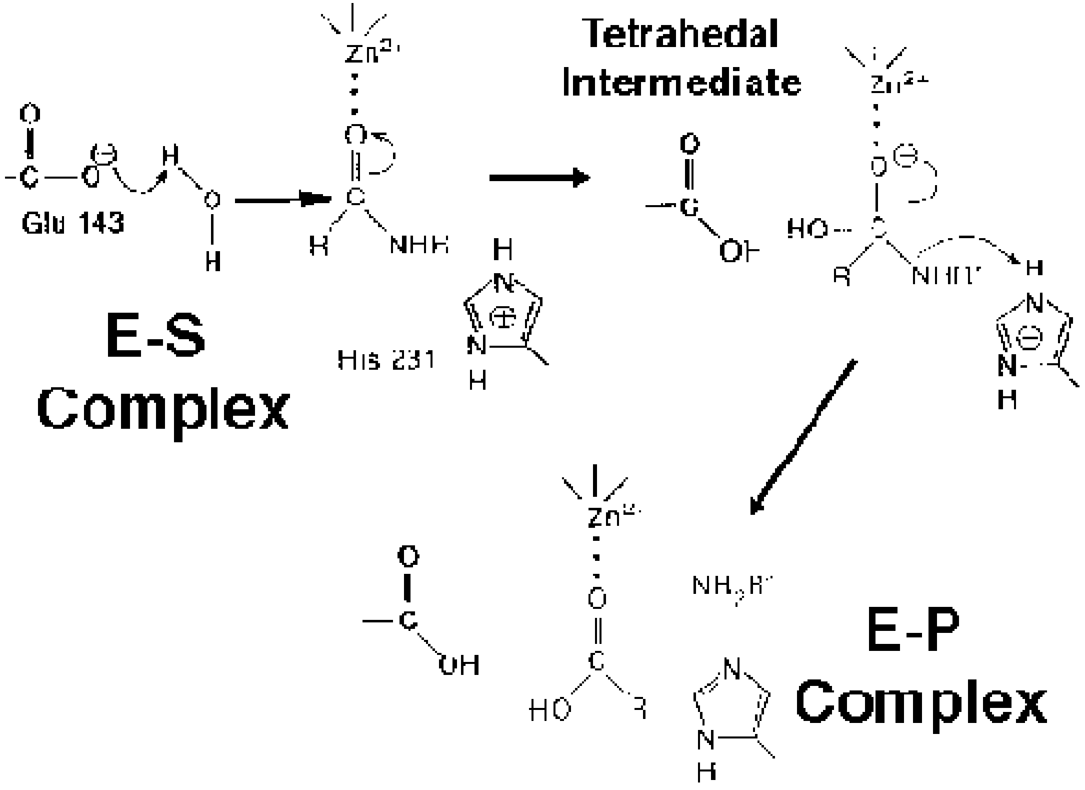
act as electrophilic catalysts, stabilizing the increased electron density or negative charge that can develop during reactions

Or

provide a powerful nucleophile at neutral pH. Coordination to a metal ion can increase the acidity of a nucleophile with an ionizable proton

$$\mathrm{M}^{2+} + \mathrm{NucH} \rightleftharpoons \mathrm{M}^{2+}(\mathrm{NucH}) \rightleftharpoons \mathrm{M}^{2+}(\mathrm{Nuc}^{-}) + \mathrm{H}^{+}$$

Metal Ion Catalysis – Thermolysin Example



Model catalytic mechanism for peptide bond hydrolysis as catalyzed by the endoprotease thermolysin, which is an enzyme found in some laundry detergents where it is used to help remove protein stains from your clothes.

Proximity and Orientation

proximity of reactants is said to raise the “effective” concentration over that of the substrates in solution, and leads to an increased reaction rate

The imidazole-catalyzed hydrolysis of *p*-nitrophenylacetate

Intermolecular reaction

K_{obs} = 55 M^{-1} min^{-1}

K_{obs} = 839 min^{-1}

(839 min-1)/(35 *M*-1 min-1) = 23.97 *M*

a concentration of imidazole of 23.9 *M* would be required in the intermolecular reaction to make it proceed as fast as the intramolecular reaction