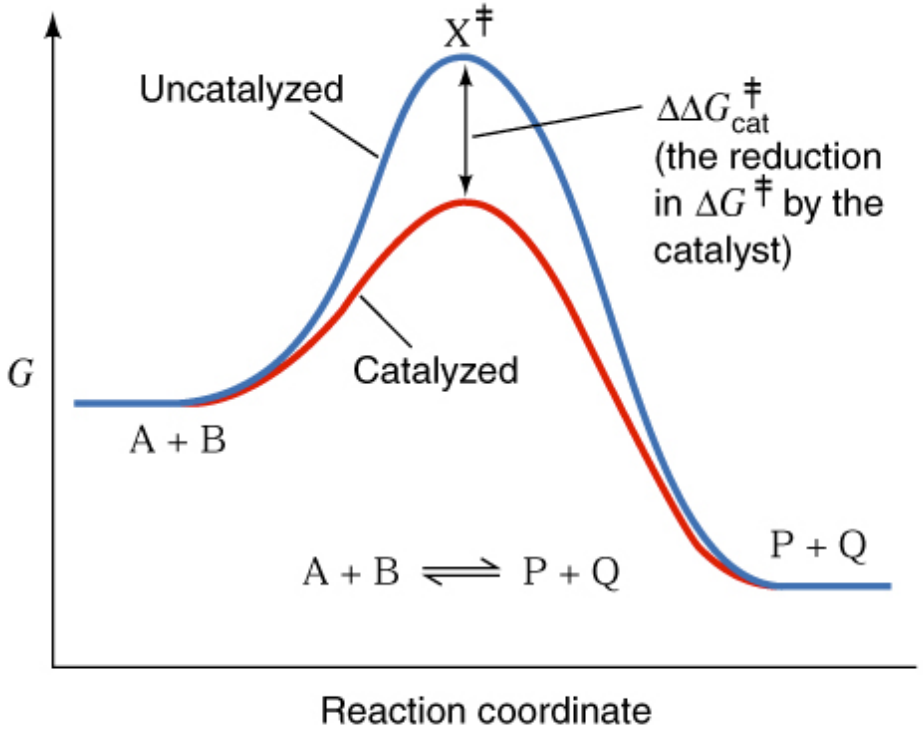
Enzyme catalysis

Factors that contribute to catalytic power of enzymes



- Acid/Base catalysis
- Covalent catalysis
- Metal ion catalysis
- Electrostatic catalysis
- Proximity and orientation
- Preferential binding of the transition state

General Acid/Base catalysis

- Distinguished from specific acid base where H^+ or OH^- accelerates reaction.
- General acid/base is where acid or base other than H^+ or OH^- accelerates reaction.
- In solution, the two distinguished by effect of buffer concentration. In general acid/base, increase in buffer increases rate of reaction.
- Acid/base groups on enzyme serve this role. Asp, Glu, His
- Example is *p*-nitrophenolacetate hydrolysis and role of imidizole in H bonding to H-O-H to make it a stronger nucleophile (next slide).

General Acid/Base catalysis

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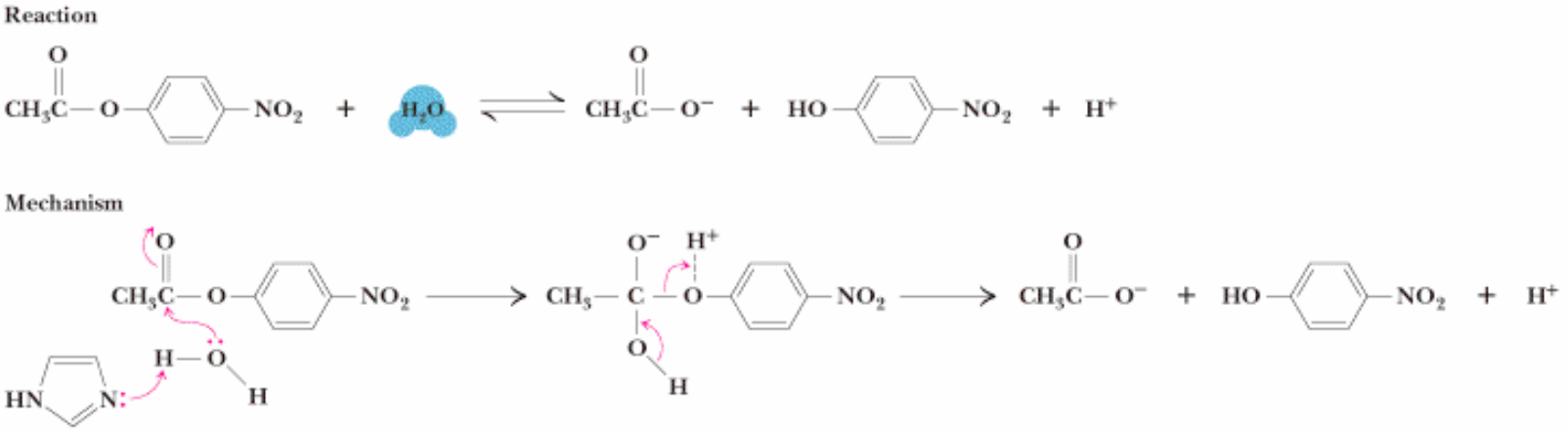
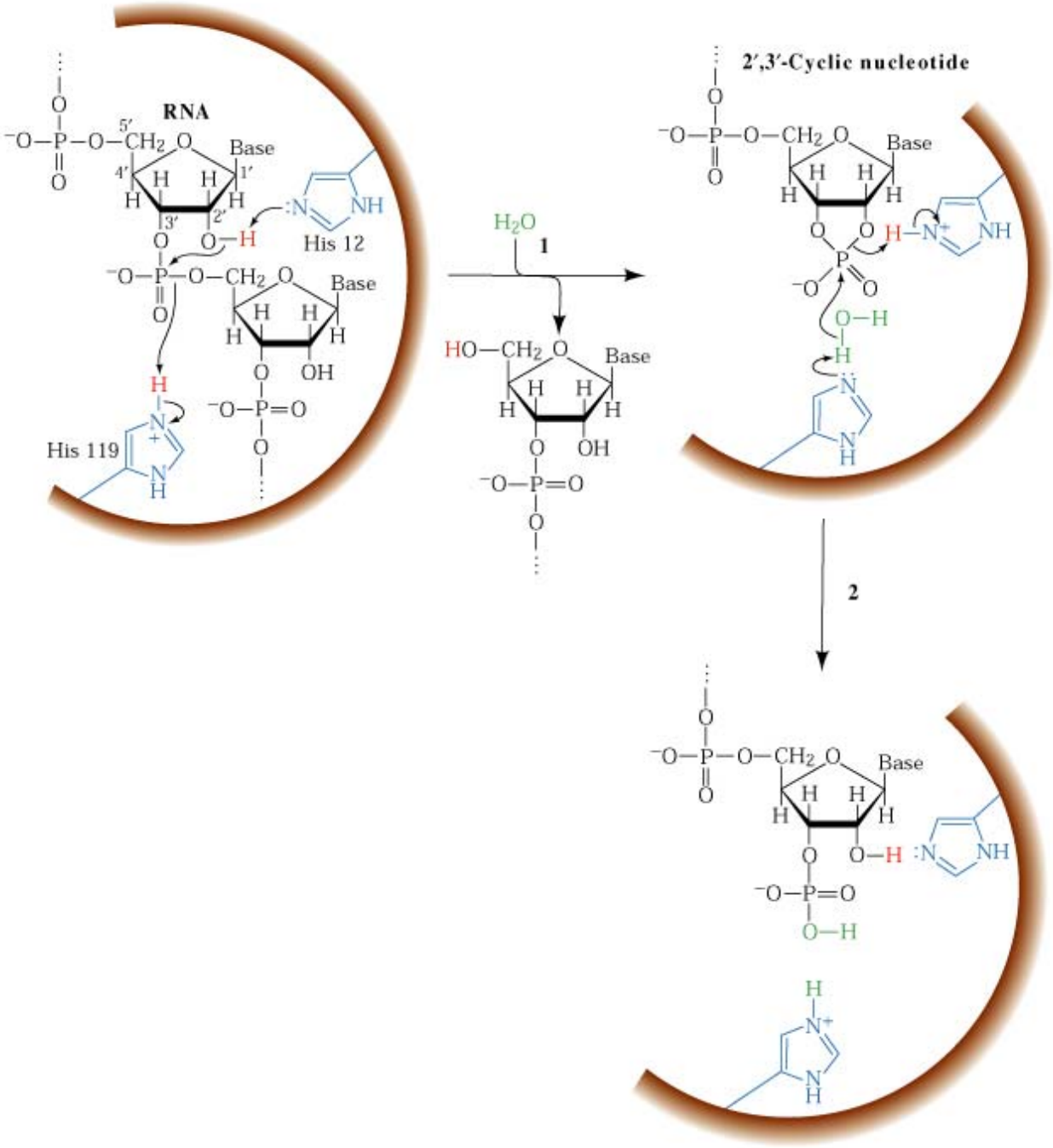
Figure 16.12

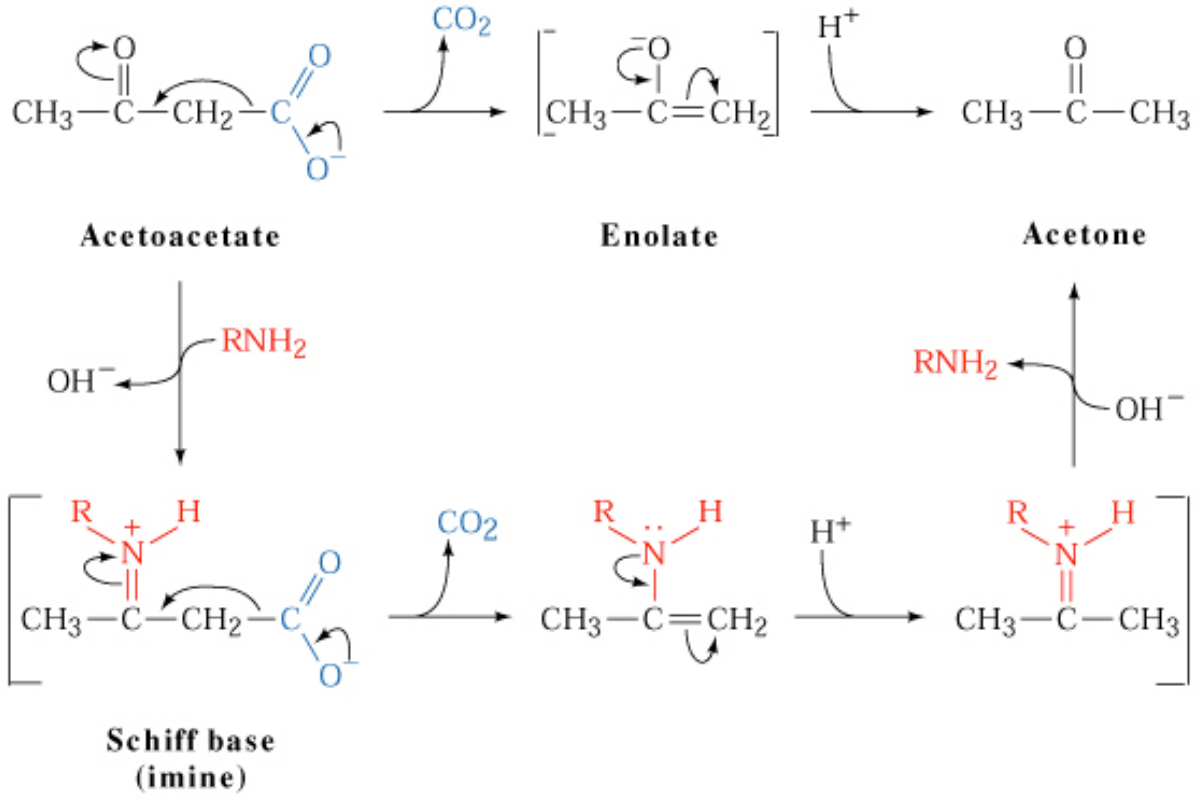


RNase A: general acid-base catalysis



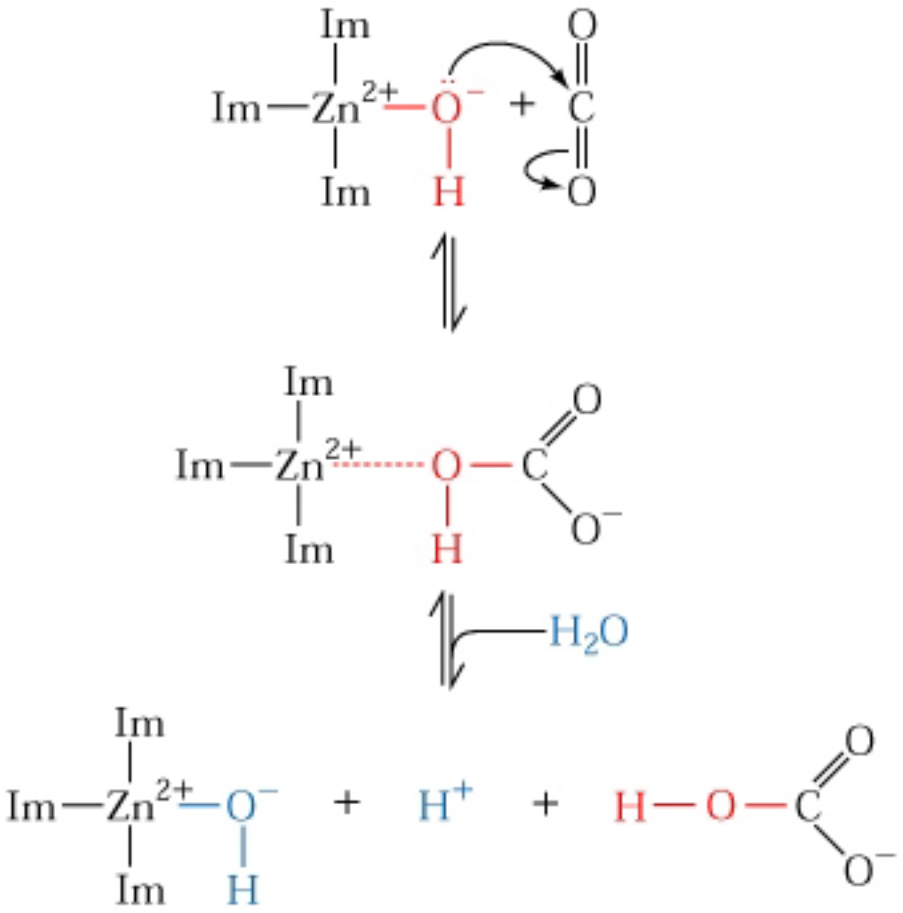
- Bovine RNase A is digestive enzyme that hydrolyzes RNA.
- Enzyme has two step mechanism with 2'3' cyclic nucleotide as intermediate
- Two his at active site. One is general acid, other is general base

Covalent catalysis



- In reaction of BX + Y → BY + X, for enzyme to catalyze reaction with covalent intermediate: BX + Enzy → Enzy-B + X + Y → Enzy + BY, it must be better nucleophile than Y and better leaving group than X. This describes a ping pong mechanism
- Variety of a.a. side chains can serve as nucleophile (-SH, -OH, -NH_2)
- In glyceraldehyde-3-P dehydrogenase, nucleophilic attack of aldehyde by SH on aldehyde forms covalent intermediate which is oxidized.
- Above shows decarboxylation of acetoacetate. Intermediate is much more stable when Schiff base is involved.

Metal ion catalysis



- Metal ions can be tightly bound in metalloenzymes or weakly associated in metal activated.
- Can act as electrophilic catalyst, stabilizing negative charge developed in reaction.
- Metal ion can also act as base accepting protons to make nucleophile stronger (water).
- Example shows Zn of carbonic anhydrase making water a stronger nucleophile.

Electrostatic catalysis

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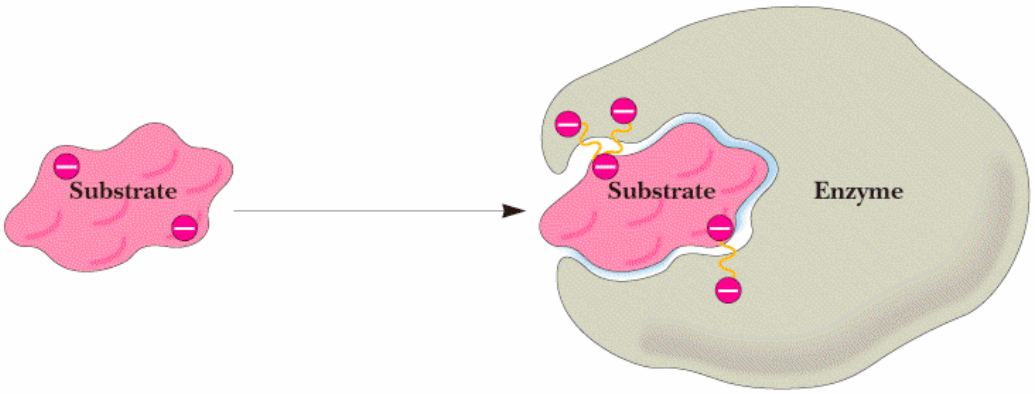
Figure 16.6



Electrostatic destabilization
in ES complex

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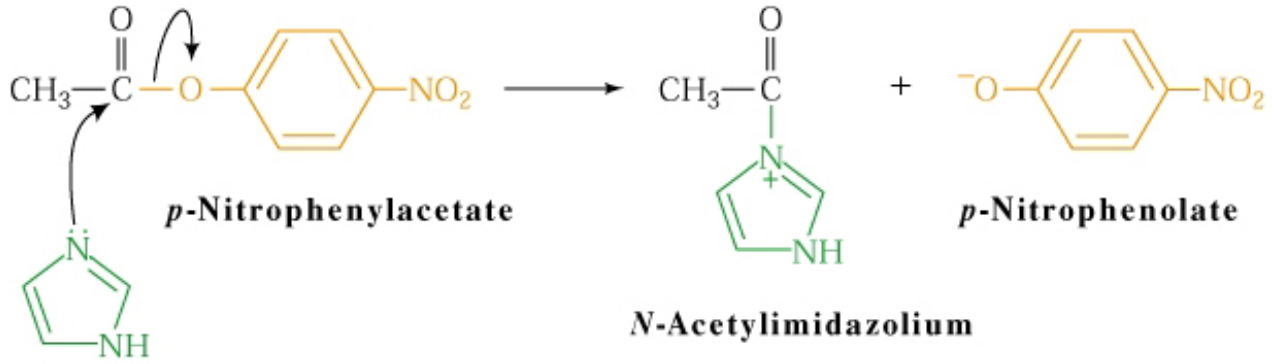
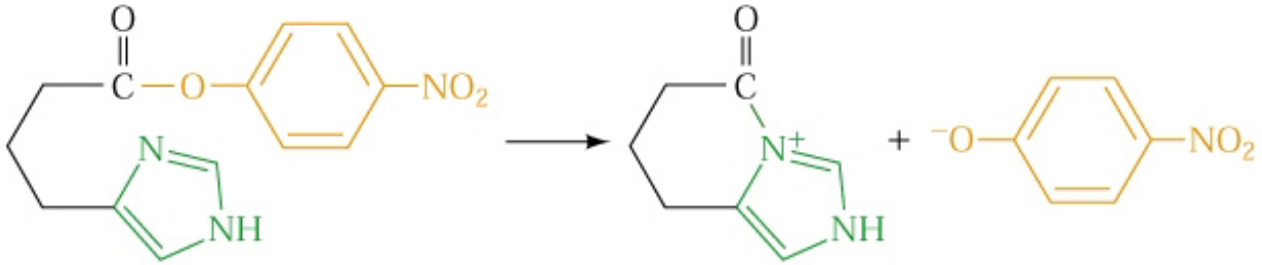
- Binding of substrate can exclude water from active site thus altering local dielectric constant of the active site.
- Make site more like organic solvent.
- Active site made to accommodate transition state, not substrate, thus there is strain. Desolvation results in groups which were solvated by water being removed to more hydrophobic environment, more reactive (higher energy). Electrostatic interactions may not be favorable as ES but more stable as EP, thus driving reaction forward.

Proximity and orientation effects

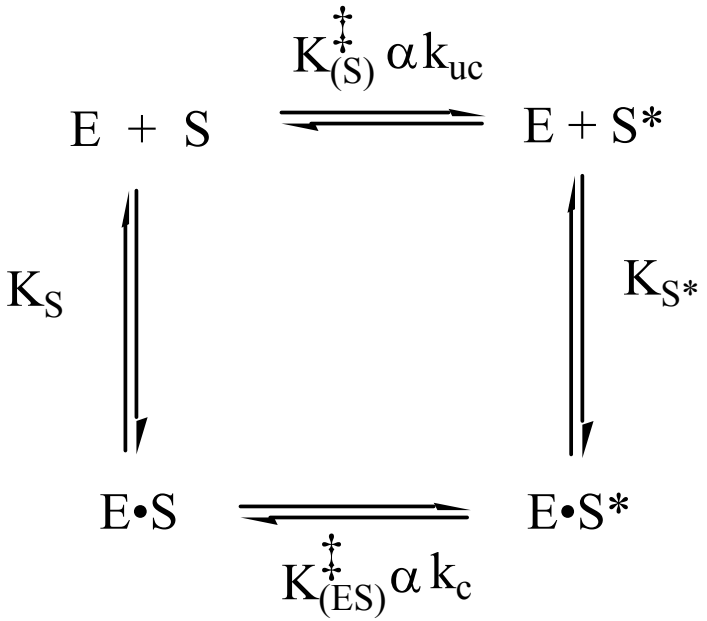




- By generating binding sites for substrates, enzymes can raise the "effective" concentration.

- Strength of proximity effect estimated by rates of similar biomolecular versus unimolecular reactions (p-nitrophenolacetate hydrolysis) where catalyst by imidazole (either free or attached).
- Rate constant for biomolecular is approx. 35 $M^{-1}min^{-1}$ whereas rate constant for unimolecular analog is 839 min^{-1}. The ratio is 24 M.
- This means that concentration of imidazole would have to be 24 M to match the rate of first order rxn
- Enzymes not only bring reactants closer together, they also orient them correctly which greatly increases the rate.

Enzyme evolved to bind transition states



k_c can be 10^{14} times greater than k_{uc} as seen with urease so K_{S*} can be 10^{14} greater than K_S

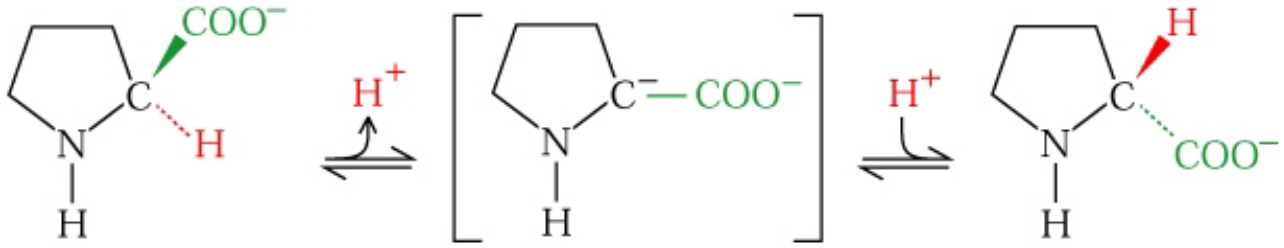
Remember that: $k=k_o\, e^{-\Delta G/RT} = k_o K^{\ddagger}$

- Enzymes speed up reactions by binding to the transition state tighter than reactants.
- Consider the following thermodynamic box where

$$K^{\ddagger}_{(S)} \times K_{S*} = K_S \times K^{\ddagger}_{(ES)}$$

- This means that enzymes can bind the transition state 10^{14} times tighter than reactants.

Transition state analogs



L-Proline | Planar transition state | D-Proline

Pyrrole-2-carboxylate | Δ-1-Pyrroline-2-carboxylate

- kc/ku may exceed 10^{16}; K_T can approach 10^{-15} M which is extremely tight binding.
- Binding of true transition state not possible due to short half life; however, a variety of compounds which resemble transition state have been studied
- Example is proline racemase which is proposed to have planar intermediate. Pyrrole-2-carboxylate binds 160 times tighter than proline.

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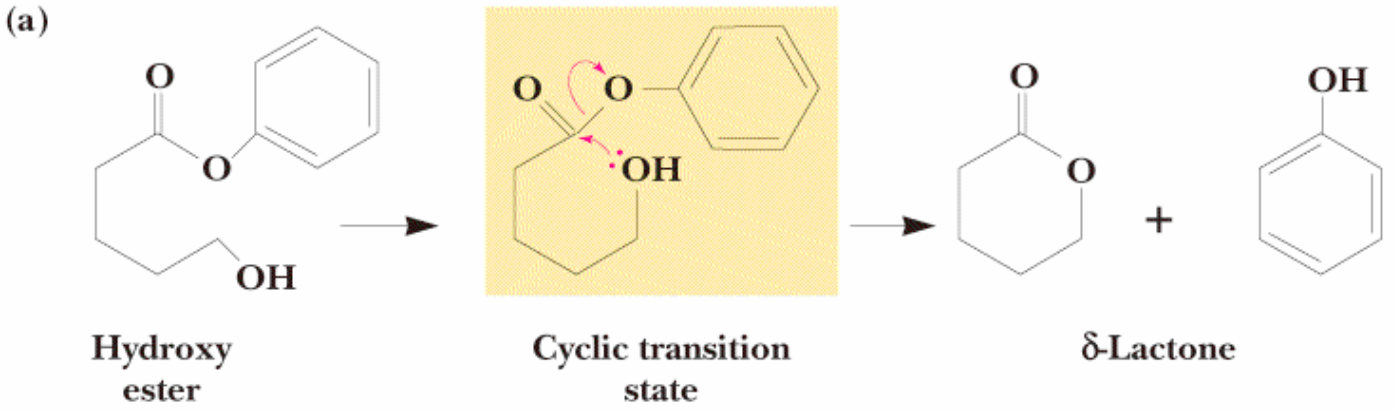
Figure 14.25



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Catalytic antibodies transition states

- Recognize foreign antigens which resembles transition by synthesis of transition state analog.
- Can accelerate rate of ester hydrolysis 1,000 fold
- Future is making 'designer enzymes