Enzyme Kinetics 2018

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Learning Objectives

- Enzyme Kinetics
- Enzyme Inhibition
- Drugs utilizing kinetics and inhibition and its clinical utility

Enzyme Kinetics

the quantitative measurement of the rates of enzyme-catalyzed reactions and the systematic study of factors that affect these rates

Catalysts

- Increase rate of reaction by factor of 10^6
- Highly selective and specific
- Not changed as a result of catalysis
- Does not change the equilibrium constant
- **Enzymes Alter Only the Reaction Rate and Not the Reaction Equilibrium**

Factors affecting reaction velocity

Substrate concentration

- The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached

Temperature

- **Bell shaped curve**

- Increase of velocity with temperature
- Decrease of velocity with higher temperature

pH

- **Bell shaped curve**

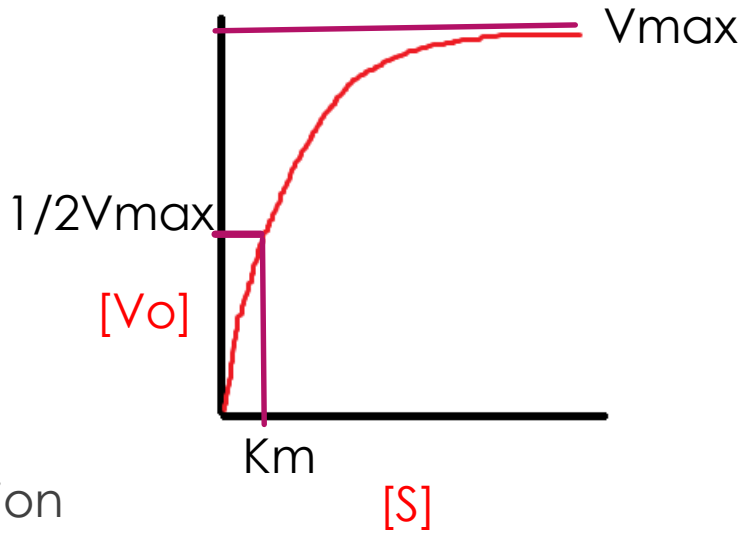
- Effect of pH on the ionization of the active site
- Effect of pH on enzyme denaturation
- Variable pH optimum

Kinetic Order of Reaction

- Sum of the molar ratios of the reactants defines the **kinetic order** of the reaction
- First order
- Second order
- Pseudofirst order reaction

Michaelis Menten Equation

$$Vo = \frac{V_{max}[S]}{Km + [S]}$$



Relationship between initial velocity and substrate concentration

Km and its importance

- The Michaelis constant ***Km*** is the **substrate concentration** at which ***Vi*** is half the maximal velocity **(*Vmax*/2)** attainable at a particular concentration of the enzyme.

Unit?

- Reflects the **affinity of the enzyme** for that substrate:
  inverse relationship

- Specific for enzyme substrate combination

- **Order of reaction**

Clinical importance of Km

- **Hexokinase vs Glucokinase**

Difference between Hexokinase and Glucokinase

	Hexokinase	Glucokinase
Substrate specificity	All hexoses	Mainly Glucose
Km	Low (high affinity) *Works at normal glucose concentration*	High (low affinity) *works only when glucose levels are elevated*
Location	Universal	Mainly liver and Beta cells of pancreas
Vmax (rate of reaction)	Low	High
Glucose-6-PO4 (Allosteric inhibition)	Inhibits the enzyme	No inhibition
Insulin	No regulation	Positive regulation

Line-Weaver Burk plot

- **Double Reciprocal Graph**
- **Linear curve**
- $$\frac{1}{V0} = \frac{Km}{Vmax\ [S]} + \frac{1}{Vmax}$$

Linear form of Michaelis menten equation to Determine Km and Vmax.

Units of Enzyme activity

- Amount of substrate converted to product per unit time under standard conditions of pH and Temperature

- **IUB unit**: Katal (µmol/min)
- **SI Unit:** (mol/sec)

Relative activities of Enzymes

- **Specific activity (Vmax/protein concentration):** Impure Enzymes
- **Turnover number**(**Vmax/moles of enzyme**): Homogenous Enzymes
- **Catalytic constant, Kcat [Vmax/No. of active sites(St)]: unit time^{-1}**
- **Catalytic efficiency: Kcat/Km** (Carbonic anhydrase, ADA, acetylylcholinesterase)

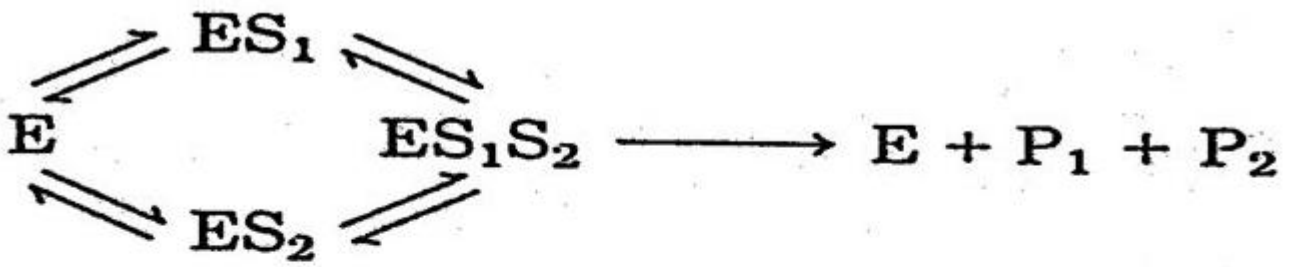
Two substrate Reactions(Bi Bi Reactions)

- **Sequential : Random**

  Ordered

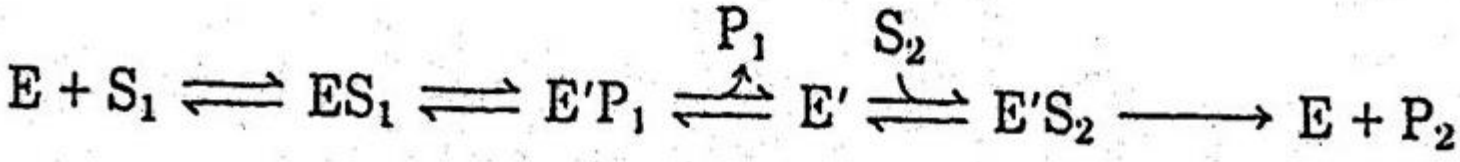
- **Ping Pong: Double displacement reactions**

Two substrate Reactions



Random

Ordered/Sequential



Ping Pong/Double Displacement

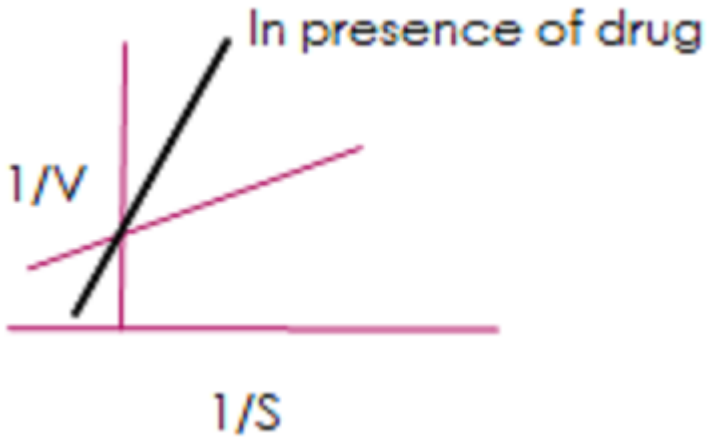
Enzyme Inhibition

Types of Inhibitions based on kinetics

- Competitive Inhibition
- Non-Competitive Inhibition
- Un-Competitive inhibition

Competitive Inhibition

- Binding at substrate binding site
- Inhibitor similar to substrate
- Km increased
- Vmax same



Clinical Application/Drugs

- **Statin Drugs**

Competitive Inhibitors of HMG CoA reductase

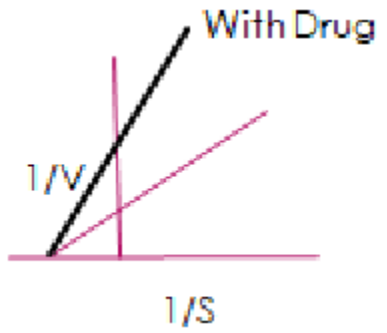
- **Sulpha Drug (Str. Analogues of PABA)**

Inhibits Folic acid synthesis in Bacteria

- **Methanol Poisoning**

Non-Competitive Inhibition

- Substrate and inhibitor binds at different sites
- Not structural analogues
- Decrease Vmax
- Km same

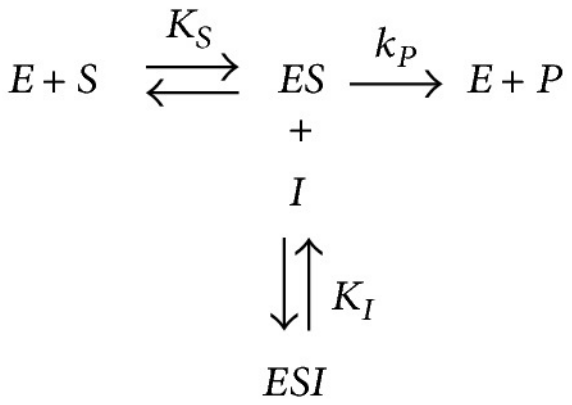


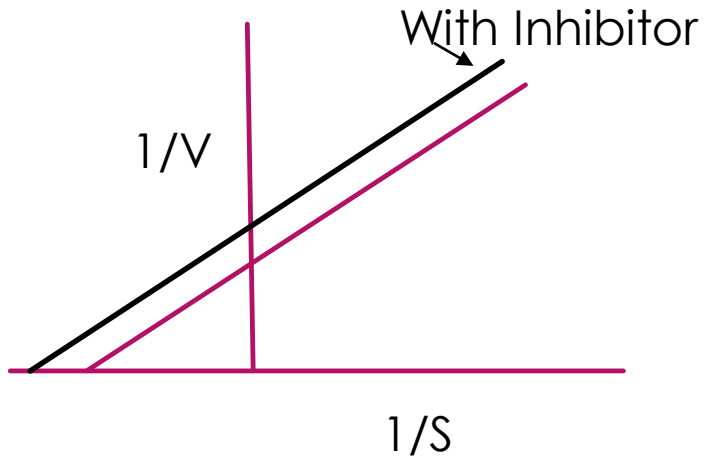
Drugs/Toxins based on Non - Competitive Inhibition

- Ferrochelatase (Inhibition by **Lead**)
- Acetylcholinesterase (**Insecticides**)
- Cytochrome oxidase(**Cyanide**)

Uncompetitive Inhibition

- Inhibitor binds to ES Complex
- Both Km and Vmax decreases





Examples of Drugs showing Uncompetitive Inhibition

- Lithium (Inositol monophosphatase)
- Phenylalanine(Placental ALP)

Classification based on Reversibility

- **Reversible**
- **Irreversible**: Chemical modification or Covalent modification

Irreversible inhibitors Poison Enzymes

- **Diisopropylflurophosphate** (nerve gas): covalently binds acetylcholinestrase
- **Aspirin**(Cox)
- **Penicillin** (bacterial transpeptidase)

Mechanism Based Inhibition

- **Suicide Inhibition**
- Contains chemical group that is transformed by catalytic machinery
- Generates highly reactive group
- Binds covalently to catalytically essential residues

Drugs based on Suicide Inhibition

- **Allopurinol** (inhibits xanthine oxidase: Oxypurinol)
- **5 fluorouracil** (inhibits thymidylate synthase: FdUMP)

Transition state Analogs & Abzymes

- Transition state analog: A molecule with shape similar to transition state
- Catalytic Antibodies
- Abzymes created using transition state analog as antigens

Clinical Scenario 1

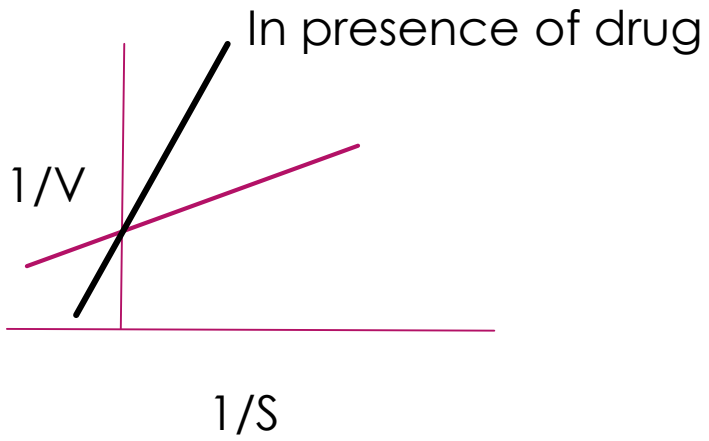
A 45-year-old man presents to emergency with bradycardia, blurred vision, vomiting, increased and salivation. He is a farmer using OPC Spray for his field and pipe ruptured. Type of inhibition?

- **(A)** Competitive
- **(B)** Noncompetitive
- **(C)** Uncompetitive
- **(D)** Irreversible

Clinical scenario 2

- **A 35 year old lady comes to OPD with evening fatigue, eyelid drooping, dysphagia and slurred speech. A drug is administered with following effect. What is true**

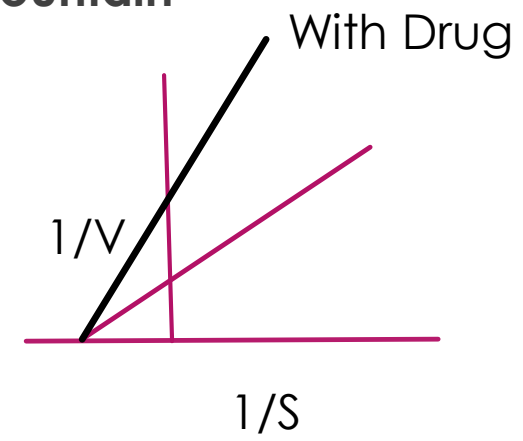
a. Competitive: Vmax same, km increased
b. Competitive : Vmax same, km decreased
c. Non-competitive: Vmax decreased, Km same
d. Non-competitive: Vmax decreased, Km decreased



Clinical Scenario 3

A patient wants to go to Manali for trekking. He took a medicine for mountain sickness with following kinetics. What is the type of inhibition?

- **A.** Competitive
- B. Noncompetitive
- C. Uncompetitive
- D. Allosteric



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- Denise R. Ferrier; Lippincott Illustrated Reviews Biochemistry, 7th Edition

Thank You!