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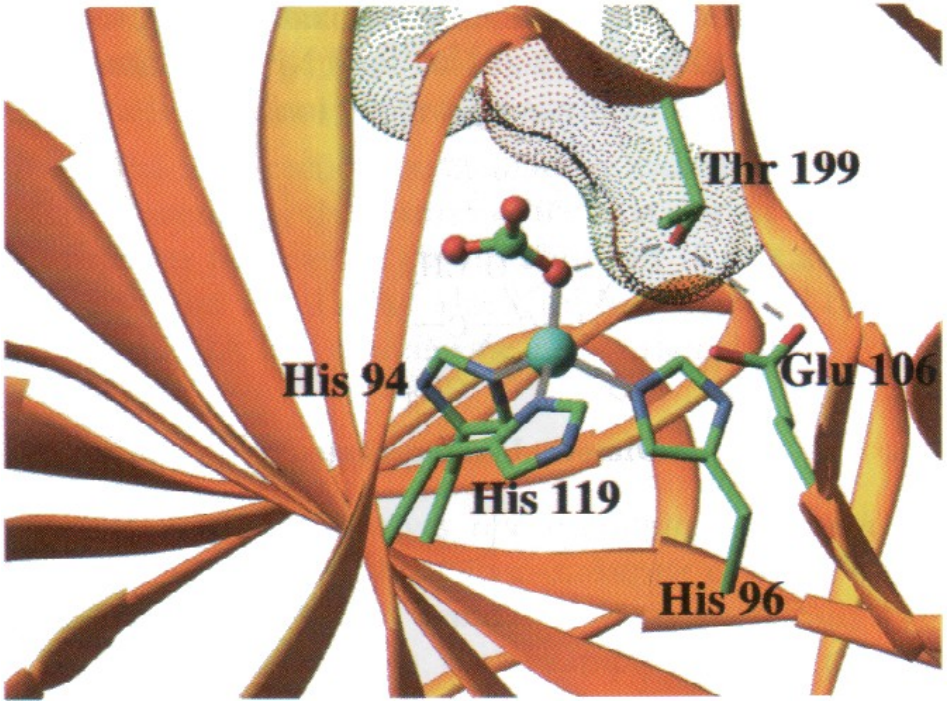
Chapter 15: Enyzmatic Catalysis

Voet & Voet: Pages 496-508



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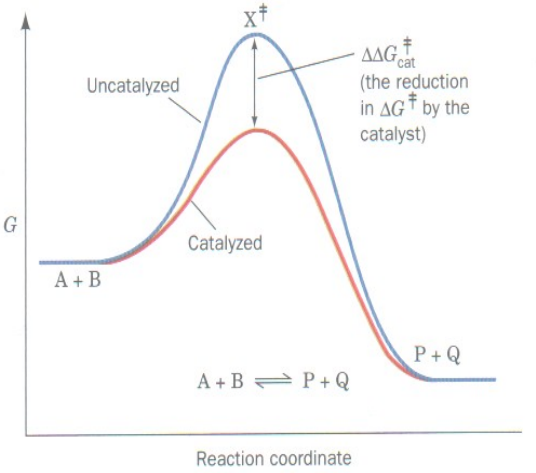
Catalytic Mechanisms

Catalysis is a process that increases the rate at which a reaction approaches equilibrium

Rate enhancement depends upon reduction of $\Delta G^{\ddagger}$ (activation barrier) relative to the uncatalyzed reaction



Two related properties make enzyme amazingly powerful catalysts

1 – specificity of substrate binding

2 – optimal arrangement of catalytic groups

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Catalytic Mechanisms

Enzymes enhance reaction rates in many ways

1) Acid-base catalysis

2) Covalent catalysis

Mechanisms 1 & 2 typically depend upon a 'catalytic' residue

3) Metal ion catalysis

Dependent upon non-covalently bound ion (enzyme or substrate)

Additional mechanisms allow the enzyme-substrate complex to lower the transition state energy through

4) Electrostatic catalysis

5) Proximity & orientation effects

6) Preferential binding of transition state complex

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1 Acid-base Catalysis

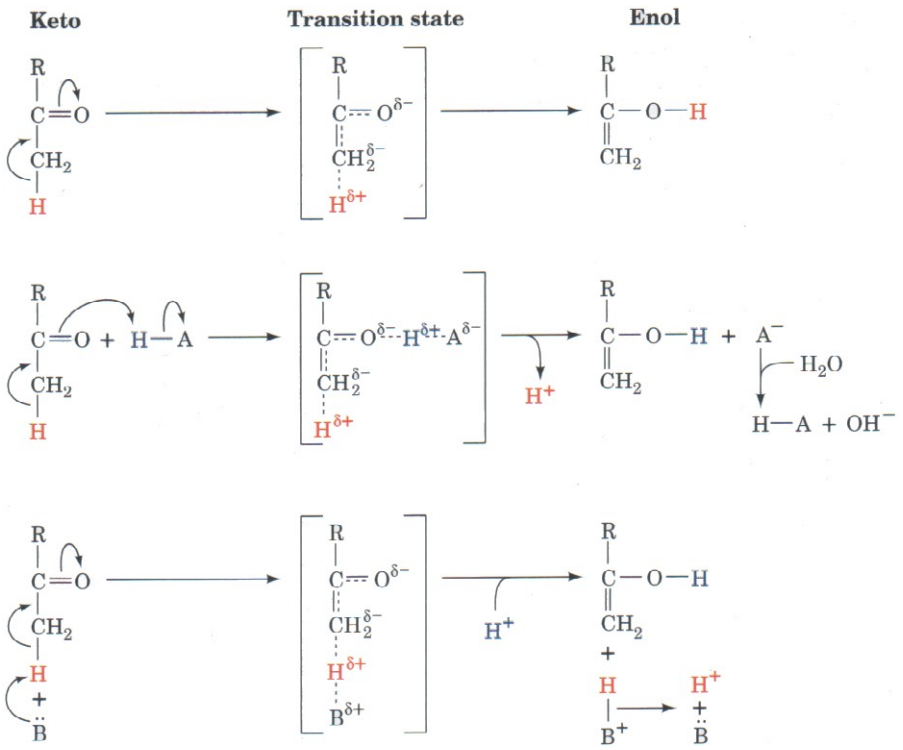
General acid catalysis involves partial proton transfer from a donor that lowers the free energy of the transition state

General base catalysis involves partial proton abstraction from an acceptor that lowers the free energy of the transition state

Biochemical reactions susceptible to acid-base catalysis include:

- peptide and ester hydrolysis
- tautomerizations
- reactions of phosphate groups
- additions to carbonyl groups

Typically involves Asp, Glu, His, Cys, Tyr & Lys residues



Many enzymes utilize a **Concerted acid-base mechanism** (ie. both acid and base catalysis)

Concerted Acid-Base Catalysis (RNase A)

Overall reaction mechanism is composed of two elementary reactions

Overall Reaction:

....pRpRpRpR.... → .....pRpRp (3' PO_4) + (5' OH) RpR....

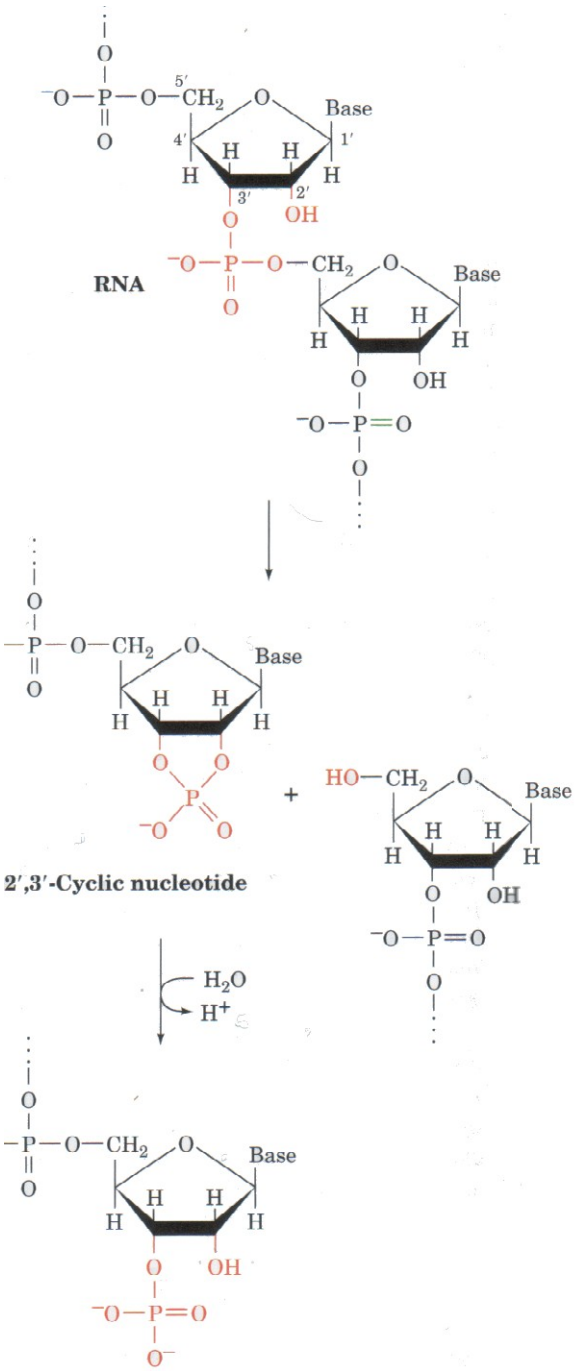
Elementary step 1:

....pRpRpR.... → .....pRp (2',3' cyclic PO_4) + (5' OH) RpR....

Elementary step 2:

.....pRp (2',3' cyclic PO_4) + H_2O → .....pRpRp (3' PO_4)

Acid-base catalysts promote reactions by increasing the strength of the nucleophile (proton abstraction) and/or the stability of the leaving group (proton donation)

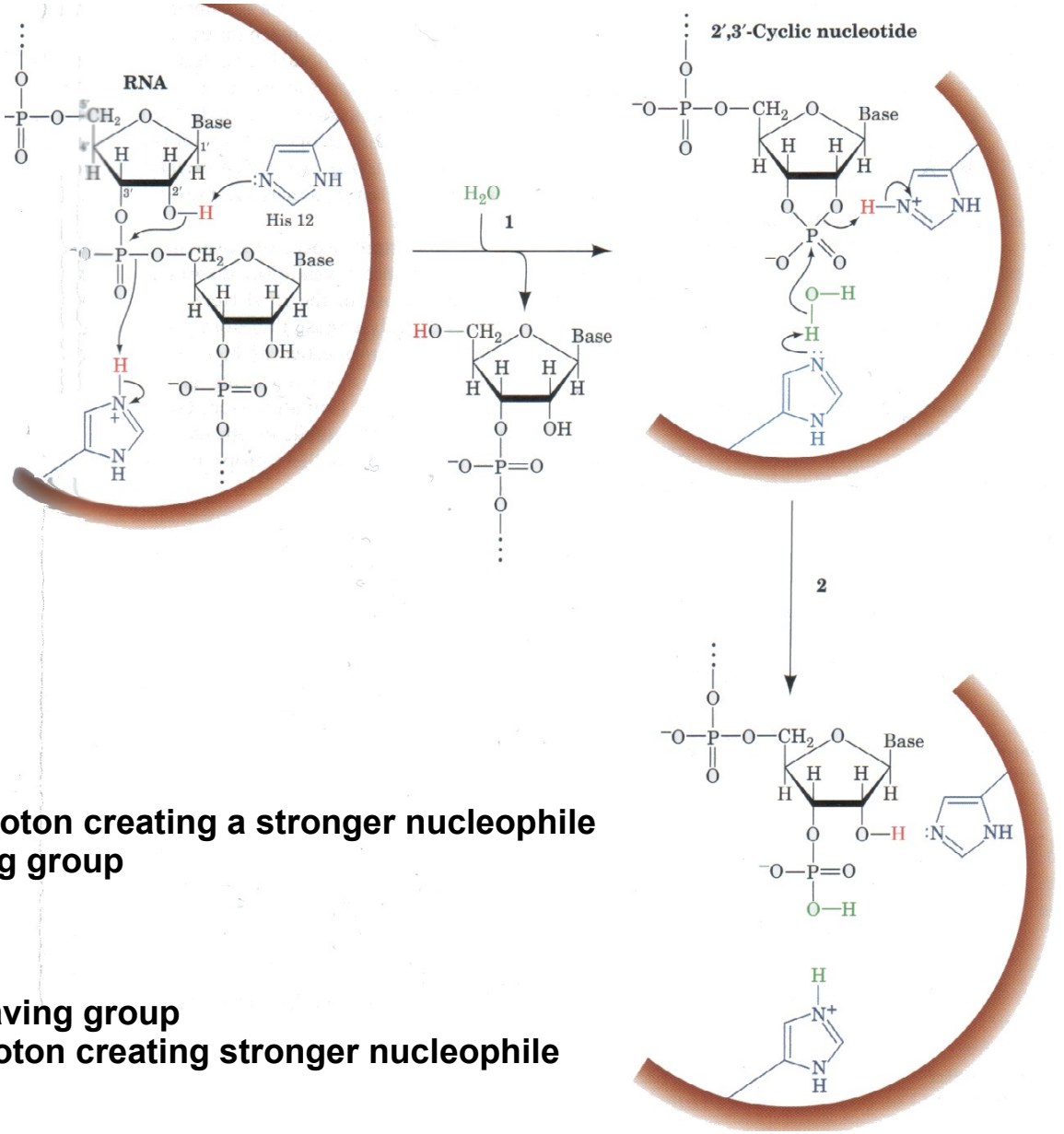


More RNase A

Enzyme kinetics, chemical modification studies and X-ray crystal structures indicate:

A) Two essential Histidine residues (H12 & H119)

B) Concerted general acid-base mechanism



Elementary Step 1:

H12 general base – abstracts 2'-OH proton creating a stronger nucleophile
H119 general acid – protonates leaving group

Elementary Step 2:

H12 general acid – protonates 2'-O leaving group
H119 general base – abstracts H_2O proton creating stronger nucleophile

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2 Covalent Catalysis

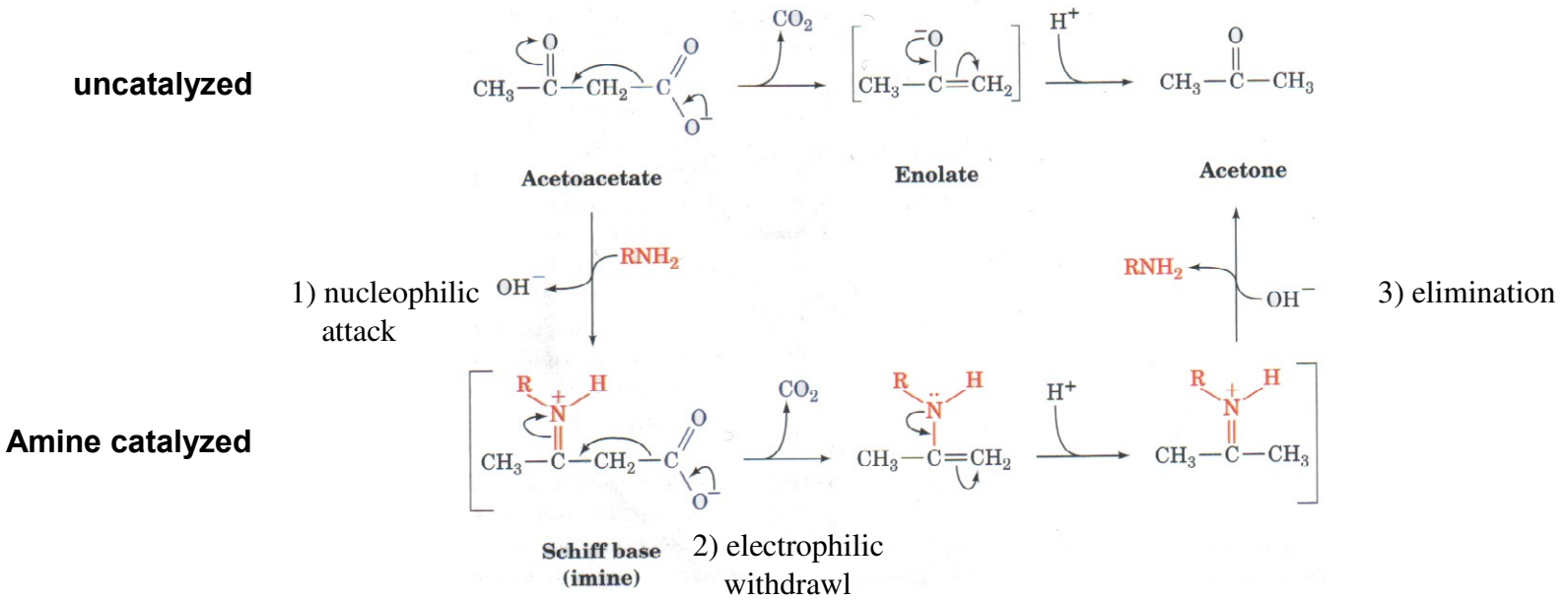
Covalent catalysis accelerates reaction rates through transient formation of enzyme-substrate covalent bond

Three stages in covalent catalysis

1) nucleophilic reaction between enzyme and substrate

2) electrophilic withdrawl of electrons from substrate

3) elimination reaction (reverse of stage 1)



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More Covalent Catalysis

Enzymatic catalysis of acetoacetate decarboxylation

1) Nucleophilic attack (neutral Lys) on carbonyl C

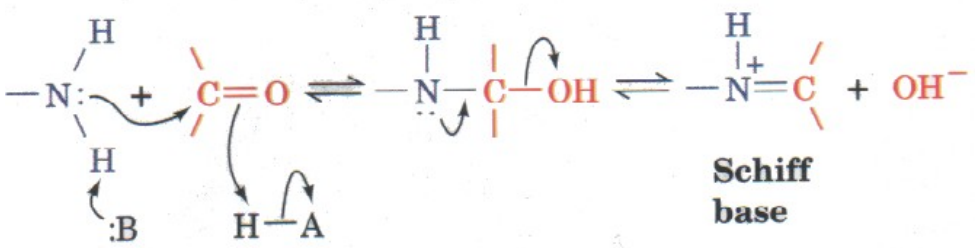
Concerted acid-base catalyzed attack

2) Electrophilic withdrawl of electrons of enolate-like transition state

Schiff base (imine) to Enamine to Schiff base with CO_2 release

3) Elimination of Schiff base

Nucleophilic attack by OH- on carbonyl C (concerted acid-base catalyzed)



Stage 1 of covalent catalysis reaction

Lys, His, Cys, Asp & Ser are nucleophile

OR

coenzymes like thiamine pyrophosphate or pyrodoxal phosphate

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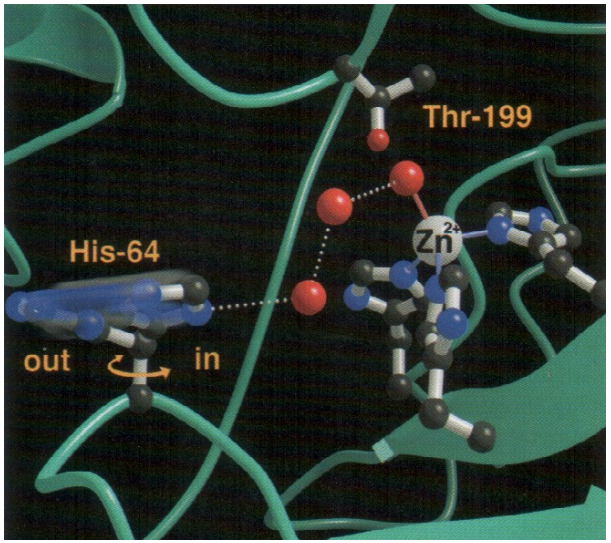
3 Metal Ion Catalysis

Two classes of metal ion dependent enzymes

1) **Metalloenzymes** contain tightly bound transition metal ions (eg. Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{3+})

2) **Metal-activated enzymes** loosely bind metal ions (eg. Alkali or alkaline metal including Na^{+}, K^{+}, Mg^{2+} and Ca^{2+})



Metal ions enhance catalysis in three major ways

1) Binding to and orienting substrates for reaction

   eg. Mg^{2+} binding to ATP

2) Mediating redox reaction through changes in oxidation state

   eg. Reduction of O_2 to H_2O through electron transfer

3) Electrostatic stabilization or shielding of negative charges

   eg. Mg^{2+} binding to ATP

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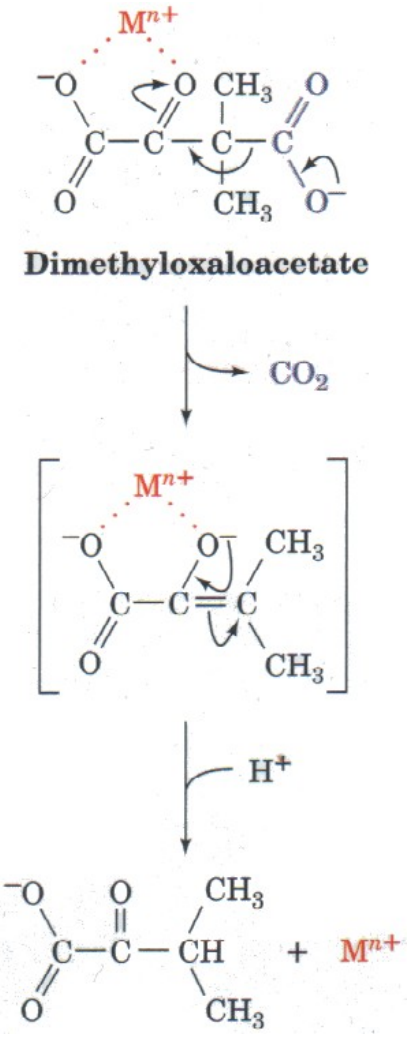
Charge Stabilization/Shielding

Metals can act as 'superacids'

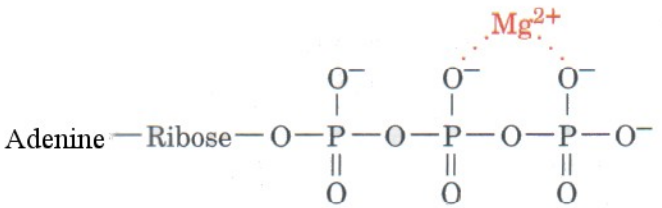
- **similar role to protons in acid catalyzed reactions**
- **more effective than protons given their [high] at neutral pH and charges > +1**



Dimethyloxaloacetate

Metals shield or reduce the effective charge on highly anionic substrate

- **facilitates nucleophile approach**



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More Stabilization

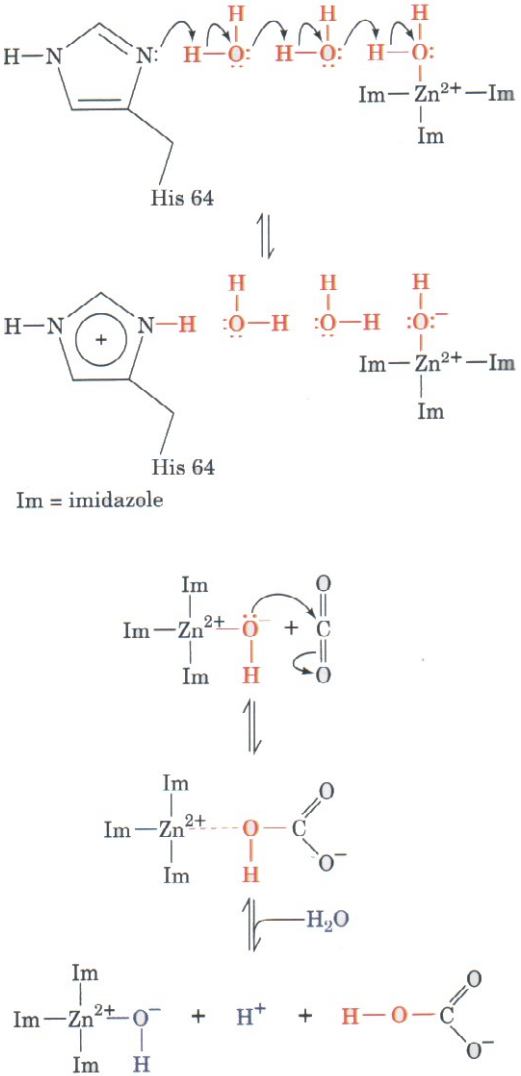
Carbonic Anhydrase: ($CO_2 + H_2O \rightarrow HCO_3 + H^+$)

(1) H64 abstracts proton from Zn^{2+} bound water molecule generating Zn^{2+} bound hydroxide ion (via proton shuttle)

- **Electrostatic stabilization of OH^-**

(2) Zn^{2+} bound hydroxide ion nucleophilically attacks bound CO_2 converting it to bicarbonate

(3) H64 releases proton to solvent to regenerate enzyme (not shown)



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4 Electrostatic Catalysis

Electrostatic catalysis – enzymes seem to arrange active site charge distributions to stabilize the transition states of catalyzed reactions

Substrate binding generally excludes water from an enzyme active site generating a low dielectric constant within the active site

- **Electrostatic interactions are stronger**
- **pk_a's can vary by several pH units due to proximity of charged groups**

Alternative form of electrostatic catalysis: Several enzymes (eg. superoxide dismutase) apparently use charge distributions to guide polar substrates to their active sites

Others actually have reaction rates greater than the apparent diffusion-controlled limit (substrate channeling)

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Proximity & Orientation Effects

Substrate binding has additional effects that enhance reaction rates

- **Most obvious is proximity & orientation**

Reactants must come together with the proper spatial relationship for a reaction to occur

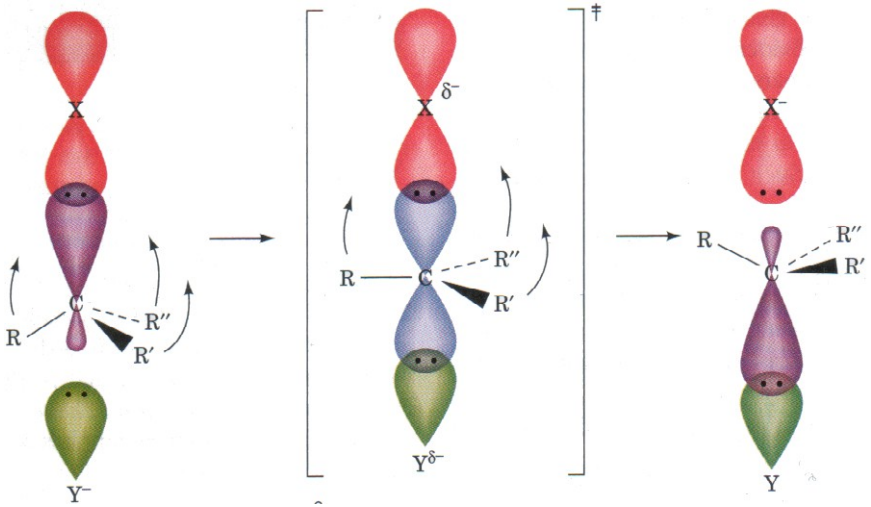
Proximity effects (minor) are most readily observed by comparing equivalent inter- and intramolecular reactions

- **Intramolecular reactions are typically 10-100 fold more rapid**

Orientation effects are more significant though difficult to quantify

Theory suggest molecules are maximally reactive when their orbitals are aligned so the electronic energy of the transition state is minimized

- **(termed stereoelectronic assistance)**



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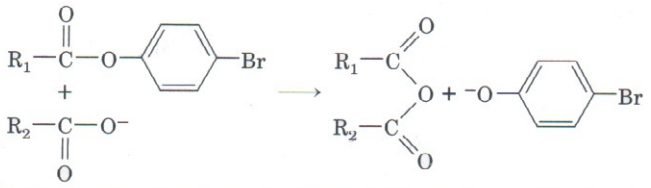
More Orientation Effects

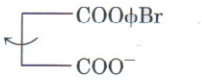
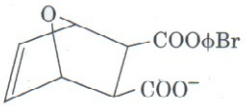
Suggested that transition state complex greatly reduces molecular motion

- **For related organic compounds, reaction rates increase as molecular motion is reduced**
- **Effects are large compared to proximity effects**

Enzymes bind substrates in a manner that both aligns and immobilizes them to optimize their reactivity

- **Free energy required to orient substrate is derived from the specific binding energy for the substrate**



Reactants[a]	Relative Rate Constant
$CH_3COO\phi Br$ + CH_3COO^-	1.0
	$\sim 1 \times 10^3$
	$\sim 2.3 \times 10^5$
	$\sim 8 \times 10^7$

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Preferential Transition State Binding

Enzymes bind the transition state with higher affinity than the substrate or product

- explains why reactions proceed and products are released
- explains why transition state analogues are excellent competitive inhibitors
- together with proximity and orientation effects, accounts for bulk of rate enhancement in many enzymes

Enzyme mechanically strain substrates towards transition states (rack mechanism)

- rate enhancement (ie. $\Delta\Delta G^{\ddagger}$) can be expressed in terms of enzyme affinity for transition state compared relative to substrate
- explains why good and bad substrates typically have similar K_m value but different k_{cat} values

A good substrate does not need to bind tightly to the enzyme but must bind tightly when activated to the transition state