Proteomics Course

LECTURE-7
Enzymes:
Basic concepts, Catalytic and Regulatory strategies

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Lecture outline

- Enzymes basic concepts
  - Enzyme kinetics, energetic, inhibition
- Catalytic strategies
- Regulatory strategies

Enzymes: Basic Concepts

Enzymes

- Enzymes are molecular catalysts
- Almost all known enzymes are proteins
- Enzymes accelerate reaction million folds
- Enzymes are highly specific, catalyzes single or closely related reactions

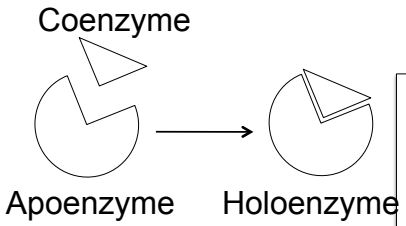
Enzymes specificity (examples)

- Trypsin
  - Specific, cleaves bond only on carboxyl side of Lys/Arg
- Thrombin
  - Hydrolysis of Arg/Gly bonds in specific peptide sequences

Enzymes classification

Enzyme class	Type of reaction
Oxidoreductase	Oxidation-reduction
Transferase	Group transfer
Hydrolase	Hydrolysis reaction
Lyase	Addition of groups to double bonds/ removal of groups to form double bonds
Isomerase	Group transfer within the molecule
Ligase	Bond formation at the expense of ATP

Enzyme activity and cofactors

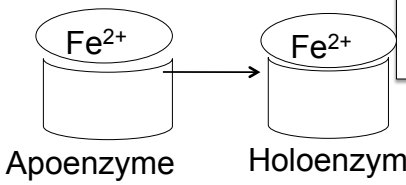


Cofactor + Apoenzyme = Holoenzyme

Metals Coenzyme

Loosely bound Tightly bound "prosthetic group"

Metal cofactor

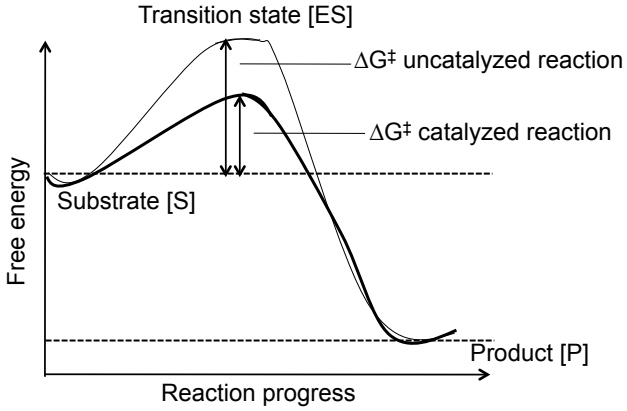


Free energy and enzyme

Enzymes: reaction rate and free energy

- ΔG must be negative for a spontaneous reaction
- Energy required to convert substrates to product (Rate of reaction)
- Enzymes cannot alter reaction equilibrium
- Accelerate attainment of equilibrium concentration

Transition state and Activation Energy

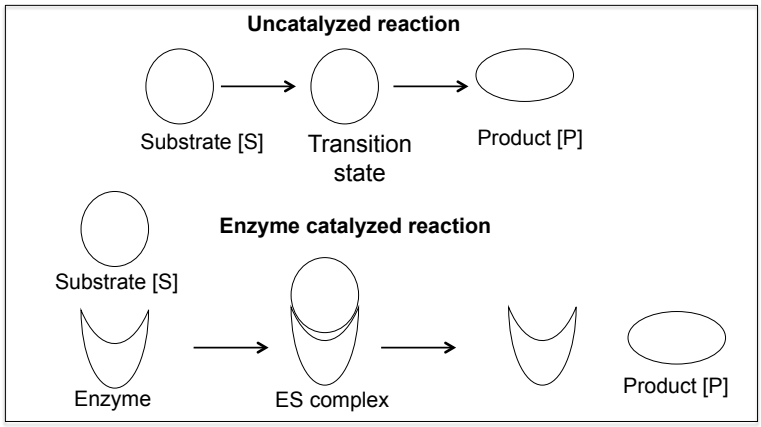


- Activation energy (ΔG‡) - difference in free energy between transition state and substrate
- Enzyme decreases activation energy

Enzyme substrate complex

Enzyme-Substrate complex

- Enzyme-substrate complex (ES) formation is first step in catalysis



Active Site of Enzymes

- Region of enzyme that binds to substrate
- Occupies only small portion of enzyme
- Extra amino acids provide scaffolding

Lock and key model
(Emil Fisher,1890)

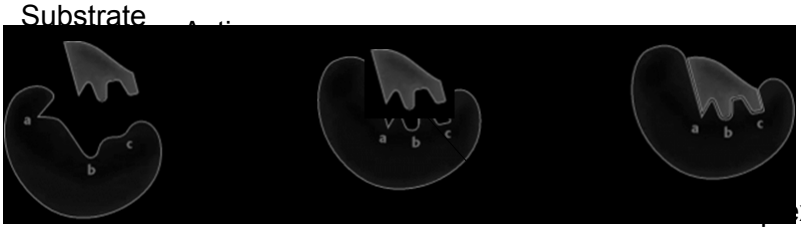
Substrate

Enzyme

Enzyme-substrate [ES] complex

Induced Fit
(Daniel Koshland,1958)

Substrate



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Enzyme

modified upon substrate approach

Enzyme kinetics

Michaelis-Menten Model

- Role of enzyme catalysis (V0): number of moles of product formed per second
- Michaelis-Menten explained kinetic characteristics
- An enzyme (E) that catalyzes substrate (S) to product (P):

$$[E] + [S] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [ES] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [E] + [P]$$

Michaelis-Menten Model

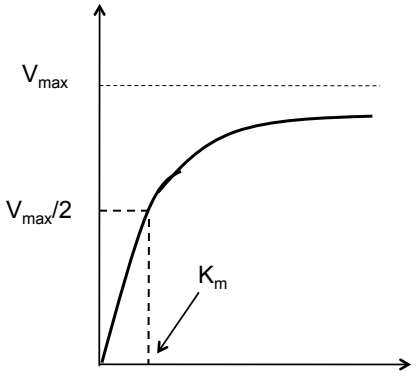
- Michaelis-Menten equation

$$V_0 = V_{max}\frac{[S]}{[S] + K_M}$$

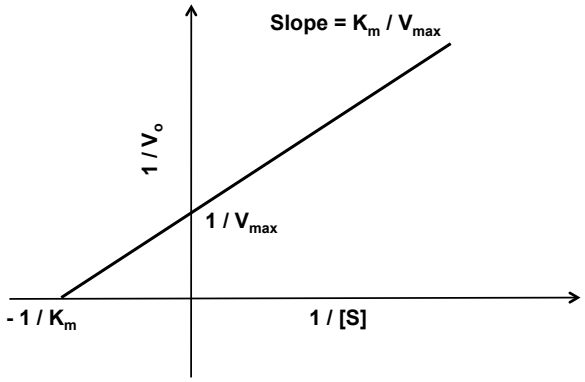
V_{max} – reaction rate when enzyme is fully saturated with S
K_M – Michaelis constant
S – substrate concentration

- K_M is a measure of strength of ES complex

Michaelis-Menten Model



Lineweaver-Burk plot



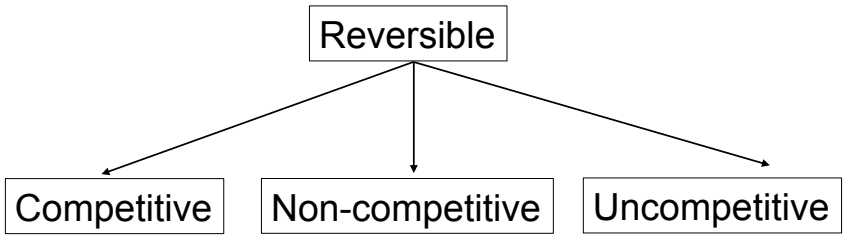
$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

Enzyme Inhibition

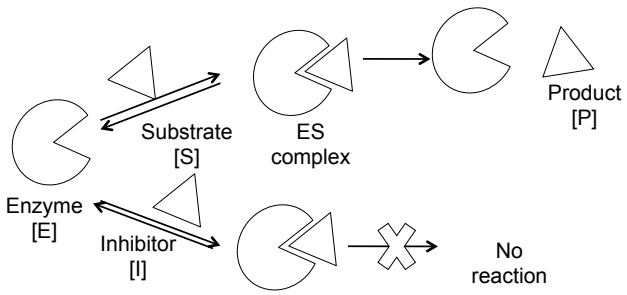
Enzyme inhibition

- Enzyme inhibition provides insights into catalysis
- Major control mechanism
- Two type of enzyme inhibition

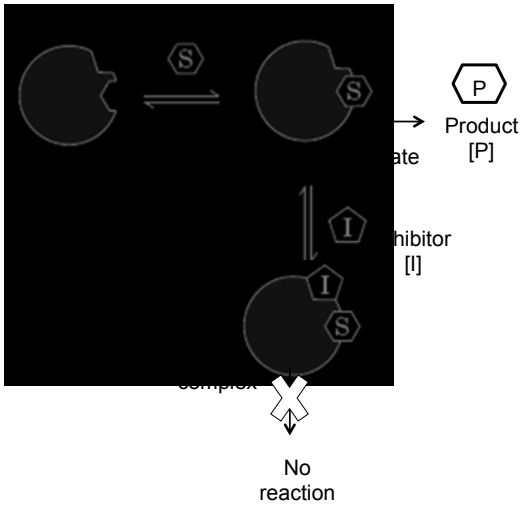
Reversible enzyme inhibition



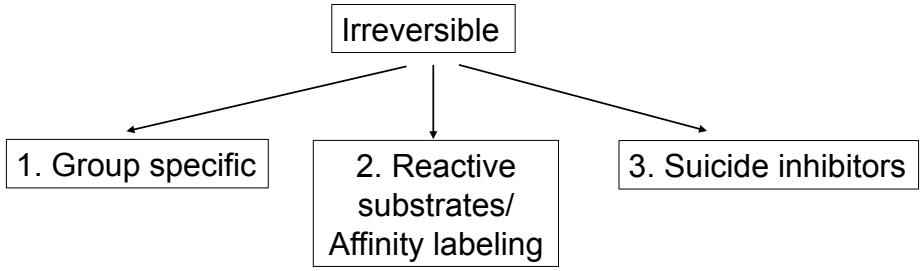
Competitive inhibition



Uncompetitive inhibition



Irreversible enzyme inhibition



Catalytic Strategies

Covalent catalysis

- Active site contains reactive group which covalently attaches to substrate
- Example – chymotrypsin

Acid-base catalysis

- A molecule plays a role of a proton donor or acceptor
- Example – carbonic anhydrase, histidine facilitates removal of H+

Metal ion catalysis

- Metal ions serve as bridge between E and S
- Increase binding energy & holding substrate in appropriate conformation for catalysis
- Example – Nucleoside Monophosphate (NMP) kinases

Regulatory Strategies

Allosteric regulation

- Allosteric proteins possess regulatory sites and multiple functional sites
- Allosteric proteins have property of cooperativity
- Allosteric proteins are information transducers

Isozymes

- Isozymes catalyze the same reaction but differ in structural characteristics
- Isozymes are expressed in distinct organelle or at distinct stages of development

Reversible covalent modification

- An effective method to control enzyme activity
- Example: phosphorylation

Enzyme activation by proteolytic cleavage

- Inactive precursor is called zymogen
- Mechanism involves cycle of active and inactive states
- Example - chymotrypsin, trypsin

Summary

- Enzymes basic concepts
- Catalytic strategies
- Regulatory strategies

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