Chapter 15. Enzymatic Catalysis
Catalytic mechanisms

- Acid-base catalysis
- Covalent catalysis
- Metal ion catalysis
- Proximity and orientation effects
- Stabilization of the transition state
Acid-base catalysis

- General acid catalysis — partial proton donation
- General base catalysis — partial proton abstraction
- Concerted general acid-base catalyzed reaction
- Asp, Glu, His, Lys, Tyr, Cys, Ser
Keto-enol tautomerization

Uncatalyzed

General acid catalyzed

General base catalyzed
pH dependence of RNAse A

\[
\log \left( \frac{V_{max}'}{K_{M}'} \right)
\]

\( pK_{E1} \)

\( pK_{E2} \)
Mechanism of RNAse A
Covalent catalysis

Transient substrate enzyme intermediate

- Nucleophilic attack by the enzyme
- Enhanced electrophilicity of E-S covalent intermediate
- Elimination of the enzyme

(a) Nucleophiles

- $\text{ROH}$ ⇌ $\text{RO}^-$ + $\text{H}^+$ Hydroxyl group
- $\text{RSH}$ ⇌ $\text{RS}^-$ + $\text{H}^+$ Sulfhydryl group
- $\text{RNH}_3^+$ ⇌ $\text{RNH}_2$ + $\text{H}^+$ Amino group
- $\text{HN}^+$ + $\text{N}^-$ Imidazole group

(b) Electrophiles

- $\text{H}^+$ Protons
- $\text{M}^{n+}$ Metal ions
- $\text{C}=\text{O}$ Carbonyl carbon atom
- $\text{C}^+=\text{N}$ Cationic imine (Schiff base)
Formation of Schiff base

More electrophilic
More $\alpha$ acidic protons
Decarboxylation of acetoacetate

Acetoacetate \[\text{CH}_3-\text{C}-\text{CH}_2-\text{CO}_2^-\]

Enolate \[\left[\text{CH}_3-\text{C}^-\text{CH}_2\right]\]

Acetone \[\text{CH}_3-\text{C}-\text{CH}_3\]

Schiff base (imine) \[\left[\text{CH}_3-\text{C}^-\text{CH}_2-\text{CO}\right]\]
Reactions catalyzed by PLP

- Transamination
- Decarboxylation
- Racemization
- β-Elimination
Transamination

Racemization

Schiff's base
Decarboxylation

$\beta$-Elimination
Thiamine pyrophosphate

Pyruvate decarboxylase

Pyruvate → Acetaldehyde

Hydroxyethylthiamine pyrophosphate

Acetaldehyde + H⁺ → + CO₂

Pyruvate + TPP (ylid form) → TPP (full form)
Metal ion catalysis

- Metalloenzymes
  - Tightly bound metal ions
  - $\text{Fe}^{2+}, \text{Fe}^{3+}, \text{Cu}^{2+}, \text{Zn}^{2+}, \text{Mn}^{2+}$ or $\text{Co}^{3+}$

- Metal-activated enzymes
  - Loosely bound metal ions
  - $\text{Na}^+, \text{K}^+, \text{Mg}^{2+}$ or $\text{Ca}^{2+}$
The roles of metal ions in catalysis

- Binding to substrate for a proper orientation
- Oxidation-reduction reactions
- Lewis acid catalysis
Metal ion as a Lewis acid

\[
\begin{align*}
\text{Mn}^{n+} &\quad \rightarrow \quad \text{CO}_2 \\
\text{Mn}^{n+} &\quad \rightarrow \quad \text{H}^+ \\
\text{Mn}^{n+} &\quad \rightarrow \quad \text{product} + \text{Mn}^{n+}
\end{align*}
\]
Carbonic anhydrase

\[ \text{H}_2\text{O} + \text{CO}_2 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]

- \( \text{Zn}^{2+} \) — coordinated by 3 His and 1 \( \text{H}_2\text{O} \)
- \( \text{H}_2\text{O} \) is polarized
- This allows a fourth His to pull a proton from the \( \text{H}_2\text{O} \) molecule
- The \( \text{HO}^- \) now attacks the carbon in a \( \text{CO}_2 \) molecule that is bound nearby
X-ray structure of carbonic anhydrase
Catalytic site of carbonic anhydrase

Activation of water

Im = imidazole
Metal as a charge shield

• Orientation effect
• Shield negative charges so as to allow the incoming nucleophile to attack
Proximity and orientation

- Bring substrates together
- Orient substrates so they have the proper orientation

Relative rate

\[
\begin{align*}
\text{p-Nitrophenylacetate} & \rightarrow \text{CH}_3\text{C}^=\text{O} + \text{p-Nitrophenolate} \\
\text{Imidazole} & \rightarrow \text{CH}_3\text{C}^=\text{O} + \text{p-Nitrophenolate}
\end{align*}
\]

Relative rate: 1

Relative rate: 24
Stereoelectronic effects

A molecule is maximally reactive only when it assumes a conformation that aligns its various orbitals in a way that minimizes the electronic energy of its transition state.

Orbital alignments in an $S_N2$ reaction
Proximity effects on the rates

\[
\begin{align*}
R_1\text{-C-O} &+ \text{Br} \\
R_2\text{-C-O}^- &\rightarrow R_1\text{-C-O}^- + \text{Br}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Reactants(^a)</th>
<th>Relative Rate Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{CH}_3\text{COO} \text{ Br}) + (\text{CH}_3\text{COO}^-)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>(\text{COO}ϕ\text{Br})</td>
<td>(~1 \times 10^3)</td>
</tr>
<tr>
<td>(\text{COO}^-)</td>
<td>(~2.3 \times 10^5)</td>
</tr>
<tr>
<td>(\text{COO} \text{ Br})</td>
<td>(~8 \times 10^7)</td>
</tr>
</tbody>
</table>

\(^a\)Curved arrows indicate rotational degrees of freedom.

Preferential transition state binding

- Substrates do not fit the binding site
- Substrate distorts during binding
- Distorted conformation $\approx$ transition state conformation
- Energy required may be generated by substrate binding
Effects of strain and distortion

\[
\text{Relative Rate} \quad 1 \quad 10^8
\]

\begin{tabular}{ll}
\hline
R & Relative rate \\
H & 1 \\
CH_3 & 315 \\
\hline
\end{tabular}
Transition state analogs

- Transition state analogs usually bind with higher affinity than substrates
- Transition state analogs are very good inhibitors
- Antibodies selected to bind to the transition state analog of a chemical reaction often act as enzymes – catalytic antibodies (abzymes)
Transition state analogs

Proline racemase

\[ \text{L-Proline} \quad \stackrel{\text{H}^+}{\leftrightarrow} \quad \text{Planar transition state} \quad \stackrel{\text{H}^+}{\leftrightarrow} \quad \text{D-Proline} \]

Pyrrole-2-carboxylate \quad \Delta-1\text{-Pyrroline-2-carboxylate}

Bind 160-fold tighter than either L-proline or D-proline
Catalytic antibodies

Ester hydrolysis

Claisen rearrangement
Lysozyme

- Cleavage of the peptidoglycan of bacterial cell walls
- Cleavage of chitin
Hen egg white lysozyme
Structure of lysozyme
Binding pocket of lysozyme

Figure 11-17c Fundamentals of Biochemistry, 2/e
Reaction rates for the substrate analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_{\text{cat}}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NAG)$_2$</td>
<td>$2.5 \times 10^{-8}$</td>
</tr>
<tr>
<td>(NAG)$_3$</td>
<td>$8.3 \times 10^{-6}$</td>
</tr>
<tr>
<td>(NAG)$_4$</td>
<td>$6.6 \times 10^{-5}$</td>
</tr>
<tr>
<td>(NAG)$_5$</td>
<td>0.033</td>
</tr>
<tr>
<td>(NAG)$_6$</td>
<td>0.25</td>
</tr>
<tr>
<td>(NAG–NAM)$_3$</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Identification of catalytic residues

• Site-directed mutagenesis
  – Glu35Gln: < 0.1% activity, only ~1.5-fold decrease in substrate affinity
  – Asp52Asn: < 5% activity, ~2-fold increase in substrate affinity

• Chemical modification of other amino acid residues with group-specific reagents does not affect the enzyme activity
Binding free energies of HEW lysozyme subsites

<table>
<thead>
<tr>
<th>Site</th>
<th>Bound Saccharide</th>
<th>Binding Free Energy (kJ · mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NAG</td>
<td>−7.5</td>
</tr>
<tr>
<td>B</td>
<td>NAM</td>
<td>−12.3</td>
</tr>
<tr>
<td>C</td>
<td>NAG</td>
<td>−23.8</td>
</tr>
<tr>
<td>D</td>
<td>NAM</td>
<td>(+12.1)</td>
</tr>
<tr>
<td>E</td>
<td>NAG</td>
<td>−7.1</td>
</tr>
<tr>
<td>F</td>
<td>NAM</td>
<td>−7.1</td>
</tr>
</tbody>
</table>

*Source: Chipman, D.M. and Sharon, N., Science 165, 459 (1969).*
Acid-catalyzed hydrolysis of an acetal to a hemiacetal
Role of strain

Oxonium ion intermediate

The δ-lactone analog of (NAG)$_4$

9.2 kJ/mole greater affinity than NAG
(It is accountable for only 40 fold rate enhancement but not ~$10^8$ fold)
Chair conformation

Half-chair conformation
Determining cleavage site and stereochemistry

Retention of chemistry (Possible double displacement mechanism)

H₂¹⁸O → lysozyme

D + E

NAc

C₁ O₁ C₄ OH

CH₂OH

H H H H
Covalent glycosyl-enzyme intermediate?

- Structural homology with all other retaining β-glycosidases that catalyze the cleavage via a covalent glycosyl-enzyme intermediate
- The lifetime of glucosyl oxonium ion in water is very short (10^{-12}s)
- The intermediate had not been observed probably because its breakdown is much faster than its formation
- The covalent intermediate was observed by ESI-MS and X-ray crystallography using a fluorinated substrate analog (NAG2FGlcF)

Electron withdrawing group
(Destabilize the oxonium ion intermediate)

Good leaving group
(Faster formation of the intermediate)

Undistorted chair conformation

Electron withdrawing group
(Destabilize the oxonium ion intermediate)
Serine proteases

• Active site serine residue
• Digestive enzymes from prokaryotes and eukaryotes
• Development, blood clotting, inflammation and other biologically important processes
Colorimetric assay of proteases

\[ \text{N-Acetyl-L-phenylalanine } p\text{-nitrophenyl ester} \]

\[ + \text{H}_2\text{O} \rightarrow \text{N-Acetyl-L-phenylalanine } + \text{H}^+ + p\text{-Nitrophenolate} \]
Identification of catalytic groups by chemical labeling

\[(\text{Active Ser}) - \text{CH}_2\text{OH} + F\overset{\text{O}}{\overset{\text{P}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{CH}(\text{CH}_3)_2}}}}}}\]

Diisopropylphosphofluoridate (DIPF)

\[\downarrow\]

\[(\text{Active Ser}) - \text{CH}_2\text{O} - \overset{\text{P}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{CH}(\text{CH}_3)_2}}}}}}\] + HF

DIP–Enzyme
Organophosphofluoridates irreversibly inactivate the active site serine

\[(\text{CH}_3)_3\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{O} \cdot \text{C} \cdot \text{CH}_3 + \text{H}_2\text{O}\]

**Acetylcholine**

\[\text{acetylcholinesterase} \rightarrow \text{Serine esterase inactivated by DIPF}\]

\[(\text{CH}_3)_3\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH} + \text{O} \cdot \text{C} \cdot \text{CH}_3 + \text{H}^+\]

**Choline**

**Nerve poisons**

**DIPF**

**Sarin**

**Insecticides**

**Parathion**

**Malathion**
Active site-directed chemical labeling

Tosyl-L-phenylalanine chloromethylketone
(Chymotrypsin inhibitor)

Tosyl-L-lysine chloromethylketone
(Trypsin inhibitor)
X-ray structure of trypsin
Asp102 is also essential

- Asp 102 is conserved in all serine proteases
- Close to Ser195 and His57
- Substitution to Asn reduced the rate by $10^4$ fold

Catalytic triad

- His 57
- Asp 102
- Ser 195
Catalytic triad

- Asp 102
- His 57
- Ser 195
- Asp 194
- Gly 193
- Ile 16
Substrate specificity

- **Chymotrypsin**
  - Scissile bond
  - Phe
  - Gly 216
  - Ser 189
  - Gly 226
  - Bulky hydrophobic residues

- **Trypsin**
  - Scissile bond
  - Lys
  - Gly 216
  - Asp 189
  - Gly 226
  - Positively charged residues

- **Elastase**
  - Scissile bond
  - Ala
  - Val 226
  - Thr 216
  - Small neutral residues
Divergent and convergent evolution
Catalytic mechanism
• His 57 is pulling on the proton from Ser 195 (general base catalysis)
• Ser 195 forms a covalent bond with the amide carbonyl (covalent catalysis)
• The oxyanion (transition state) is stabilized by two hydrogen bonds (electrostatic catalysis, or preferential binding to the transition state)
• His 57 is now donates a proton to the transition state (general acid catalysis), resulting in decomposition of the transition state
• The carboxy-terminal half of the protein is released (replaced H₂O and a reversal of the first 4 steps of catalysis)
Transition state stabilization

Oxyanion hole

Gly 193

Ser 195
Evidence for the tetrahedral intermediate

Trypsin

Bovine pancreatic trypsin inhibitor (BPTI)
Acyl-enzyme and tetrahedral intermediates

(a) Acyl-enzyme intermediate (pH 5)

(b) Tetrahedral intermediate (pH 9)
The role of the catalytic triad

• Implausible old postulation: “Charge relay system”: pKa (His) ~6 ; pKa (Ser) ~15

• New theory: “Low-barrier hydrogen bonds (LBHBs)”: Sharing of a hydrogen atom
Zymogens (Proenzymes)

Essential for preventing premature activation
Other classes of proteases

- **Cysteine proteases**
  - Example: Papain
  - Key features: Cys and His residues

- **Aspartyl proteases**
  - Example: Renin
  - Key features: Asp residues

- **Metalloproteases**
  - Example: Thermolysin
  - Key features: His, Zn$^{2+}$, and Glu residues
HIV Protease

 BINDING POCKET

 HIV Protease Inhibitor

 Indinavir

 Peptide Substrate
Other HIV protease inhibitors

HIV protease substrate

Saquinavir

Ritonavir

$K_I = 0.40 \text{ nM}$

$K_I = 0.015 \text{ nM}$
The blood coagulation cascade

**INTRINSIC PATHWAY**

- Factor XI
- Factor XIa
- Factor IX
- Factor IXa
- Factor VIIIa
- Factor VIII
- Factor Xa
- Factor X
- Prothrombin
- Thrombin

**EXTRINSIC PATHWAY**

- Vascular injury
- Tissue factor
- Factor VIIa – Tissue factor
- Factor VII – Tissue factor
- Factor IX
- Factor X
- Factor Xa
- Factor Va
- Factor Va
- Fibrinogen
- Fibrin
- Cross-linked fibrin
- Factor XIII
- Factor XIIIa

*Serine protease*
Reaction catalyzed by coenzymes

- Nicotinamide (NAD$^+$)
- Pyridoxal phosphate (PLP)
- Thiamine pyrophosphate
- Flavin
- Biotin
- Folate
- Coenzyme A
- Others
Biotin

(a) Biotin

Valerate side chain

(b) Carboxybiotinyl-enzyme

Lys residue
Pyruvate carboxylase catalyzes the conversion of pyruvate to oxaloacetate with the consumption of ATP and the release of ADP and inorganic phosphate (P_i). The reaction is driven by bicarbonate (HCO_3^-) as a source of carbon.

Phase I:
- ATP is converted to carboxyphosphate and ADP.
- Carboxyphosphate reacts with biotin-enzyme to form carboxybiotinyl-enzyme.

Phase II:
- Pyruvate and carboxybiotinyl-enzyme react to form pyruvate enolate.
- Pyruvate enolate then reacts with biotin-enzyme to form oxaloacetate.
Biotin-streptavidin interaction

- Binding constant = $4 \times 10^{14}$
- Extensively used for affinity isolation/purification
Flavin adenine dinucleotide (FAD)

- Flavin adenine dinucleotide (FAD) (oxidized or quinone form)
- FADH · (radical or semiquinone form)
- FADH₂ (reduced or hydroquinone form)

Charge-transfer complex
Covalent adduct
Redox-active disulfide
Folate

2-Amino-4-oxo-6-methylpterin

*p*-Aminobenzoic acid

Glutamates ($n = 1-6$)

Pteric acid

Pteroylglutamic acid (tetrahydrofolate; THF)

Folate

$\xrightarrow{NADPH, NADP^+} 7,8$-Dihydrofolate (DHF)

$\xrightarrow{NADPH, NADP^+} \text{Tetrahydrofolate (THF)}$
Coenzyme A (CoA)
Fatty acid metabolism

1. Acyl-CoA dehydrogenase (AD) converts acyl-CoA to fatty acyl-CoA.
   - \( \text{CH}_3-(\text{CH}_2)_n-\text{C}-\text{H}_2-\text{C}-\text{SCoA} \)
   - \( \text{FADH}_2 \)

2. Enoyl-CoA hydratase (EH) converts fatty acyl-CoA to trans-\( \Delta^2 \)-enoyl-CoA.
   - \( \text{H}_2\text{O} \)
   - \( \text{CH}_3-(\text{CH}_2)_n-\text{C}==\text{C}-\text{H}_2-\text{C}-\text{SCoA} \)

3. 3-L-Hydroxyacyl-CoA dehydrogenase (HAD) converts trans-\( \Delta^2 \)-enoyl-CoA to 3-L-hydroxyacyl-CoA.
   - \( \text{NAD}^+ \)
   - \( \text{CH}_3-(\text{CH}_2)_n-\text{C}==\text{C}-\text{H}_2-\text{C}-\text{SCoA} \)
   - \( \text{NADH} + \text{H}^+ \)

4. \( \beta \)-Ketoacyl-CoA thiolase (KT) converts 3-L-hydroxyacyl-CoA to \( \beta \)-ketoacyl-CoA.
   - \( \text{CoASH} \)
   - \( \text{CH}_3-(\text{CH}_2)_n-\text{C}-\text{SCoA} + \text{CH}_3-\text{C}-\text{SCoA} \)

5. ETF: ubiquinone oxidoreductase converts \( \beta \)-ketoacyl-CoA to ETF-ubiquinone oxidoreductase.
   - \( \text{QT} \)
   - \( \text{Q} \)

6. ETF: ubiquinone oxidoreductase converts ETF-ubiquinone oxidoreductase to ETF-ubiquinone.
   - \( \text{Q} \)
   - \( \text{QH}_2 \)

7. ETF: ubiquinone oxidoreductase converts ETF-ubiquinone to ETF.
   - \( \text{Q} \)
   - \( \text{QH}_2 \)

8. Mitochondrial electron transport chain converts ETF to water.
   - \( \text{H}_2\text{O} \)
   - \( 1/2 \text{O}_2 \)
   - \( 2\text{ADP} + 2\text{Pi} \)
   - \( 2\text{ATP} \)
Drug discovery process

- Basic research (Years 0-3): thousands of substances are being developed, examined and screened
- Development (Years 4-10): 10-20 substances are tested, both *in vitro* (within an artificial environment) and *in vivo* (within a living body)
  - Preclinical trials: Animal
  - Clinical trials: Human
    - Phase I – Small group of healthy volunteers (safety, tolerability, pharmacokinetics, pharmacodynamics)
    - Phase II – Larger group of healthy volunteers and patients (dose requirement, efficacy, toxicity); single blind tests
    - Phase III – Randomized controlled multicenter trials on large patient groups (Efficacy comparison with the standard treatment); double blind test
- New Drug Application (NDA) to FDA
- Market
- Postmarketing safety surveillance (Phase IV clinical trials)
Definitions

• Pharmacology – Science of drugs, including their composition, uses and effects

• Pharmacokinetics – Determination of the fate of substances administered externally to a living organism (ADME; Absorption, Distribution, Metabolism and Excretion)

• Pharmacodynamics – Biochemical and physiological effects of a drug and its mechanism of action

• Toxicology – Study of the adverse effects of substances on living organisms
Drug discovery

- Lead compounds
- Structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs)
- Structure-based drug design (aka. rational drug design)
- Combinatorial chemistry and high-throughput screening
Bioavailability

- Stability
- Absorption through the gastrointestinal tract
- Binding to other substances
- Detoxifying enzymes in the liver
- Excretion by the kidneys
- Passing from the capillaries to its target tissues
- Blood-brain barrier
- Plasma membrane and intracellular membranes
Lipinski’s “rule of five”

A compound is likely to exhibit poor absorption or permeation if:

- M.W. > 500D – Low solubility
- More than five H-bond donors (OH and NH$_2$) – Too polar to pass through cell membrane
- More than ten H-bond acceptors (N and O atoms) – Too polar to pass through cell membrane
- Octanol-water partition coefficient logP > 5 – Poor aqueous solubility

“Most effective drug are neither too lipophilic nor too hydrophilic”
Origins of differences in reactions to drugs

- Genetic differences
- Disease states
- Other drugs
- Age
- Sex
- Environmental factors

Personalized drugs – Pharmacogenomics
Cytochrome P450 (CYP)

- Superfamily of heme-containing enzymes found in bacteria, archaea and eukaryotes
- ~ 100 isozymes in human
- Broad substrate specificity
- Oxygenation of external compounds (xenobiotics) to more water-soluble forms – Detoxification and elimination of harmful substances
- Polymorphism – Variants of CYP cause altered rates of drug metabolism
Minor metabolic reaction of acetaminophen

Larger dose of acetaminophen could result in fatal hepatotoxicity due to the glutathione depletion and the subsequent reaction of the acetaminquinone with the sulfhydryl groups of cellular proteins.
“There is no safe drugs”