BIOCHEMISTRY

RR-8.0

Contents

ENZYMES : PART 1

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Basics of Enzymes

00:01:00

Definition : Specialized proteins that act as biological catalyst.

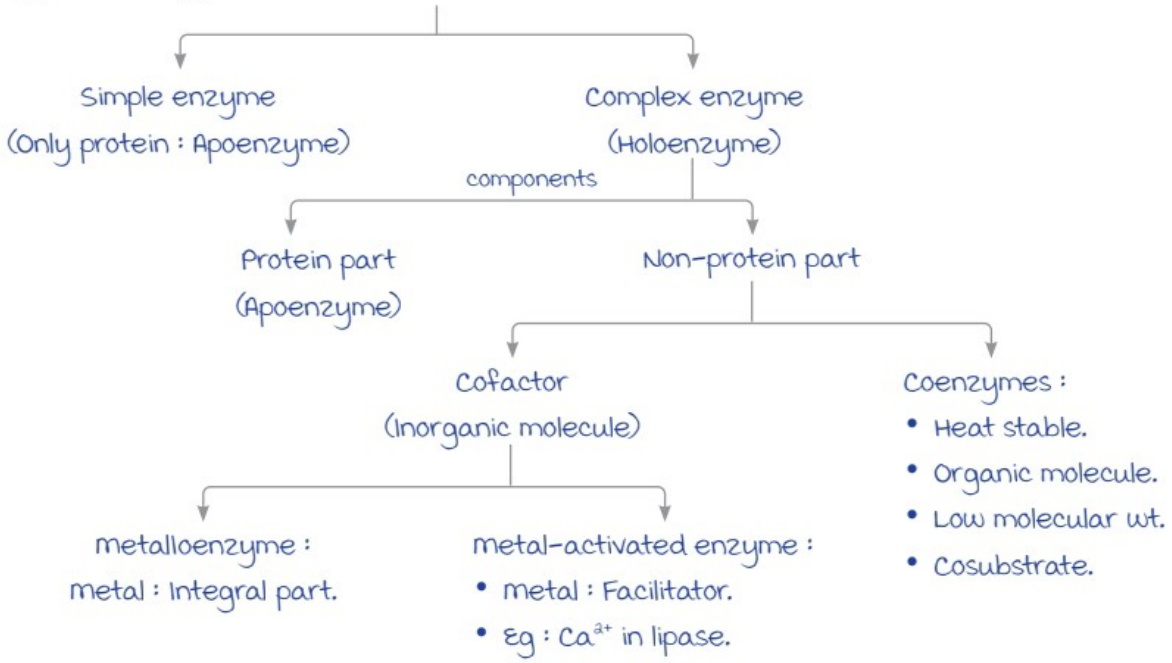
- Exception : Ribozymes (RNA).

Ribozyme	Location	Function
Peptidyl transferase	28 Sr RNA	Translation
Sn RNA	Spliceosome	RNA splicing
Group II introns	-	
Ribonuclease P	-	Post-translational modification of tRNA

Properties of Enzymes :

1. Made of protein.
2. 16% by weight : Nitrogen.
3. Heat labile.
4. Precipitated by protein precipitating agents.

Types of Enzymes :



Prosthetic group : Co-enzyme/Co-factor tightly integrated into apoenzyme.

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Coenzymes :

Vitamin	Active form	Reaction involved
Thiamine	Thiamine di/ pyrophosphate (TDP/TPP)	• Oxidative decarboxylation • Transketolase
Riboflavin	FAD ; FMN	Dehydrogenase (Succinyl, acyl CoA)
Niacin	NAD^+ $NADP^+$	Most dehydrogenases
Panthothenic acid	CoA ; Acyl carrier protein	All reactions with acetyl CoA; Succinyl CoA; Fatty acid synthase complex
Pyridoxine	Pyridoxal phosphate	• Transamination • Transulfuration • Decarboxylation } Amino acid metabolism • ALA synthase • Glycogen phosphorylase
Folic acid	THFA	All 1 Carbon reactions
Cobalamin	Methyl B12	Methionine synthase
	Adenosyl B12	Methyl malonyl CoA mutase
Lipoate	Lipoamide	Oxidative decarboxylation
Ascorbic acid	-	Hydroxylation (Prolyl & lysyl)

Cofactors :

Metal	Reaction catalysed
Zinc	• Carbonic anhydrase • Carboxypeptidase A & B • Alcohol dehydrogenase • ALA dehydratase • Cytosolic SOD (Scavenging enzyme)
Magnesium	ATP/PO_4 group involved : • Kinase • Mutase • Phosphatase • Enolase
Iron	Heme iron : • Complex III & IV of ETC (Cytochrome) • No synthase, Peroxidase, Catalase
	Non-heme iron : • Complex I & II of ETC (Fe-S cluster)
Manganese	• Kinase • Mitochondrial SOD • Phosphatase
Molybdenum	Xanthine oxidase

Zn deficiency features
• Acrodermatitis enteropathica • Visual disturbances (↓Retinol dehydrogenase) • Alopecia • Diarrhoea • Perioral, acral rash

Purine catabolism
Mb deficiency ↓ Hypouricemia

metal	Reaction catalysed
Potassium	• Na^+ – K^+ ATPase • Pyruvate Kinase
Copper	• Tyrosinase (Melanin production) • Complex IV of ETC (Cytochrome C oxidase) • Lysyl oxidase (Covalent cross linking of Collagen)

Cu deficiency
• Depigmentation • Neutropenia • X-ray : Similar to scurvy

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Note : Vitamin C leads to defective collagen D/t ↓lysyl hydroxylase.

Classification of Enzymes

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Class	Class name	Details & examples.
I	Oxidoreductases :	
	a. Dehydrogenase	NAD^+ FAD required as election acceptor (Oxidative decarboxylation)
	b. Oxygenase	• Monooxygenase : Phenylalanine/Tyrosine/Tryptophan hydroxylase, Cytochromes • Dioxygenase : Homogentisate oxidase
	c. Oxidase	Complex IV ETC
	d. Peroxidase	Glutathione peroxidase ($H_2O_2 \rightarrow H_2O$)
	e. Catalase	$H_2O_2 \rightarrow H_2O$; Enzyme marker of peroxisome
II	Transferase	Transfers functional group Eg → Kinases (Hexo/glucokinase) → Phosphorylases (Glycogen phosphorylase)
III	Hydrolase	• Breaks covalent bonds by adding H_2O • Eg : All digestive enzymes, arginase, phosphatase
IV	Lyase	• Breaks covalent bond without H_2O/atom elimination • Eg : Aldolase, fumarase, aconitase, enolase, simple decarboxylase
V	Isomerase	Eg → Isomerase (Produces isomers) → Racemase (D&L isomers) → Mutase (intramolecular PO_4 transfer)
VI	Ligase	• Coupling of molecules with breakdown of ATP • Eg → Synthetase → Carboxylase (Requires biotin)
VII	Translocase	• Transfer of ions/molecules across membrane • Eg: H^+ pump/Ca^{a+} channel

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Applied biochemistry
Defective synthesis of tetrahydrobiopterin (BH_4) from GTP : • ↓ phenylalanine hydroxylase : Non-classical phenylketonuria • ↓ tyrosine & tryptophan hydroxylase : Neurological symptoms (D/t ↓ catecholamines & serotonin)
• Resistant seizures in neonate : Supplement Vit. B6 (PLP) : – Dopamine, serotonin, epinephrine & norepinephrine require PLP as co-factor (simple decarboxylation)
• Fatigue in chronic alcoholics : D/t Vit. B1 deficiency (↓NADPH ⟶ ↓ATP)
• Polished rice consumption ⟶ Beri-Beri (D/t vit B1 deficiency)
• Raw egg consumption ⟶ Fatigue, hypoglycemia, organic aciduria. – Avidin (Raw egg) inhibits biotin (Vit. B7) & hence, all carboxylase reactions.

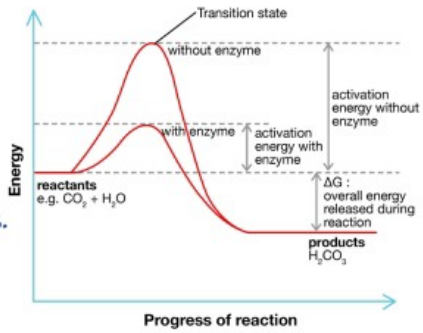
Enzyme Mechanism of Action

00:41:42

- Substrate binding : Active site.
- Site for regulator/modifier Allosteric site.

Free energy change (ΔG) :

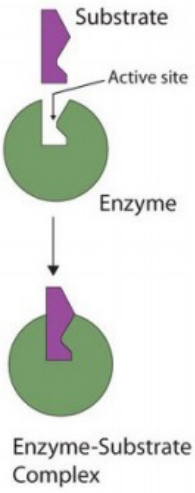
- Free energy change =
  ΔG = Energy of reactants – Energy of products.
- Enzyme ⟶ ↓ activation energy.
  ↳ No change in ΔG.

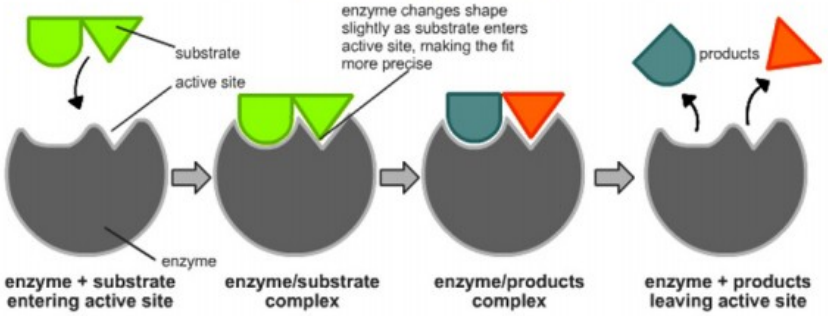


Enzyme-substrate complex :

Emil-Fischer's template theory :
Lock and key mechanism.

Koshland's induced fit theory :
Conformational change in active site induced by substrate.





ENZYMES : PART 2

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Enzyme Kinetics

00:00:12

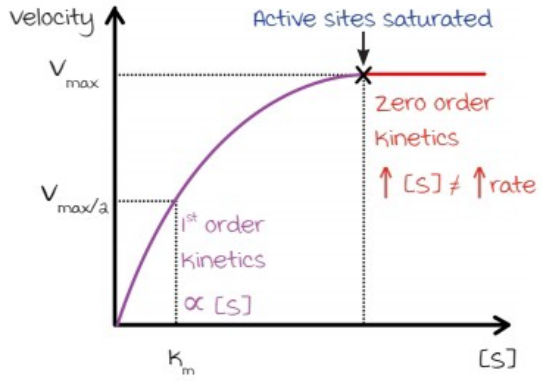
Equilibrium Constant (K_{eq}) :

Independent of enzyme action.

$$K_{eq} = \frac{[Products]}{[Substrates]}$$

Factors Affecting Rate of Reaction :

1. Substrate concentration :

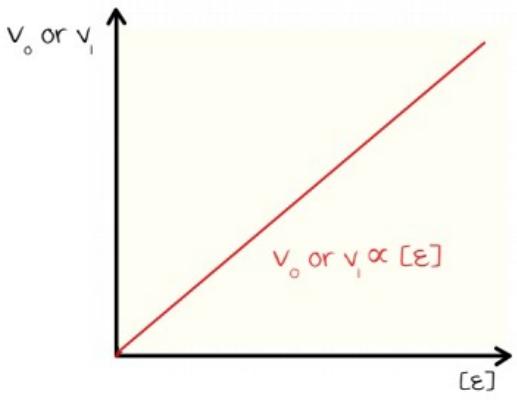


- Hyperbolic curve.
- Michaelis Menten equation :

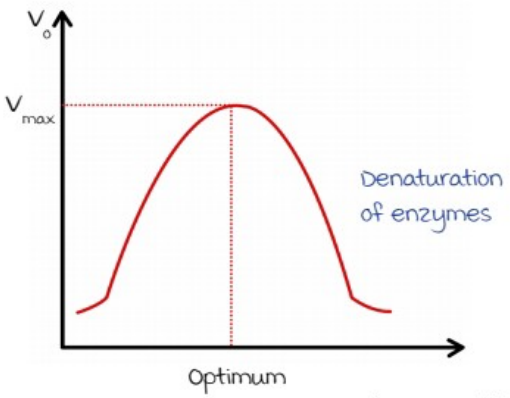
$$V_i = \frac{V_{max} \times [S]}{K_m + [S]}$$

- K_m (Michaelis constant) :
  - [S] at $V_{max}/2$.
  - $\propto \frac{1}{\text{Enzyme affinity to substrate}}$
- Ideal substrate : $\downarrow K_m$.

2. Enzyme concentration :



3. Temperature & pH :



- Temperature (35 to 40 °c).
- pH (5 to 9).

- Q_{10} : 10°c ↑ = 2 x rate of reaction.

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Catalytic Constant :

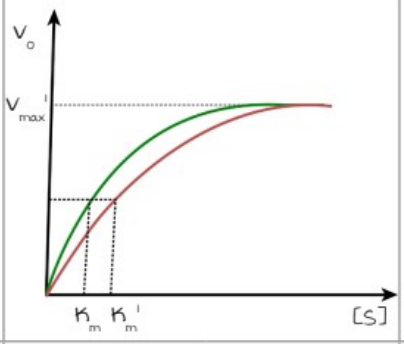
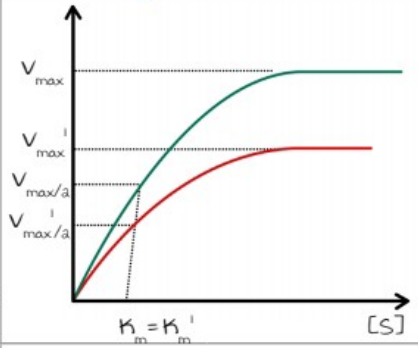
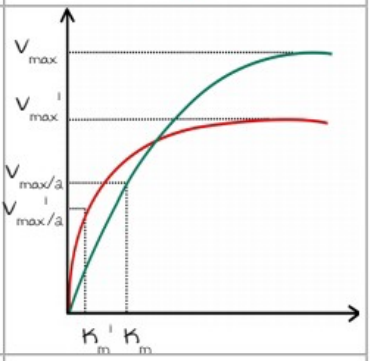
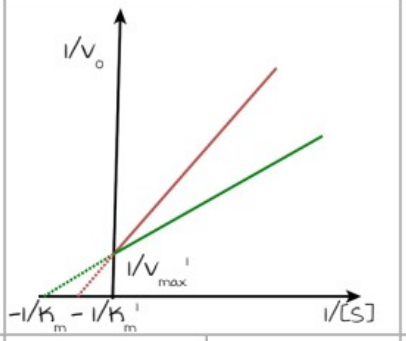
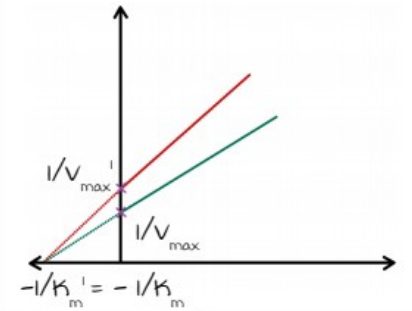
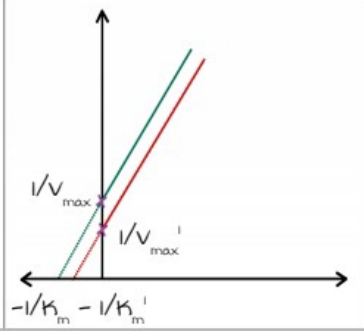
AKA turnover number.

$K_{cat} = \frac{[V_{max}]}{[\varepsilon_t]}$; ε_t = Total enzyme concentration.

Catalytic efficiency = $\frac{K_{cat}}{K_m}$

Enzyme Inhibition

00:12:00

	Competitive inhibition	Non-competitive inhibition	Uncompetitive inhibition
Features	• Inhibitor : Structural analogue of substrate • Compete for same site as substrate	• Inhibitor : Not a structural analogue • Distinct binding site • Usually irreversible	Inhibitor binds to enzyme-substrate complex.
Effect on V_{max} & K_m			
Line weaver Burk plot			

Examples

Inhibitor	Enzyme	Inhibitor	Enzyme	
Methotrexate	Dihydrofolate reductase	Cyanide CO	Cytochrome c oxidase	Phenylalanine inhibits placental alkaline phosphatase
Statins	HMG CoA reductase	Fluoride	Enolase	
Dicumarol	Vit K epoxide	Iodoacetate	Glyceraldehyde 3-P-DH	
Ethanol	Alcohol DH	Fluoroacetate	Aconitase	
Malonate (Poison)	Succinate DH			

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Applied biochemistry

1. Folic acid supplemented when patient is on methotrexate.
2. Dicumarol : Anticoagulant.
   - Inhibits Vit. K dependent γ carboxylation of clotting factors II, VII, IX & X.
3. Hooch tragedy : Methanol poisoning $\xrightarrow{\text{Antidote}}$ Ethanol (Competitively inhibits formaldehyde).
4. Fluoride oxalate used in estimation of blood glucose (Gray tube).

Suicide Inhibition :

Unreactive inhibitor $\xrightarrow{\text{Binds to enzyme}}$ Reactive inhibitor (Irreversible).

Suicide inhibitor	Enzyme
Allopurinol	Xanthine oxidase
Difluoromethyl ornithine	Ornithine decarboxylase
Aspirin	Cyclooxygenase

Regulation of Enzyme Activity 00:22:53

Enzyme Quantity :

- Heme $\xrightarrow{\text{Represses}}$ ALA synthase gene.
- Dietary cholesterol $\xrightarrow{\text{Represses}}$ HMG CoA reductase gene.

Porphyria : Take detailed drug history

Drugs metabolized by cytochrome

↓

↓Heme levels

↓

↑ALA synthase (Gene induction)

↓ Accumulation of intermediates

Aggravates porphyria.

Covalent Modification :

1. Zymogen activation (Irreversible) :
   - Gastrointestinal enzyme (Eg : Trypsinogen → Trypsin).
   - Clotting factors.