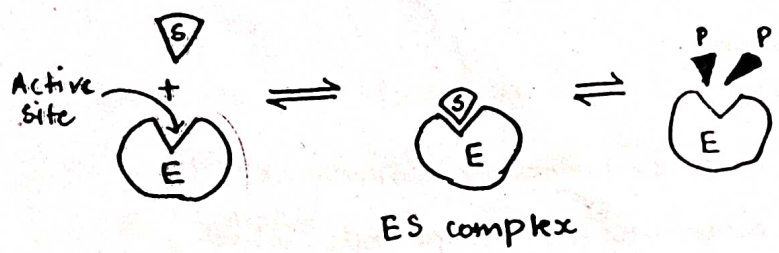


CHAPTER-1 Structure of enzyme : methods to determine amino acid residues at active site [Physical & Chemical]

- ~ Enzyme catalysed rxns take place within the confines of a pocket on the enzyme → ACTIVE SITE
- ~ The molecule that is bound in the active site and is acted upon by the enzyme → SUBSTRATE



Bonds formed between E & S are weak bonds - van Der Waals forces, hydrogen bonds, electrostatic attraction etc

- ~ Active site is made up of only a small portion of the total volume of enzyme.
- ~ Is lined by a.a residues with substituent grps that bind to the substrate & catalyze its chemical transformation.
- ~ Active site is usually at or near the surface of enzyme as it must be available for substrate molecules to bind & react.
- ~ The active site includes both polar & non polar amino acids residues → gives rise to arrangement of hydrophilic & hydrophobic microenvironment not found elsewhere on enzyme molecule.
- ~ Also, amino acid residues involved in active site architecture may actually be widespread in primary structure, only brought together because of secondary & tertiary structure of protein. ∴ Catalytic function of enzyme depends upon 3-D arrangement of binding & catalytic residues and the microenvironment in which these residues occur.
- a.a not involved in catalytic action, are/may be involved contributing to shape of active site.

ENZYMODOLOGY - Introduction & Revision

1) Enzymes - proteins
made up of RNAs

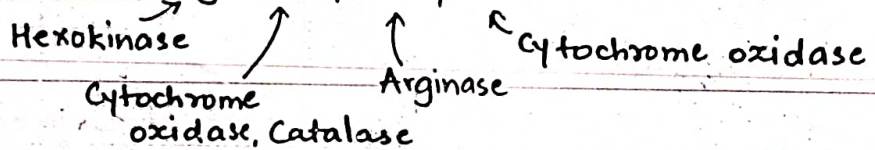
Exception: Riboenzymes or Ribozymes - are

2) Are biological catalysts - essential for biochemical functioning of cell & overall metabolism.

3) Are high molecular weight proteins

4) May or may not be associated with cofactors or coenzymes. [are inorganic or organic small molecular weight compounds which assist enzymes during catalysis.]

- Cofactor - inorganic ions - Mg^{+2} , Fe^{+2} , Mn^{+2} , Cu^{+2}



- Coenzymes :- complex organic or metalloorganic molecule

eg: Thiamine pyrophosphate, Flavin adenine dinucleotide (FAD)

- Enzymes may require both cofactor & coenzymes or any one.

- When covalently attached to enzyme \rightarrow PROSTHETIC GROUP.

\rightarrow Apoenzyme + Cofactor/Coenzymes \rightarrow Holoenzyme

5) Enzymes - proteins - made up of amino acids

Primary structure (2D) - amino acid chain $[a_1 - a_2 - a_3 \dots a_n]$

Secondary structure (2D) - Folding \rightarrow non covalent interactions

Tertiary structure (3D) - further folding \rightarrow " \rightarrow

Quaternary structure (3D) - may or may not be present \rightarrow

↑ subunit 1 ↓ subunit 2

Enzymes → are long chains of amino acids w are held together with peptide bonds.

- Linear structures fold in 3-D pattern to form globular or fibrous structures

→ water insoluble, structural protein
Ex: keratin

↓
water soluble, functional proteins
Ex: (Hb)

- Most of the water loving [hydrophilic] groups of constituent amino acid are displayed on surface of globular / fibrous protein whereas, water hating [hydrophobic] groups are embedded.

1) Enzymes are classified in six major classes:-

1) Oxidoreductases → transfer of H^+ ion.

2) Transferases → Group transfer reactions.

3) Hydrolases → hydrolysis reaction [use H_2O to cleave chemical bonds].

4) Lyases → forms a double bond either by addition of groups or removal of groups.

5) Isomerases → transfer of groups within molecules to yield isomeric forms.

6) Ligases → catalyze joining of molecules by forming a new chemical bond, coupled to cleavage of ATP or similar cofactor.

7) Translocases - newly added class of enzyme - enz w move ions or molecules across membranes

8) Ribozymes :- [ribonucleic acid enzymes] are capable of catalyzing

specific biochemical reactions similar to action of protein enzymes.

eg: ribosome / hammer head ribozyme

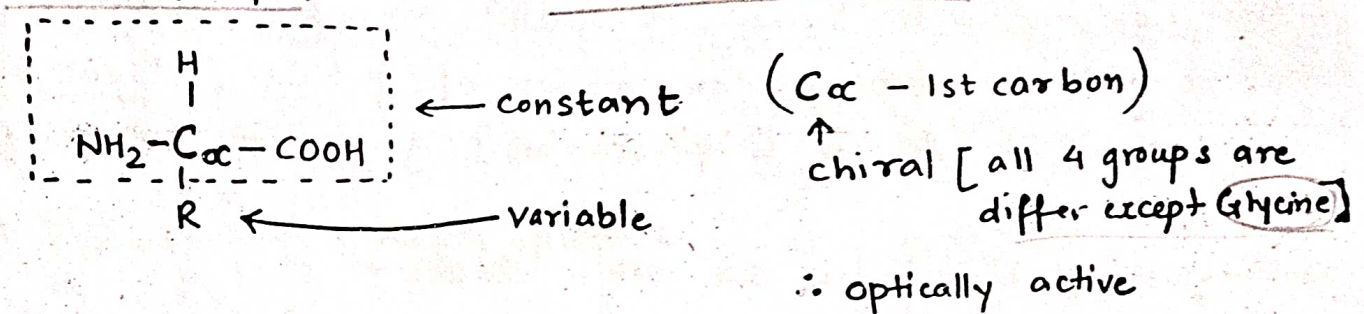
RNA world hypothesis → ∴ RNA can act as genetic material and

can have catalytic functions, RNA may have been important

in evolution of pre-biotic self replicating systems

AMINO ACIDS - OVERVIEW

- 20 naturally occurring amino-acids
- are organic compounds w contain an amino group (-NH₂) and carboxyl group (-COOH) within the same molecule.

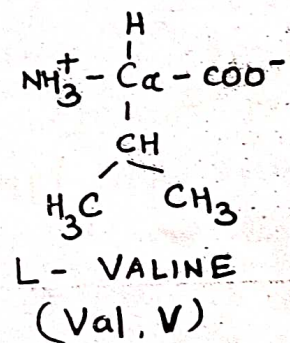
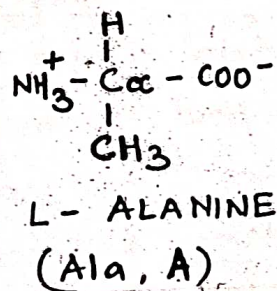
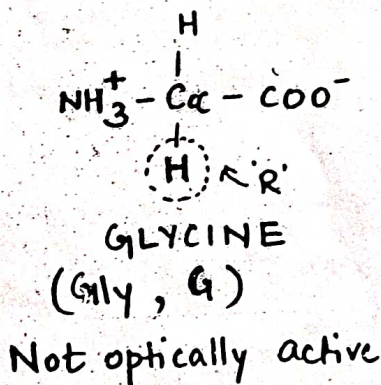


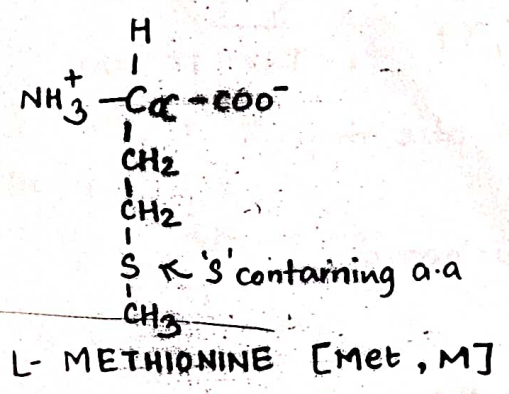
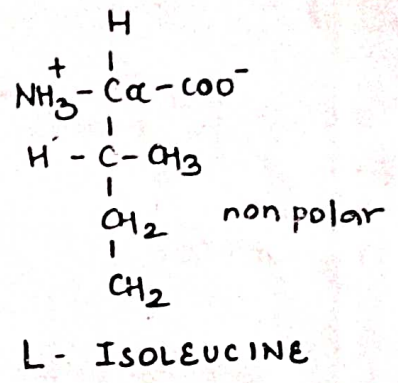
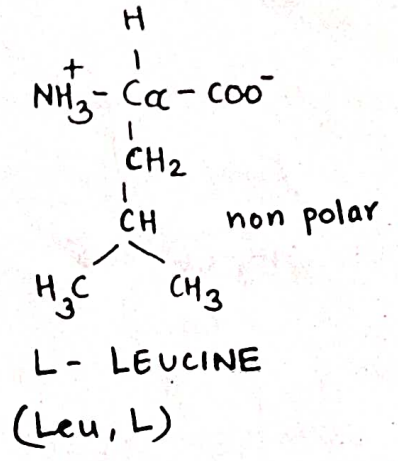
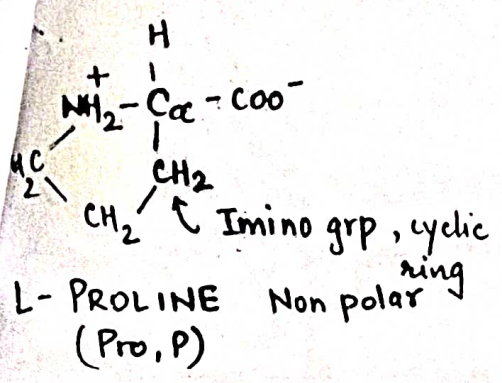
Amino acids → L- stereoisomers!

Sugars → D

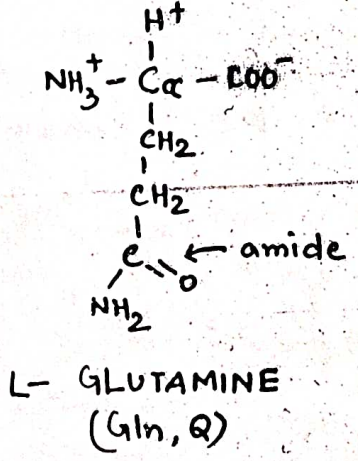
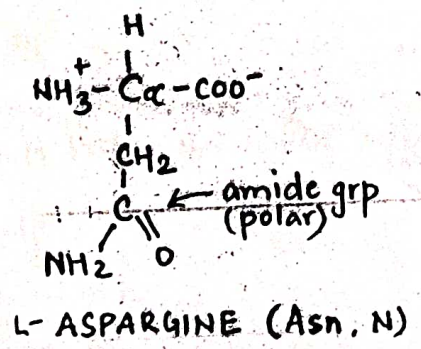
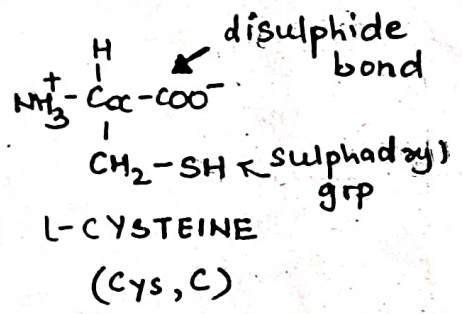
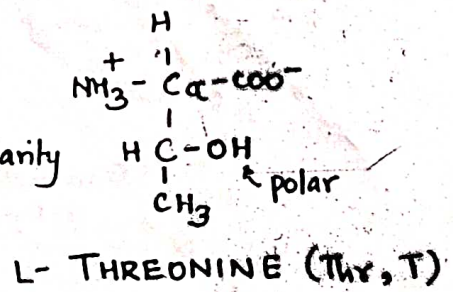
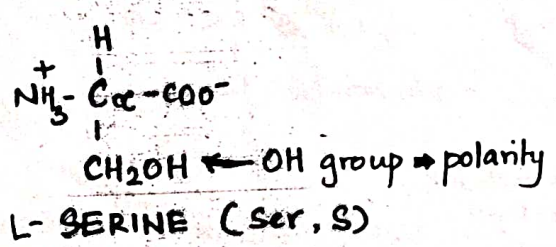
- Depending upon the properties of "R" group a.a can be classified as :-
- (1) Polar or non polar
 - (2) Aliphatic or aromatic
 - (3) Charged (Acidic/Basic) or Uncharged or neutral

Non polar aliphatic 'R' groups :- (Non polar/hydrophic)

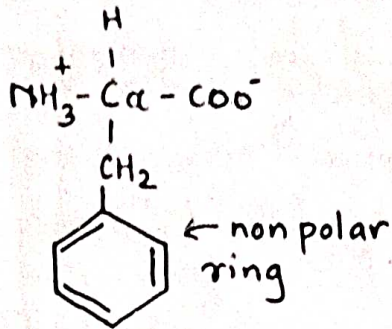




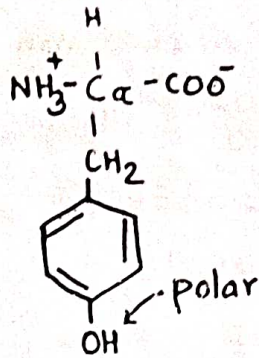
POLAR, UNCHARGED 'R' group:-



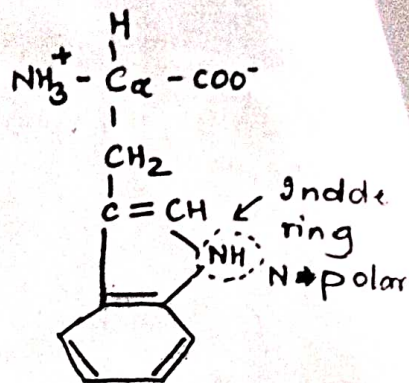
AROMATIC R groups :-



L-PHENYLALANINE (Phe, F)



L-TYROSINE (Tyr, Y)



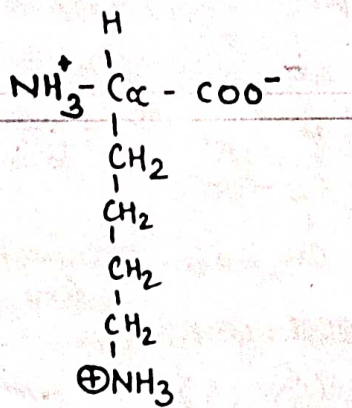
L-TRYPTOPHAN [Trp, W]

All aromatic a.a absorb UV light due to aromatic ring structures. (~280 nm)

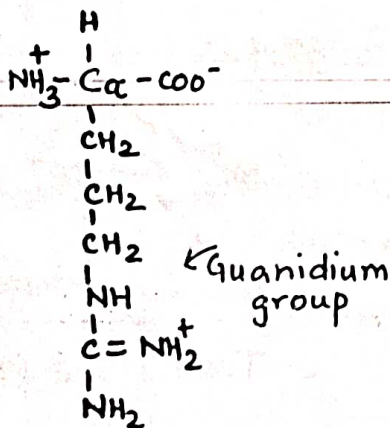
Trp > Tyr > Phe

[absorbance]

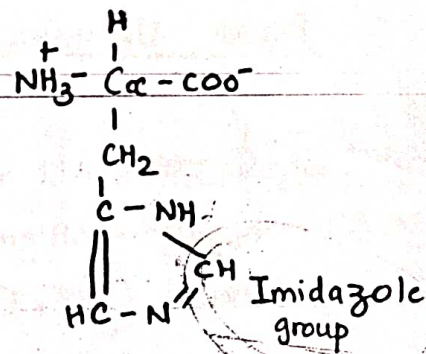
POSITIVELY CHARGED 'R' GROUPS → 'R' grps have positive charge at pH 7



L-LYSINE (Lys, K)

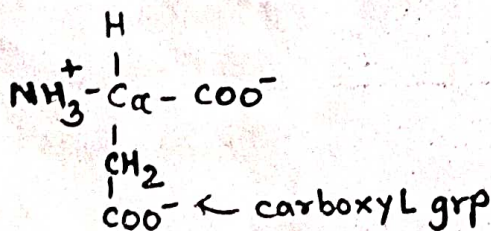


L-ARGININE (Arg, R)



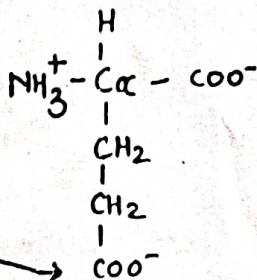
L-HISTIDINE (His, H)

NEGATIVELY CHARGED R GRP :- pH=7, 'R' grps have negative charge



ASPARTATE (Asp, D)

Aspartic Acid



GLUTAMATE (Glu, E)

Glutamic Acid

→ Essential amino acids → Human diet Lysine, Methionine, Threonine, Phenylalanine, Tryptophan, Valine, Leucine, Isoleucine, Histidine, Arginine

PVT TIM HALL

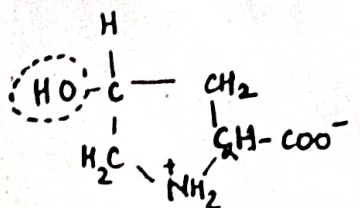
→ Uncommon amino acids → > 300 a.a ; are derivatives of natural amino acids.

Eg: 4-hydroxyproline - plant cell wall protein, collagen

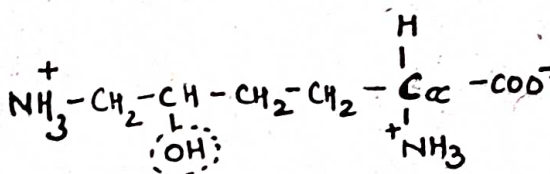
5-hydroxylysine - collagen

γ -carboxyglutamate - constituent of prothrombin

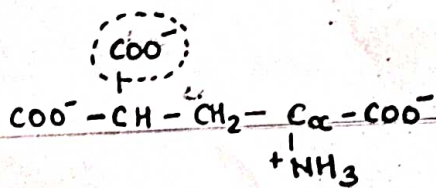
6-N-Methyllysine - constituent of myosin



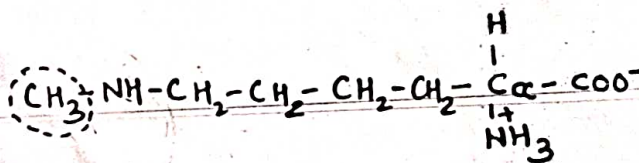
4-hydroxyproline



5-hydroxylysine



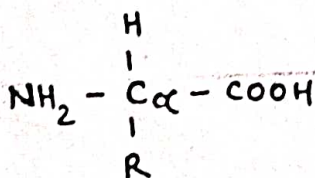
γ -carboxyglutamate



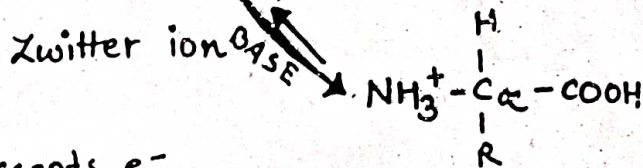
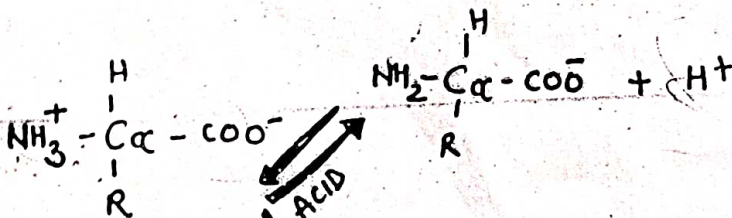
6-N-Methyllysine

→ Amino acids can act as Acids & Bases ~ weak acid (COO^-)
weak base (NH_3^+)

When a.a lacking ionizable 'R' grp is dissolved in water at neutral pH → it exists as Zwitterion (dipolar ion) ∴ can behave as acid &/or base.



Non-ionic form of a.a



Acid - donates H^+ / accepts e^-

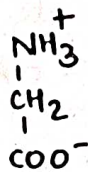
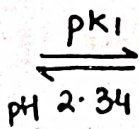
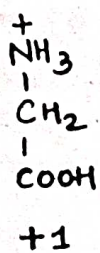
Base - accepts H^+ / releases OH^-

→ pKa & pI of a.a

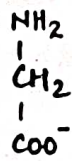
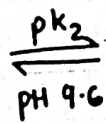
pKa is a measure of the tendency of a group to give up a proton.

pI - isoelectric point is the pH at which the a.a has a neutral charge.

* Glycine



Neutral



-1

Titration curve
against base

$$\text{pI} = \frac{\text{pK}_1 + \text{pK}_2}{2}$$

Buffering zone : ± 1 unit of pKa values

∴ for Glycine

2.34 ± 1 unit of pH

9.60 ± 1 unit of pH

} can be used as
buffer