Catalytic Mechanisms

Acid-Base Catalysis

Covalent Catalysis

Metal Ion Catalysis

Electrostatic Catalysis

Proximity and Orientation Effects

Preferential Binding of the Transition State Complex

Acid-Base Catalysis

General acid - partial transfer of a proton from a Brønsted acid lowers the free energy of the transition state
rate of reaction increases with decrease in pH and increase in [Brønsted acid]

Specific acid - protonation lowers the free energy of the transition state
rate of reaction increases with decrease in pH

General base - partial abstraction of a proton by a Brønsted base lowers the free energy of the transition state
rate of reaction increases with increase in pH and increase in Brønsted base

Specific base - abstraction of a proton (or nucleophilic attack) by OH^- lowers the free energy of the transition state
rate of reaction increases with increase in pH

Concerted general acid-base catalyzed reactions

Covalent Catalysis

Rate acceleration through transient formation of covalent bond between substrate and catalysis

Nucleophilic and electrophilic stages

Schiff base formation by amino group lysine residue

Other functional groups include:
imidazole of histidine
thiol of cysteine
carbonyl of aspartic acid
hydroxyl of serine

Coenzymes that serve in covalent catalysis:
thiamine pyrophosphate
pyridoxal phosphate

Metal Ion Catalysis

Metalloenzymes - tightly bound ions

Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{3+}, Ni^{3+}, Mo^{6+}

Metal-activated enzymes - loosely bound ions

Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}

Preferential binding and orientation

Oxidation-reduction

Stabilizing or shielding negative charges

Electrostatic Catalysis

Charge distribution around active site stabilizes transition state

(similar to metal ion effects)

Catalysis through Proximity and Orientation Effects

Intermolecular vs intramolecular constraints

Catalysis by Preferential Transition State Binding

Interactions that preferentially bind the transition state increase its concentration and proportionally increase the reaction rate

Use of transition state theory leads to the prediction that enzymatic binding of a transition state by two hydrogen bonds that cannot form in the Michaelis complex should result in a ~10^6 rate enhancement based on this effect alone

This effect has led to the development of transition state analogs (rational drug design), producing a molecule that binds with greater affinity to the enzyme than the actual substrate, but does not get processed!

Lysozyme

Enzyme destroys bacterial cell walls

Hydrolyzes β(1 → 4) glycosidic linkages from *N*-acetylmuramic acid (NAM) to *N*-acetylglucosamine (NAG)

Hydrolyzes β(1 → 4) linked poly (NAG) (chitin)

Enzyme structure

Hen egg white - 14.6 kDa, 129 amino acids, 4 disulfide bridges

catalyzed rate ~10^8-fold >> uncatalyzed reaction

prominent cleft along one face of protein molecule

substrates bind along cleft with key contacts

D ring (NAM) assumes half-chair to H-bond with Gln57

Catalytic mechanism

Glu35 and Asp52 are catalytic residues

Phillips mechanism:

Enzyme binds hexasaccharide unit, residue D distorted towards half-chair to minimize CH_2OH interactions

Glu35 transfers H^+ to O1 of D ring (general acid), C1-O1 bond cleaved generating resonance-stabilized oxonium ion at C1

Asp52 stabilizes planar (transition state binding catalysis) oxonium ion through charge-charge interactions (electrostatic catalysis), S_N1 mechanism

Enzyme releases hydrolyzed E ring with attached polysaccharide, yielding glycosyl-enzyme intermediate, H_2O adds to oxonium ion to form product and reprotonated Glu35, retention of configuration is result of enzyme cleft shielding one face of oxonium ion

Catalytic residues were identified by chemical (group specific reagents) and molecular mutagenesis

Serine Proteases

Chymotrypsin, trypsin, and elastase

Synthesized as inactive proenzymes (chymotrypsinogen)

Formation of key acyl-enzyme intermediate

Catalytic residues are Ser195 and His57

X-ray Structure

~240-residue monomeric proteins, 4 disulfide bridges

Two folded domains with antiparallel β-sheets (barrel-like) and little helix

Catalytic triad - His57 and Ser195 located at substrate binding site along with Asp102, which is buried in solvent-inaccessible pocket

Chymotrypsin - prefers bulky Phe, Trp, or Tyr in hydrophobic pocket

Trypsin - prefers Arg and Lys in binding pocket (Ser189 replaced by Asp)

Elastase - prefers Ala, Gly, Val in its depression site

Catalytic Mechanism

Bound substrate is attacked by nucleophilic Ser195 forming transition state complex (tetrahedral intermediate), His57 takes up H^+, which is facilitated by Asp102

Tetrahedral intermediate decomposes to acyl-enzyme intermediate by His57 (general acid)

Acyl-enzyme intermediate is deacylated by reverse of above steps, release of carboxylate product, H_2O is nucleophile and Ser195 is leaving group

Enzyme prefers binding transition state to either Michaelis complex or acyl-enzyme intermediate forms

Catalytic triad serves to form low-barrier hydrogen bonds in the transition state (assisted by hydrophobic environment)

Zymogens

Inactive (proenzyme) forms

Enzyme inhibitors (pancreatic trypsin inhibitor) or zymogen granules prevent activation

Active sites are distorted

Glutathione Reductase

Oxidation-reduction enzyme

Uses coenzymes $NADP^+$/NADPH and $FAD/FADH_2$

In humans, this is a dimer of 478-amino acid subunits linked by disulfide bond

Two-stage reaction

1st stage:

$$\mathbf{E + NADPH + H^+ \rightleftharpoons EH_2 + NADP^+}$$

Oxidized E binds NADPH, immediately reduces FAD, producing $NADP^+$.

E contains redox-active disulfide bond (Cys58-Cys63), which has accepted an electron pair in EH_2 to form a dithiol (one S^- is in a charge transfer complex with $FADH^-$)

2nd stage:

$$\mathbf{EH_2 + GSSG \rightleftharpoons E + 2GSH}$$

GSSG binds to EH_2

Cys58 nucleophile attacks one S of GSSG yielding mixed disulfide, promoted by His467' acting as general base

One GSH is kicked-off by protonation by His467' (general acid)

Cys63 nucleophile attacks Cys58 to form redox-active disulfide, kicking off second GSH