ENZYME
&
CLINICAL ENZYMEOLOGY

DR PIYUSH B. TAILOR
Associate Professor
Department of Biochemistry
Govt. Medical College,
Surat, Gujarat
What is true about enzyme?

A. All are protein in nature
B. They are consumed in reaction
C. Increase Activation energy
D. Increase velocity of reaction
Which of The following is Enzyme?

A. Ribozyme
B. Abzyme
C. Thrombin
D. All of Above
Enzymes

- All Protein, Exception (Ribozyme)
- Increase Reaction Velocity
- Lowering Activation energy.
- Increase rates by $10^3$-$10^8$
- Allow reactions to occur under much milder condition
  - Low Temperature
  - Low Atmospheric pressure
  - At physiological pH
Ribozyme
Abzyme
• Which of following clotting factor work as serine protease enzyme?

A. Fibrinogen (Clotting Factor I)
B. Calcium (Clotting Factor IV)
C. Proaaccelerin (Clotting Factor V)
D. Antihaemophilic factor (Clotting Factor VIII)
E. Fibrin stabilizing factor (Clotting Factor XIII)
Clotting factor - Serine Protease

INTRINSIC PATHWAY

Damaged Surface

Kininogen Kallikrein

XII → XIIa → XI → X → IX → IXa → VIIIa → Xa → X

EXTRINSIC PATHWAY

Trauma → VIIa → Tissue Factor → Xa → X

FINAL COMMON PATHWAY

Prothrombin (II) → Fibrinogen (I) → Thrombin (IIa) → Fibrin (Ia) → Cross-linked fibrin clot
All Clotting factor are “Serine Protease” enzyme

Clotting factor which is **Not enzyme**

1. Clotting Factor I = Fibrinogen
2. Clotting Factor IV = Calcium
3. Clotting Factor V = Proaccelerin (Labile Factor)
4. Clotting Factor VIII = Anti Haemophilic Factor

• They work like “**cofactor**”

**C.F. XIII - Fibrin stabilizing factor - Transglutaminase**
Which of the following is not a cofactor?

A. Thiamin Pyrophosphate
B. Pyridoxime phosphate
C. Biotin
D. Fibrinogen
Holo-enzyme means

A. Apoenzyme + Co-Enzyme
B. Apoenzyme + Co-factor
C. Apoenzyme + Metalloenzyme
D. All of Above
Holo-enzyme

Holo-enzyme = Apoenzyme + Non-protein component

- Apoenzyme = Enzyme (Protein moiety)
- Non – Protein Component
  - Co-enzyme = Organic molecule
    1. Co-Substrate (Loosely bound)
      - NADH, NADPH, FMN, FAD, Coenzyme A
    2. Prosthetic (tightly bound)
      - TPP, PLP, Biotin
  - Co-factor = Inorganic molecules
    - Metal ion e.g. Zn, Fe, Cu, Mn, Mg
Features of Co-enzymes

- Heat stable
- Low molecular weight
- After completion of reaction, come out from reaction.
- And participate in another reaction
Thiamine Pyrophosphate (TPP) is required as a cofactor in,
A. Carboxylation  
B. Decarboxylation  
C. Transamination  
D. Transketolase
Thiamine Pyrophosphate (TPP) is required as a cofactor in:

A. Carboxylation = Biotin
B. Decarboxylation = TPP
C. Transamination = PLP
D. Transketolase = TPP
Oxidoreductase type of reaction require (as co-enzyme), EXCEPT

A. Riboflavin
B. Niacin
C. Pantothanic acid
D. Folic acid
Oxidoreductase type of reaction require (as cofactor), EXCEPT

A. Riboflavin = FAD, FMN = Oxidoreductase
B. Niacin = NAD, NADP = Oxidoreductase
C. Pantothanic acid = Coenzyme A = Acyl carrier
D. Folic acid = THF = Oxidoreductase
   & One Carbon carrier
Co-Enzyme, Cofactor & Prosthetic from Vitamins

- Thiamine = TPP = Decarboxylation & Transketolase
- Riboflavin = FMN, FAD = Oxida-reduction
- Niacin = NAD, NADP = Oxida-reduction
- Pyridoxin = PLP = Transamination
- Biotin = Biocytin = Carboxylation
- Folic acid = THF = Carrier of One Carbon
- Pantothenic acid = Coenzyme A = Acyl Carrier
- Vitamin B12 = Methylcobalamine = Isomerization & H₂ group transfer
Severe Iron deficiency can affect all of following, EXCEPT

A. Glutathione synthesis  
B. Uric acid Synthesis  
C. ATP synthesis  
D. Collegen synthesis
Severe Iron deficiency can affect all of following, EXCEPT

A. Glutathione synthesis = Glutathione synthetase (Mg)
B. Uric acid Synthesis = Xanthine Oxidase (Fe)
C. ATP synthesis = Cytochrome Oxidase (Fe)
D. Collegen synthesis = Lysyl hydroxylase (Fe)
                  = Lysyl oxidase (Cu)
Cofactor = Metalloenzyme

- **Magnesium (Mg)**
  - Hexokinase
  - Phosphofructokinase
  - Enolase
  - Glutathione Synthetase

- **Manganese (Mn)**
  - Hexokinase
  - Enolase

- **Molybdenum (Mo)**
  - Xanthine oxidase
  - Sulfite oxidase

- **Iron (Fe)**
  - Xanthine Oxidase
  - Cytochrome oxidase
  - Peroxidase
  - Catalase
  - Lysyl Hydroxylase

- **Potassium**
  - Pyruvate Kinase

- **Copper (Cu)**
  - Cytochrome oxidase
  - Lysyl oxidase
  - Tyrosinase
  - Ceruloplasmin (Ferroxidase)

- **Zinc (Zn)**
  - Lactate dehydrogenase
  - Carbonic anhydrase
  - Alkaline phosphatase
  - Alcohol dehydrogenase
  - Glutamate dehydrogenase

- **Selenium**
  - Glutathione Peroxidase

- **Nickel**
  - Urease
Anaerobic Glycolysis can be inhibited due to deficiency of

A. Magnesium
B. Manganese
C. Zinc
D. Potassium
E. All of Above
Anaerobic Glycolysis can be inhibited due to deficiency of:

A. Magnesium = HK, PFK, Enolase
B. Manganese = HK, Enolase
C. Zinc = LDH
D. Potassium = PK
E. All of Above
• Non protein part of the enzyme is called
  A. Apo-enzyme
  B. Co-enzyme
  C. Holo-enzyme
  D. Abzyme
Units of Enzyme

1 unit enzyme = Amount of enzyme that convert
1 micro mol of substrate per min into product

1 katal enzyme = Amount of enzyme that convert
1 mole of substrate per second into product

• 1 U = 1/60 micro katal = 16.67 nano katal.
Velocity (Turn over) of Enzyme = \( V_0 \)

Velocity of Enzyme Measure = micro mole / min

Catalase Velocity : 5 million micro mole / min

One catalase molecule convert approx. 5 million molecules of H\(_2\)O\(_2\) into H\(_2\)O + O\(_2\) per minute

Fastest to Slowest Velocity (Turnover) Enzyme

Catalase > Carbonic andydrase
  > Acetylcholinesterase > Amylase
    > LDH > Trypsin > Chymotrypsin
      > DNA polymerase > Lysozyme
Velocity of the enzyme

A. indicate turn over of substrate to product
B. is indicated in micromole per min
C. indicate conc. of substrate required for half Vmax
D. indicate number of unit of enzyme require for substrate.
IUB (International Union of Biochemistry)
Classification of Enzyme

Enzyme Code = Four Digits

1. First (main class) = Type of Reaction
2. Second (subclass) = Type of Group involved
3. Third (sub-subclass) = denotes Substrate
4. Fourth = Individual enzyme name & serial number

E.C. 1. Oxidoreductases
E.C. 2. Transferases
E.C. 3. Hydrolases
E.C. 4. Lyases
E.C. 5. Isomeraes
E.C. 6. Ligases
Which of the following enzymes is considered as NAD+ dependant Oxidoreductase?

1. Isocitrate dehydrogenase
2. Alpha Keto Glutarate dehydrogenase
3. Succinate dehydrogenase
4. Malate dehydrogenase
5. Lactate dehydrogenase
6. Glucose 6 Phosphate dehydrogenase

A. 1, 2, 3, 4
B. 1, 2, 4, 5
C. 1, 2, 5, 6
D. 1, 2, 3, 6
Which of the following enzymes is considered as NAD+ dependant Oxidoreductase?

1. Isocitrate dehydrogenase = NAD+
2. Alpha Keto Glutarate dehydrogenase = NAD+
3. Succinate dehydrogenase = FAD
4. Malate dehydrogenase = NAD+
5. Lactate dehydrogenase = NAD+
6. Glucose 6 Phosphate dehydrogenase = NADP+

A. 1, 2, 3, 4
B. 1, 2, 4, 5
C. 1, 2, 5, 6
D. 1, 2, 3, 6
1. Oxidoreductases

- Catalyses a variety of oxidation-reduction reaction
- With help of NADH, NADPH, FADH$_2$, FMN
- Common names
  - Dehydrogenases - Oxidases
  - Peroxidases - Reductases
HMP Shunt

Nonoxidative phase

Glucose 6-phosphate → transketolase, transaldolase → 6-Phosphogluconate

Oxidative phase

NADP⁺ → NADPH → 2 GSH (glutathione reductase) → NADPH → NADP⁺ → 6-Phosphogluconate → NADPH → CO₂ → Ribulose 5-phosphate → Ribose 5-phosphate → Nucleotides, coenzymes, DNA, RNA

Fatty acids, sterols, etc. → reductive biosynthesis → Precursors
Oxidoreductase

1. **NAD+ dependent**
   1. Pyrivate dehydrogenase
   2. Isocitrate dehydrogenase
   3. Alpha Ketoglutarate dehydrogenase
   4. Malate dehydrogenase

2. **NADP+ dependent**
   1. Glucose 6 Phosphate dehydrogenase
   2. 6 Phosphogluconate dehydrogenase

3. **FAD+ dependent**
   1. Succinate dehydrogenase
Oxidoreductase

4. Other
   1. Xanthine oxidase
   2. Tyrosinase
   3. Phenylalanine hydroxylase
   4. Homogentisate oxidase
   5. Peroxidase
   6. Catalase
2. **Transferases**

- **Transfer of**
  - Amino
  - Carboxyl
  - Phosphoryl
  - Methyl
  - Acyl
  - Glycosyl

- **Kinase transfer**
  - Phosphate group

- **Example**
  - GPT (ALT)
  - GOT (AST)
  - Hexokinase
  - Glucokinase
  - Pyruvate Kinase
  - Transketolase
  - Transaldolase
  - Transcarboxylase.
Alanine Amino-transferase
Alanine Transaminase (ALT)
Glutamate Pyruvate Transaminase (GPT)

Alanine + Alpha Ketoglutarate ⇌ Pyruvate + Glutamate
Aspartate Amino-transferase
Aspartate Transaminase (AST)
Glutamate Oxaloacetate Transaminase (GOT)

Aspartate + Alpha Ketoglutarate $\rightleftharpoons$ Oxaloacetate + Glutamate
3. Hydrolases

- Cleavage of C-C, C-O, C-N & other Covalant bonds
- By *addition of water*.
- **Example = All Digestive Enzyme**
  - Protease (Trypsin, Chymotrypsin, Pepsin, Collagenase)
  - Esterase
  - Amylase
  - Lipase
  - Phosphatase
  - Urease
  - Arginase
- *Amylase, Lipase, Protease, Cellulase = Present in Detergent*
3. Hydrolases

Catalyze cleavage of bonds by addition of water, such as:

\[
\text{NH}_2\text{C} \vdash \text{O} \vdash \text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3
\]
4. Lyases

- Cleavage of C-C, C-O, C-N & other Covalent bonds
- By atomic elimination and Generating double bond.
- Without adding water
- Example
  - Aldolase
  - Enolase
  - Fumarase
  - Arginosuccinase
  - Pyruvate decarboxylaes
  - HMG CoA lyase
Glucose

ATP

ADP

hexokinase, glucokinase

Glucose-6-phosphate

phosphohexose isomerase

Fructose-6-phosphate

ATP

ADP

phosphofructokinase-1

Fructose-1,6-bisphosphate

aldolase

Glyceraldehyde-3-phosphate

triosephosphate isomerase

Dihydroxyacetone phosphate
Pyruvate → Acetaldehyde → Ethanol

Pyruvate decarboxylase

TPP, Mg^{2+}

Acetaldehyde dehydrogenase

NADH + H^{+} → NAD^{+}

alcohol dehydrogenase

CO_{2}
Urea Cycle

1. Carbamoyl Phosphate Synthase
   CO₂ + NH₃ → Carbamoyl Phosphate

2. Ornithine carbamoyltransferase
   Carbamoyl Phosphate → Citrulline

3. Arginosuccinate synthase
   L-Aspartate → Arginosuccinate

4. Argininosuccinate lysase
   Arginosuccinate → Urea + H₂O

5. Arginase
   Urea → L-Ornithine

L-Arginine → L-Fumarate
5. Isomerases

- Optical, Geometric or Positional changes of substrate
- Example
  - Racemases
  - Epimerases
  - Triose phosphate isomerase
Odd carbon fatty acid → Propionyl CoA

Propionyl CoA + CO₂ + H₂O → D-Methylmalonyl CoA

Biotin + ATP → ADP + Pi

D-Methylmalonyl CoA → Methylmalonyl-CoA racemase

Methylmalonyl-CoA isomerase → Vitamin B12

Succinyl-CoA

Citric acid cycle intermediate

Methylmalonic Acidemia
6. Ligases

- Link two substrate Usually with help of ATP
- Example
  - Synthetase
  - Acetyl CoA carboxylase
  - DNA Ligase
Enzymes which move a molecular group from one molecule to another are known as
A. Oxido-reductase
B. Transferase
C. Hydratase
D. Lyase
Active Site for Enzyme

- Special pocket of Enzyme molecules
- Three-dimensional surface
- Complementory Amino acid side chain in Enzyme & Substrate
Substrate entering the active site of the enzyme

Enzyme/substrate complex

Enzyme/product complex

Products leaving the active site of the enzyme
Mode of action of Enzymes

- Reactions have an energy barrier
- That energy barrier separate substrates and products.
- Energy barrier = free energy of activation
If any enzymatic reaction require high “free energy of activation”, it means

A. It is slow reaction
B. It is fast reaction
C. It generates more energy
D. None
Michaelis-Menten Equation

\[ E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P \]

\[ v_o = \frac{V_{max} [S]}{K_m + [S]} \]
Velocity (Turn over) of Enzyme (reaction)

Velocity of Enzyme Measure = micro mole / min

\( V_{\text{max}} = \text{Maximum velocity of the reaction.} \)

Catalase Velocity : 5 million micro mole / min

One catalase molecule convert approx. 5 million molecules of H2O2 into H2O + O2 per minute

Fastest to Slowest Velocity (Turnover) Enzyme

Catalase > Carbonic anhydrase

> Acetylcholinesterase > Amylase

> LDH > Trypsin > Chymotrypsin

> DNA polymerase > Lysozyme
**$K_m$ (Michaelis constant)**

- Substrate concentration required to achieve $\frac{1}{2}V_{\text{max}}$
- Reflects Affinity of Enzyme for substrate.
- Low $K_m$ = Less Substrate required for $\frac{1}{2}V_{\text{max}}$
  = Indicate High Affinity
- High $K_m$ = High Substrate required for $\frac{1}{2}V_{\text{max}}$
  = Indicate Low Affinity
\[ K_{cat} = \text{Turnover number of 'S' to 'P'} \]

\[ K_m = \text{Affinity of Enzyme towards substrate} \]

\[ \frac{K_{cat}}{K_m} = \text{Catalytic Efficiency of Enzyme} \]
What is true about “Faster catalytic reaction”?

A. Less “Free energy of activation” require
B. Less Vmax
C. Less Kcat/km for enzyme
D. Less Km value for enzyme
• Faster Reaction
  • Less “Free energy of activation” require
  • Easily reach Transition state
  • High Kcat/km

• Slow Reaction
  • More “Free energy of activation”
  • Difficult to reach Transition state
  • Low Kcat/km
**Type of Reaction**

- **Zero order:** Rate is independent of $[S]$, i.e., proportional to $[S]^0 (= 1)$.

- **First order:** Rate is proportional to $[S]$, i.e., $[S]^1 (= [S])$.

- **Second order:** Rate is proportional to $[S] \times [S]$ (i.e., $[S]^2$).
In first order kinetic, velocity of reaction is
A. directly proportional to [S].
B. Inversely proportional to [S].
C. not depend on [S].
D. proportion to [S]^2
Hexokinase Vs Glucokinase

- Non Specific
- Act on any hexose sugar
- For Glycolysis
- Present in every cell
- Low km
- High affinity
- Activated even with low concentration of glucose

- Specific
- Act on only Glucose
- For Glycogen synthesis
- Present in hepatic cell
- High km
- Low affinity
- Activated with only high concentration of glucose
Koshland’s Induce Fit Theory

- After binding of substrate to enzyme at specific site, there will be more conformational change in the enzyme.
- e.g. Hand Gloves.

**INDUCED-FIT THEORY**
Fischer’s Template Theory

- Active site of the enzyme is complementary to the substrate.
- E.g. Lock & Key
Factors Affecting Enzyme Reaction Activity and It’s Velocity
1. **Substrate concentration**

- Velocity increases with \([S]\)
- Until \(V_{\text{max}}\) is reached.
- High \([S]\) = enzyme Saturated with substrate.
2. Temperature

- Optimum temperature (Human) = 35° - 40°C.
- Enzymes denature = above 40°C temperature.
- Maximum reaction velocity at Optimum temperature.

In PCR

- Enzyme to act at 70-90°C
- Enzyme from Hot spring Bacteria
- Thermus Equaticus = Optimum temperatures of 70°C.
- Taq Polymerase = PCR
Effect of Temperature on Enzyme activity

![Graph showing the effect of temperature on enzyme activity. The graph indicates that enzyme activity increases with temperature up to a certain point and then decreases, with a peak around 40°C and a heat inactivation zone above 80°C.](image)
$Q_{10}$ Temperature Coefficient

- The rate of change in Reaction velocity as increase the temperature by 10 °C
- Biological Value of $Q_{10} = 2 - 3$
3. Enzyme concentration

- Velocity (Rate of the reaction) is directly proportional to [E] at all [S].

- Half [E] = $V_o$ & $V_{max}$ are reduced to half.
Figure 3-22a
Molecular Cell Biology, Sixth Edition
© 2008 W.H. Freeman and Company
Substrate concentration is always more than Km in

A. Zero order kinetic
B. First order kinetic
C. Second order kinetic
D. Third order kinetic
**First Order Reaction**

- $[S] << K_m$
- $V \propto [S]$

**Zero Order Reaction**

- $[S] >> K_m$
- $V$ remains constant $[S]$
- $V = V_{\text{max}}$

At high concentrations of substrate ($[S] >> K_m$), the velocity of the reaction is zero order—that is, it is constant and independent of substrate concentration.

At low concentrations of substrate ($[S] << K_m$), the velocity of the reaction is first order—that is, it is proportional to substrate concentration.
4. **Product concentration**

- Increase Product Conc. = Velocity Slow.
- Higher Product conc. = Inhibits reaction.

![Diagram showing the relationship between time and concentration of A and B molecules.](image)
Enzyme activity increase with, Except

A. Increase substrate concentration
B. Increase enzyme concentration
C. Increase temperature (not more than optimum)
D. Increase product concentration
5. pH

- $[\text{H}^+]$
  - Change Active site & Bonds
  - Configuration change
  - Change Velocity
  - Can denature enzyme

**Different Optimum pH for Different enzyme.**
What change can occur at active site, because of change in pH?
Different enzyme with its optimum pH
6. **Enzyme activation**

- In presence of certain metallic ions, some enzyme shows higher activity.
  - Salivary amylase = chloride
  - Lipase = calcium
- Pro-enzyme (Zymogen) to active form
  - Trypsinogen = Trypsin
  - Chymotrypsinogen = Chymotrypsin
- Lysosomal enzymes

**Protein Activation**

- Coagulation factors
- Complementary components
7. Enzyme Inhibition
Inhibitors

- Reduce the rate of enzymic reactions
- Work at low concentrations
- Block the enzyme but they do not usually destroy it
- Many drugs and poisons are inhibitors of enzymes
The effect of enzyme inhibition

**Reversible inhibitors**

1. Competitive inhibitors
2. Non–competitive inhibitors

**Irreversible inhibitors**

- Combine with functional groups at active site
- Irreversibly
Compatitive Inhibition

- Inhibitor = Structurally resemble to substrate.
- Compete with the substrate molecules for the active site.
- Inhibitor action is proportional to its concentration
Cholesterol Regulation

Glucose → Pyruvate → Acetyl CoA → HMG CoA → Mevalonic acid → Cholesterol

Insulin activates Protein phosphatase and Protein kinase A.

HMG CoA reductase catalyzes the conversion of HMG CoA to Acetyl CoA.
Competitive Inhibition

- HMG CoA-reductase
  - Active site
- HMG-CoA (substrate)
  - Lovastatin (competitive inhibitor)
Statin use in hypercholesterolemia, because it makes
A. Competitive inhibition as it is analogues to HMG CoA
B. Competitive inhibition as it is analogues to HMG CoA reductase
C. Non-competitive inhibition as it is analogues to HMG CoA
D. Non-competitive inhibition as it is analogues to HMG CoA reductase
Vmax decrease in,

A. Competitive inhibition
B. Non-Competitive inhibition
C. Un-Competitive inhibition
D. Suicide inhibition
E. B & C
F. All of Above
In Competitive Inhibition
Km Increases & Vmax remains unchanged

Maximal velocity, $V_{\text{max}}$, is the same in the presence of a competitive inhibitor.

Michaelis constant, $K_m$, is apparently increased in the presence of a competitive inhibitor.

(Apparent $K_m$ in the presence of a competitive inhibitor)
PABA (metabolit)

Sulfonamid (antimetabolit)
Sulfa Drug - Antimetabolite

pteridine + H₂N(para-aminobenzoic acid) → pteridine + PABA + glutamic acid → folic acid

para-aminobenzoic acid (PABA)

Sulfonamide base structure

pteridine + H₂N₁₁ → pteridine + sulfa drug + glutamic acid → folic acid

1st enzyme "fooled"

2nd enzyme inhibited

C. Ophardt, c. 2003
Methotrexate = Folic Acid Analogues

Folic acid

Méthotrexate
Vitamin K analogues

• **Dicoumarol and Warfarine**
  • Structurally analogues to Vitamin K.
  • Inhibit formation of reduce vitamin-k
  • So, decrease formation of carboxylation of glutamic acid of the prothrombin
  • Subsequently, activation of clotting factor is inhibited
  • So both drug work as Anti-coagulant.
Alcohol Metabolism

Ethanol metabolism:

- Ethanol (CH₃CH₂OH) is converted to acetaldehyde (CH₃CHO) by alcohol dehydrogenase using NAD⁺.
- Acetaldehyde is converted to acetic acid (CH₃COOH) by aldehyde dehydrogenase using NAD⁺.
- Acetic acid is converted to acetyl-SCoA (CH₃COSCoA) using NAD⁺.

Methanol metabolism:

- Methanol (CH₃OH) is converted to formaldehyde (HCHO) by alcohol dehydrogenase using NAD⁺.
- Formaldehyde is converted to formic acid (HCOOH) by aldehyde dehydrogenase using NAD⁺.
Example: Competitive Inhibition

**Sulphonamides**
- Sulphonamide is analogues to PABA.
- Antibacterial agent
- In bacteria, PABA + Pteroyl glutamic acid = Folic acid (require for bacterial growth)
- Drug is non-toxic to human cells.

**Statin Drugs (Atorvastatin, Simvastatin)**
- HMG CoA analogues
- Inhibit HMG CoA reductase
- Decrease serum cholesterol level

**Methotrexate**
- Folic acid analogues
- Inhibit *dehydrofolate reductase* enzyme.
- Inhibit purine – pyrimidine synthesis
- Inhibit cell division.
- Use as Chemotherapy (Anti-cancer drug)
Example: Competitive Inhibition

**Isonicotinic acid Hydrazide (INH)**
- Analogue to Pyridoxal.
- Drug for tuberculosis (AKT)
- Inhibit Pyridoxal kinase enzyme
- which convert Pyridoxal into PLP.

**Dicoumarol and Warfarine**
- Structurally analogues to Vitamin K.
- Anti-coagulant.

**Methanol**
- Ethanol is analogues to methanol
- Methanol converted into formaldehyde & formic acid.
- Enzyme Alcohol dehydrogenase
- Formaldehyde causes sudden death & blindness.
- Ethanol has high affinity for ADH than Methanol
- Ethanol is use as antidote in methanol poisoning.
1. **Non-competitive**
   - Inhibitor is **not analogue** to substrate.
   - Inhibitor & substrate **bind at different sites**.
   - Inhibitor can **bind either free enzyme or ES complex**.
   - Not influenced by the conc. of the substrate.

   - \( V_{max} = \text{Decrease} \)
     - Inhibition can not overcome by substrate
   - \( K_m = \text{Not changed} \)
     - Not infer with substrate for active site
Non-competitive
Un-competitive

- Inhibitor bind to ES complex
- Both \( K_m \) and \( V_{max} \) decrease
1. Non-competitive:

Examples

- **Cyanide** combines with the Iron in Cytochrome oxidase
- **Lead** inhibit Ferrochelate of heme synthesis.
- **Heavy metals**, Ag or Hg, combine with –SH groups.
- **Fluoride** inhibit Enolase, Glycolysis.
- **Di-isopropyl fluoro phosphatase inhibit acetylcholine-esterase.**

These can be removed by using a chelating agent such as EDTA

**BAL (British Anti Lewisite, Dimercaprol)**

- Use as antidote for heavy metal poisoning
- It have several –SH group to neutralized heavy metal
Suicide Inhibition

- Inhibitor = Structural analogues
- Make Competitive inhibition
- Than converted to more effective inhibitor
- Which inhibit it’s own enzymatic reaction.
- **Allopurinol** = Drug for gouty arthritis.
- **Aspirin**
- **5-Fluro-Uracil**

- **Difluro Methyl Ornithine (DFMO)** = Inhibit Ornithine decarboxylase
Hypoxanthine $\xrightarrow{\text{Xanthine Oxidase}}$ Xanthine

Xanthine oxidase

Inhibition

Uric acid

Allopurinol $\xrightarrow{\text{Xanthine Oxidase}}$ Alloxanthine
Vmax decrease in,
A. Competitive inhibition
B. Non-Competitive inhibition
C. Un-Competitive inhibition
D. Suicide inhibition
E. B & C
F. All of Above
Vmax decrease in,

A. Competitive inhibition = unchange
B. Non-Competitive inhibition = decrease
C. Un-Competitive inhibition = decrease
D. Suicide inhibition = unchange
E. B & C
F. All of Above
COX + Aspirin (acetylsalicylate) → Acetylated, inactivated COX + Salicylate
FIGURE 1. Algorithm of the biochemical pathway shows that the formation of prostaglandins occurs via both cyclooxygenase enzymes (COX-1 and COX-2).
Fluorouracil (5-FU)

\[ \text{dUMP} \rightarrow \text{dTMP} \rightarrow \text{Thymidylate synthase} \]

\[ \text{Dihydrofolate reductase (DHFR)} \]

\[ \text{Tetrahydrofolate (methyl donor)} \]

\[ \text{Dihydrofolate} \]

\[ \text{Folic acid} \]

\[ \text{Methotrexate (MTX)} \]
Feedback inhibition

Product E inhibits Enzyme 1

Active configuration

Inactive configuration
8. Allosteric Regulation

• **EFFECTOR (MODIFIER)**

• Bind to site other than active site.

• Affect Both
  • Affinity (Km) OR
  • Catalytic activity (Vmax).

• **Negative effectors**

• **Positive effectors.**

• *Allosteric enzyme play role as regulatory enzyme (Key enzyme, Rate limiting enzyme) in cycle of reaction.*
9. **covalent modification**

- Most frequently by the addition or removal of phosphate groups.
- Phosphorylation reactions = use ATP
  1. Phosphorylation – Dephosphorylation
  2. Adenylation – Deadenylation
  3. Ribosylation
  4. Uridylation
  5. Methylation
covalent modification
• Active in Dephosphorylate form
  – PFK-1
  – Pyruvate Kinase
  – Glycogen synthase
  – HMG CoA reductase

• Active in Phosphorylate form
  – Fructose 1-6 bisphaphatase
  – Glycogen phosphorylase
  – Glucose 6 phosphatase
• **Active in Dephosphorylate form**
  
  • *(Decrease Glucose) (Insulin)*
    
    – PFK-1
    
    – Pyruvate Kinase
    
    – Glycogen synthase
    
    – HMG CoA reductase

• **Active in Phosphorylate form**

• *(Increase Glucose) (Glucagon)*

  – Fructose 1-6 bisphosphatase
  
  – Fructose 2-6 bisphosphatase
  
  – Glycogen phosphorylase
  
  – Glucose 6 phosphatase
Regulation of Glycolysis by Covalent modification

Glycolysis is less active

Fructose 6-phosphate

Fructose 2,6-bisphosphate

Glycolysis is more active (PFK-1 is activated)

Low blood sugar

Glucagon

Protein kinase A

PFK-2

FBPase-2

Protein phosphatase-1

Insulin

High blood sugar

Gluconeogenesis is more active

Fructose 6-phosphate

Fructose 2,6-bisphosphate

Gluconeogenesis is less active (FBPase-1 is inhibited)
Which of following enzyme is active in phosphorylated form?

A. Hexokinase
B. Phosphofructokinase – 1
C. Fructose 1 – 6 bisphosphatase
D. Glycogen synthase
Which of following enzyme is active in phosphorylated form?  (Increase Glucose)
A. Hexokinase  (decrease Glucose)
B. Phosphofructokinase – 1  (decrease Glucose)
C. Fructose 1 – 6 bisphosphatase  (increase Glucose)
D. Glycogen synthase  (decrease Glucose)
10. Induction & 11. Repression

- Regulate the amount of enzyme activity.
- Efficiency of enzyme = Not affected.
- Act at Gene level.
- **Altering rate of enzyme synthesis.**
  - Increase enzyme synthesis = **Induction**
  - Decrease enzyme synthesis = **Repression**
- Induction / Repression = Slow (hours to days)
- Allosteric regulation = Fast (seconds to minutes)
Example

- ALA synthase (key enzyme of Heme synthesis)
- Autoregulated by the heme
- Heme act as repressor molecule on ALA synthase gene
Induction of ALA synthase, due low haemoglobin level can increases,

A. ALA synthase $Vo$
B. ALA synthase $K_{cat}/K_{m}$
C. ALA synthase concentration
D. All of Above
Induction of ALA synthase, due low haemoglobin level can increases,

A. ALA synthase $V_0$
B. ALA synthase $K_{cat}/K_m$
C. ALA synthase concentration
D. ALA synthase $K_m$
12. Compartmenalisation

- Certain enzymes are present
  - In mitochondria &
  - In cytoplasm.
- Some of Cycle occurs in **Both Mitochondria & Cytoplasm**, 
  - Urea cycle
  - Heme synthesis
  - Gluconeogenesis.
Iso-enzyme
and
Clinical Enzymology
What is matching with Isoenzyme?

A. Same Chemically form
B. Same Physical characteristic
C. Does same catalytic reaction
D. Has single polypeptide unit
<table>
<thead>
<tr>
<th>ISOENZYMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalyze the same reaction</td>
</tr>
<tr>
<td>Two or more polypeptide chains</td>
</tr>
<tr>
<td>Different polypeptide chains are products of different genes</td>
</tr>
<tr>
<td>Differ in AA sequence and physical properties</td>
</tr>
<tr>
<td>May be separable on the basis of charge</td>
</tr>
<tr>
<td>Are tissue specific</td>
</tr>
<tr>
<td>“They are physical distinct forms of the same enzyme activity”</td>
</tr>
</tbody>
</table>
Identification of Iso-enzymes

1. Electrophoresis
2. Heat stability
3. Inhibitors
4. Substrate specificity, Km value
   - e.g. Hexokinase & Glucokinase
5. Cofactor requirement
   - e.g. Mitochondrial ICD – NAD$^+$ dependent Cytoplasmic ICD – NADP$^+$ dependent
6. Tissue location
7. Specific antibody
<table>
<thead>
<tr>
<th>Type of LDH</th>
<th>Composition</th>
<th>Fraction of LDH in %</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH 1</td>
<td>HHHHH</td>
<td>20-30%</td>
<td>Myocardium</td>
</tr>
<tr>
<td>LDH 2</td>
<td>HHHHM</td>
<td>30-40%</td>
<td>RBC</td>
</tr>
<tr>
<td>LDH 3</td>
<td>HHMM</td>
<td>20-25%</td>
<td>Lung</td>
</tr>
<tr>
<td>LDH 4</td>
<td>HMMMP</td>
<td>10-15%</td>
<td>Kidney &amp; Pancrease</td>
</tr>
<tr>
<td>LDH 5</td>
<td>MMMMM</td>
<td>5-15%</td>
<td>Skeletal muscle &amp; Liver</td>
</tr>
<tr>
<td>Type of CK</td>
<td>Composition</td>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>CK- 1 (CK-BB)</td>
<td>BB</td>
<td>Brain</td>
<td></td>
</tr>
<tr>
<td>CK- 2 (CK-MB)</td>
<td>MB</td>
<td>Myocardium</td>
<td></td>
</tr>
<tr>
<td>CK- 3 (CK-MM)</td>
<td>MM</td>
<td>Skeletal Muscle</td>
<td></td>
</tr>
</tbody>
</table>
CK-2 & CK-3 in normal subject &

After 24 hours of Myocardial Infarction

Creatine Kinase isoenzymes in blood

Patient 24 hrs after myocardial infarction
ENZYME ACTIVITY AFTER MYOCARDIAL INFARCTION

Time since onset of symptoms (days)

Enzyme activity (x upper reference value)

- Troponin
- CK-MB
- 'Heart-specific' LD
- Total CK

Graph shows enzyme activity over time post myocardial infarction.
In Acute myocardial infaction, which of following enzyme rises?

A.  LDH- 1 & CK – MM
B.  LDH- 2 & CK – MM
C.  LDH -1 & CK – MB
D.  LDH- 2 & CK – MB
### Plasma Enzymes Changes After Myocardial Infarction

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Abnormal activity (hour)</th>
<th>Peak value (hour)</th>
<th>Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Troponin I (not enzyme)</td>
<td>4 - 6</td>
<td>12 – 24</td>
<td>10 - 14</td>
</tr>
<tr>
<td>CK-MB</td>
<td>4 - 6</td>
<td>12 - 24</td>
<td>1.5 - 3</td>
</tr>
<tr>
<td>Total CK</td>
<td>6 - 12</td>
<td>18 - 30</td>
<td>2 - 5</td>
</tr>
<tr>
<td>AST(GOT)</td>
<td>6 - 12</td>
<td>20 - 30</td>
<td>2 - 6</td>
</tr>
<tr>
<td>LDH-1</td>
<td>8 - 18</td>
<td>30 - 48</td>
<td>5 - 14</td>
</tr>
</tbody>
</table>
In Acute myocardial infaction, which enzyme is considered as specific marker for diagnosis?
A. LDH
B. AST
C. CK-MB
D. Cardiac Troponin – I
Isoenzymes of Alkaline Phosphatase

Depending on number of sialic acid residue

1. Alpha – 1 ALP (10%) Biliary Canaliculi
2. Alpha – 2 heat labile ALP (25%) Hepatic cells
3. Alpha – 2 heat stable ALP (1%) Regan Isoenzyme Placental cell
4. Pre – beta ALP (50%) Bone disease
5. Gamma – ALP (10%) Intestinal cells
6. Leucocyte ALP Leucocyte
   • Decrease in chronic myeloid leukemia
   • Increase in lymphoma
<table>
<thead>
<tr>
<th>Organ</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>CK-MB, AST (GOT), LDH</td>
</tr>
<tr>
<td>Liver</td>
<td>ALT, AST, LDH, Alkaline Phosphatase, Gamma Glutamyl Transferase</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Lipase, Amylase</td>
</tr>
<tr>
<td>Muscle</td>
<td>Aldolase, CK-MM, CK-Total, AST</td>
</tr>
<tr>
<td>Bone</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>Prostate</td>
<td>Acid Phosphatase (Prostate isoform – inhibited by Tartrate)</td>
</tr>
<tr>
<td>RBC</td>
<td>LDH, Acid Phosphatase (Erythrocyte isoform – inhibited by formaldehyde &amp; cupric ion)</td>
</tr>
<tr>
<td>Diagnostically Important Enzyme</td>
<td>Principal Sources</td>
</tr>
<tr>
<td>------------------------------------------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>Liver</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST) I (cytosol) &amp; II (mitochondria)</td>
<td>Liver, Gall Bladder, Erythrocytes Skeletal muscle, Heart, Kidney,</td>
</tr>
<tr>
<td>Gamma Glutamyl Transferase</td>
<td>Hepatobiliary tract, Kidney</td>
</tr>
<tr>
<td>5’ Nucleosidase</td>
<td>Hepatobiliary tract</td>
</tr>
<tr>
<td>Alkaline Phosphatase (ALP)</td>
<td>Bone, Gall Bladder, Liver, Intestinal mucosa, Placenta, Kidney</td>
</tr>
<tr>
<td>Acid Phosphatase</td>
<td>Prostate, Erythrocytes</td>
</tr>
<tr>
<td>Amylase</td>
<td>Pancreas, Salivary glands, Ovaries</td>
</tr>
<tr>
<td>Lipase</td>
<td>Pancreas</td>
</tr>
</tbody>
</table>
For diagnosis of Acute Viral Hepatitis, Which of the following enzyme is specific?

A. ALT
B. AST
C. Alkaline Phosphatase
D. Gamma Glutamyl transfarase
For diagnosis of Acute Viral Hepatitis, Which of the following enzyme is specific?

A. ALT = Liver
B. AST = Liver, Gall Bladder, Heart
C. Alkaline Phosphatase = Bone, Liver, Gall Bladder
D. Gamma Glutamyl transfarase = Liver (induce by Drug & Alcohol), Kidney
Enzyme as Therapeutic Agents

1. Streptokinase & Urokinase
   • Lysis intravascular clot
   • Use in myocardial infarction

2. Pepsin & Trypsin
   • Use in patient having indigestion

3. Asparaginase
   • Used as anticancer drugs.
Enzyme as Diagnostic Agents

1. Glucose oxidase & Peroxidase (GOD-POD)
2. Urease
3. ELISA test
4. Restricted Endonuclease
Thank you