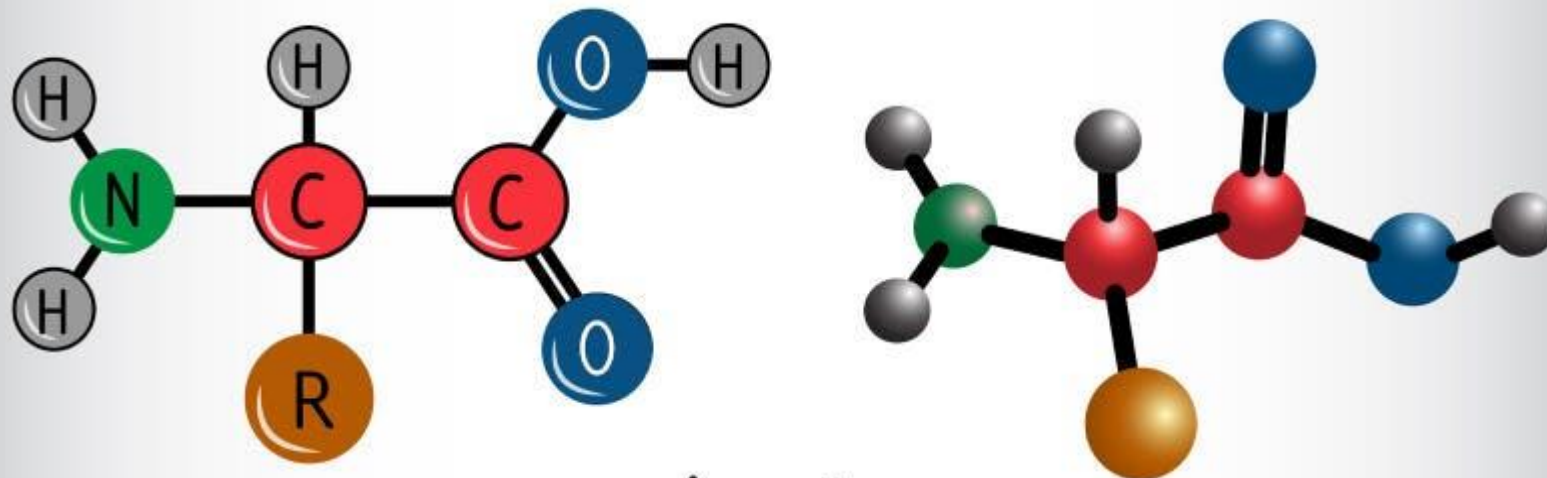
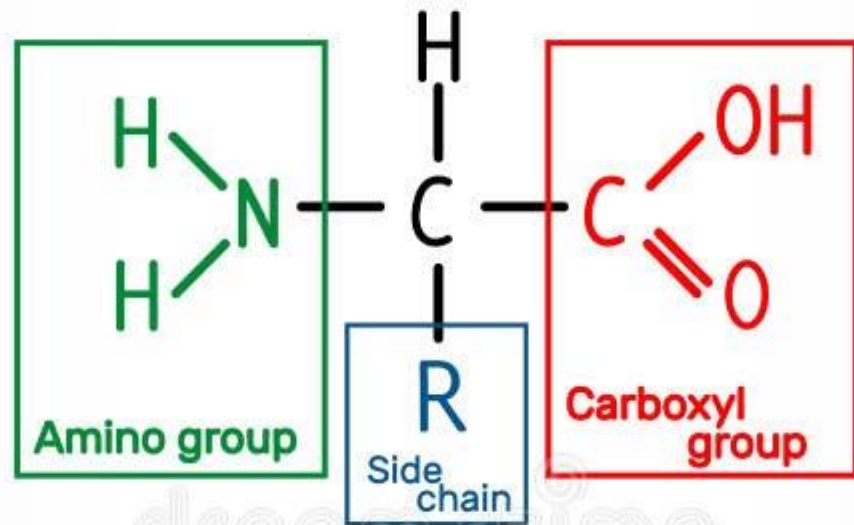


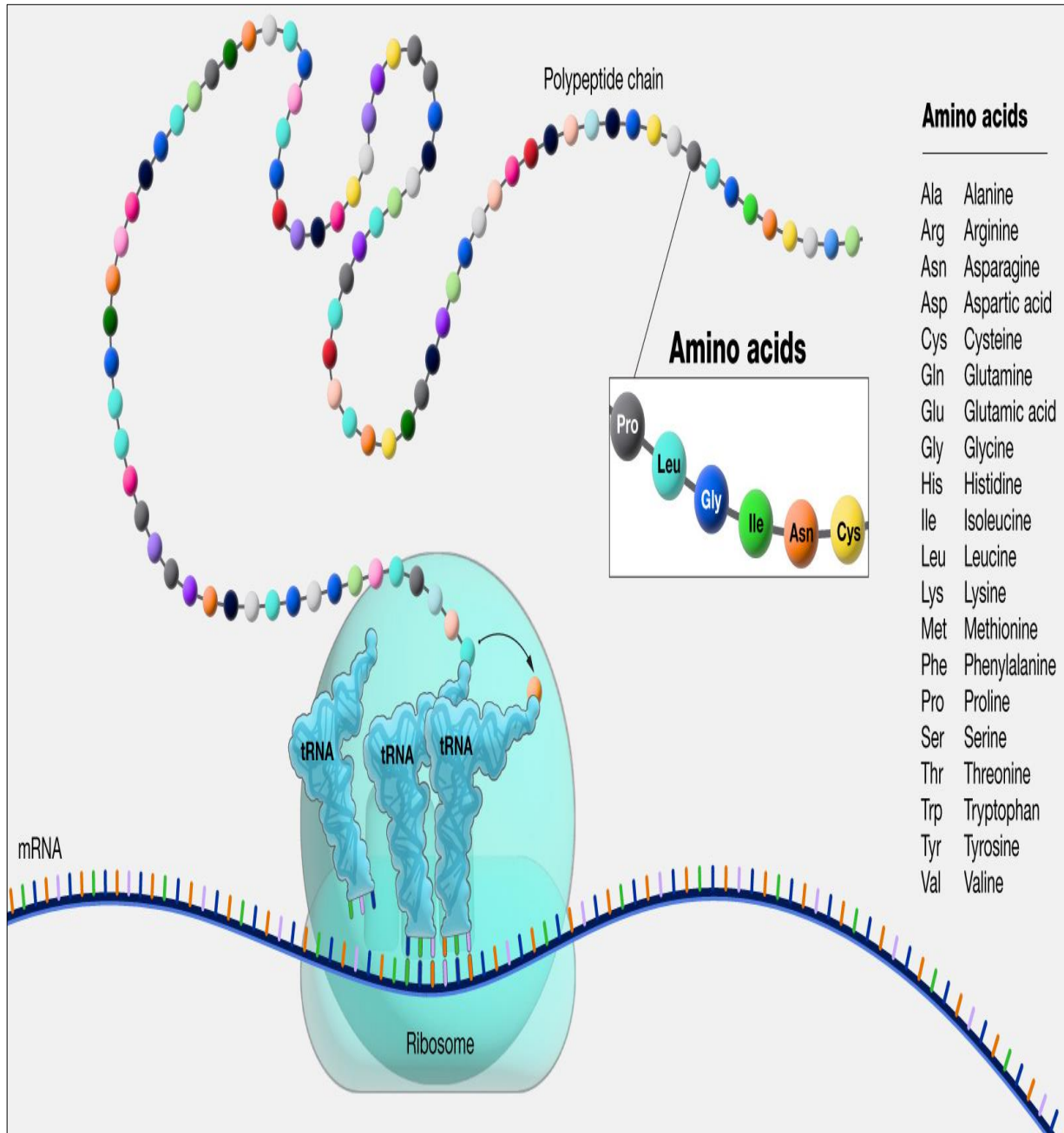
Amino acids



COURSE Fundamentals of Biochemistry

Unit: 2

Ms. Sarojmoni Sonowal
Assistant Professor
Department of Zoology
Pub Kamrup College

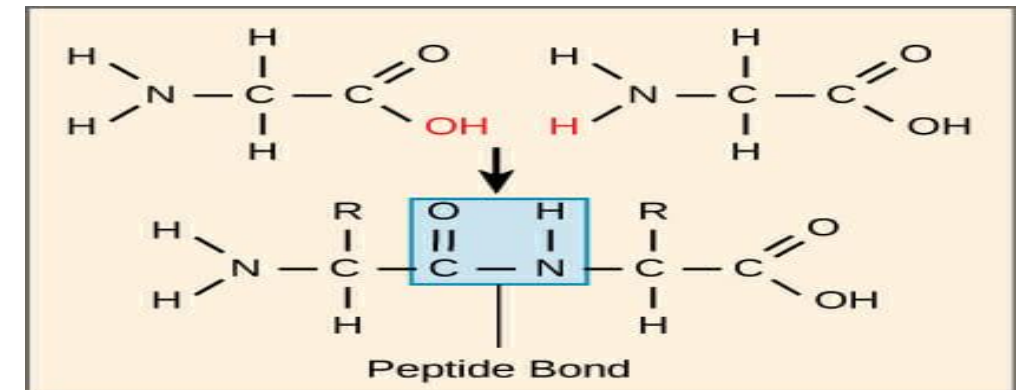


AMINO ACIDS

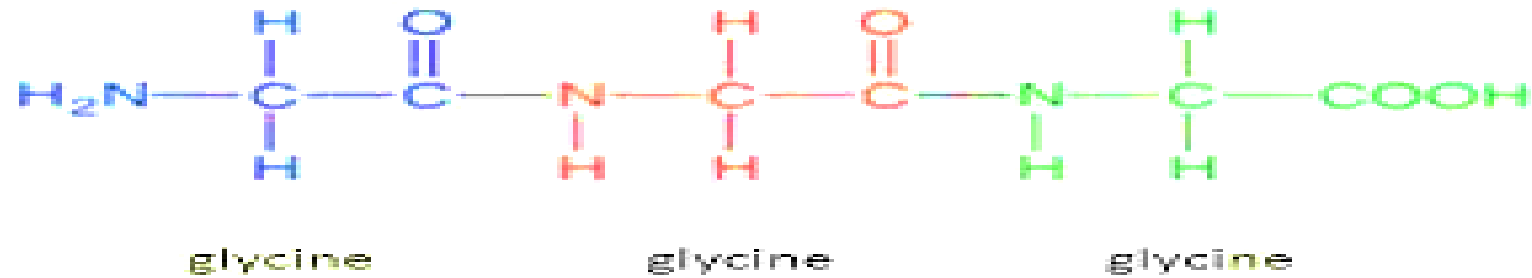
What are amino acids?

Amino acids are **organic compounds** containing an amino group ($-\text{NH}_2$), a carboxyl group ($-\text{COOH}$), a hydrogen atom and a variable side chain (R group) attached to the same α -carbon.

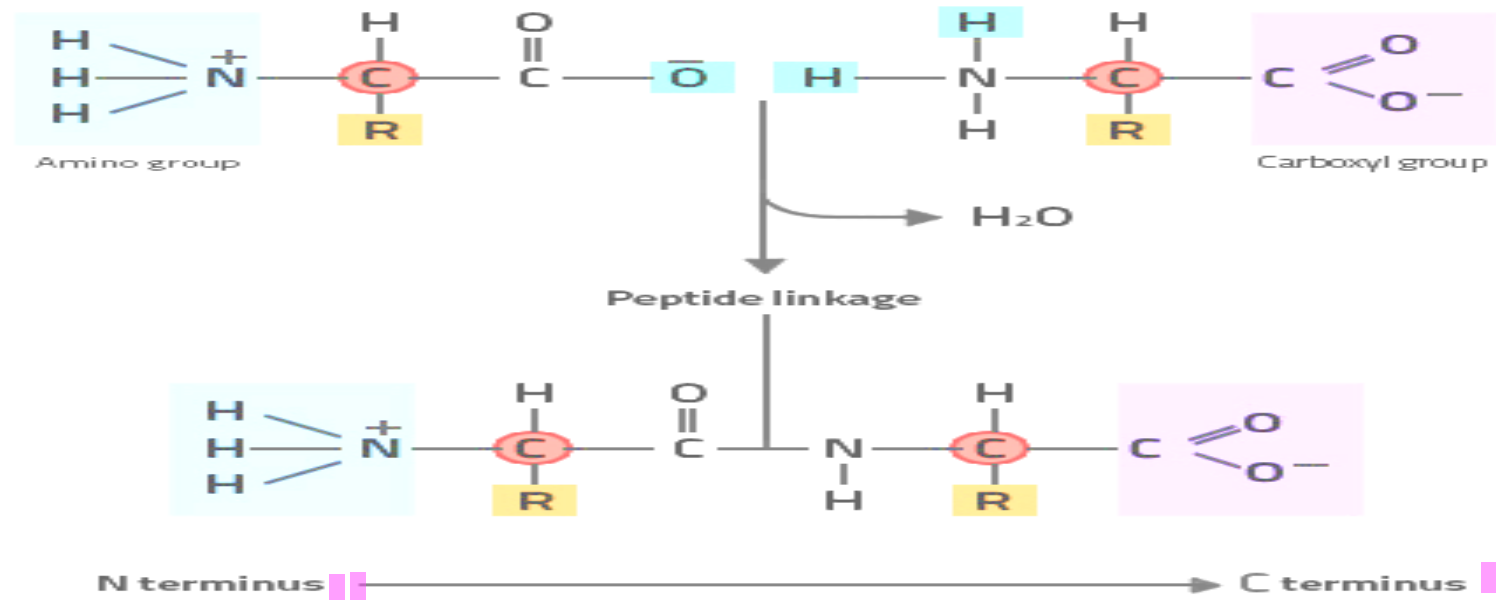
- Amino Acids are **the building units of proteins**
(Proteins are polymers of amino acids)
- Each amino acid residue linked to its neighbor by a specific type of covalent bond ---“**Peptide bond**”.



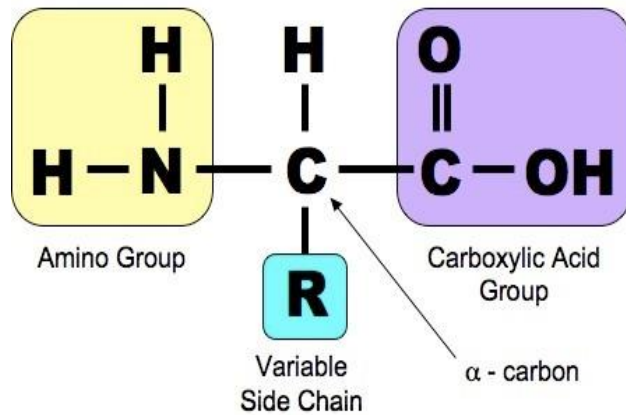
Tripeptide



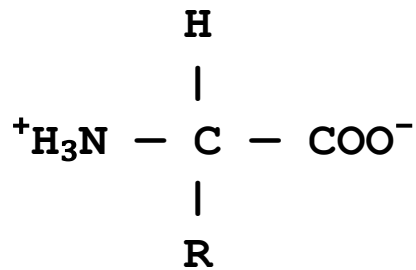
Peptide bond



The general structure of an α -amino acid is:



At physiological pH, the structure is usually present as:



The central carbon is called the **α -carbon (C_α)**.

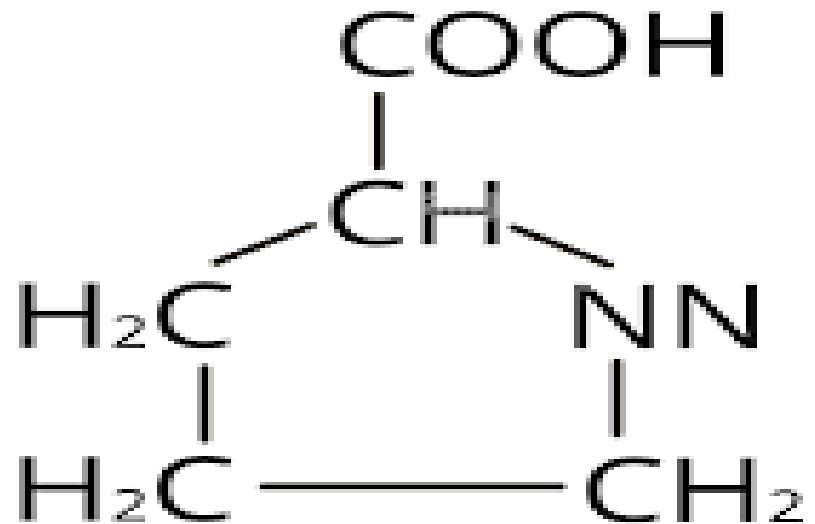
Four groups attached to C_α

1. Amino group
2. Carboxyl group
3. Hydrogen atom
4. Variable R group

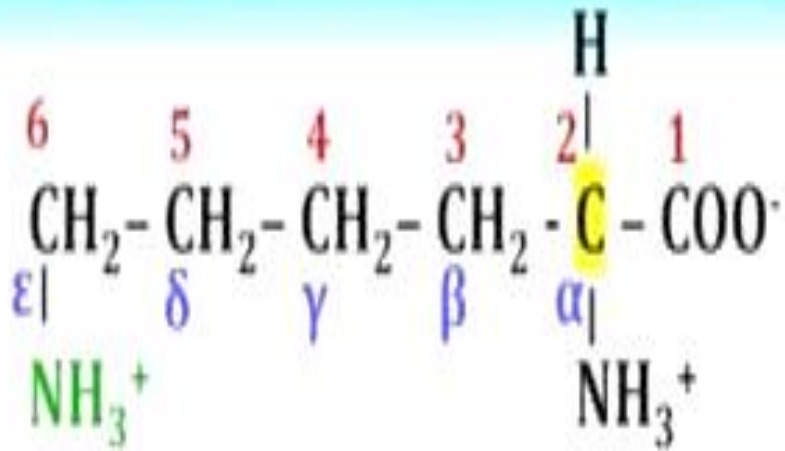
The **R** group determines the chemical properties of the amino acid.

**General Structure of amino acid
is common to all except one of the
amino acid
(i.e. **PROLINE**)--- EXCEPTION**

Proline



Amino acids: Numbering



The α -carbon

The carbon to which the amino and carboxyl groups are directly attached is called the **α -carbon**.

The **additional carbons in an R** group are commonly designated as:

Beta,
gamma,
delta,

epsilon and so forth proceeding out from the alpha carbon.

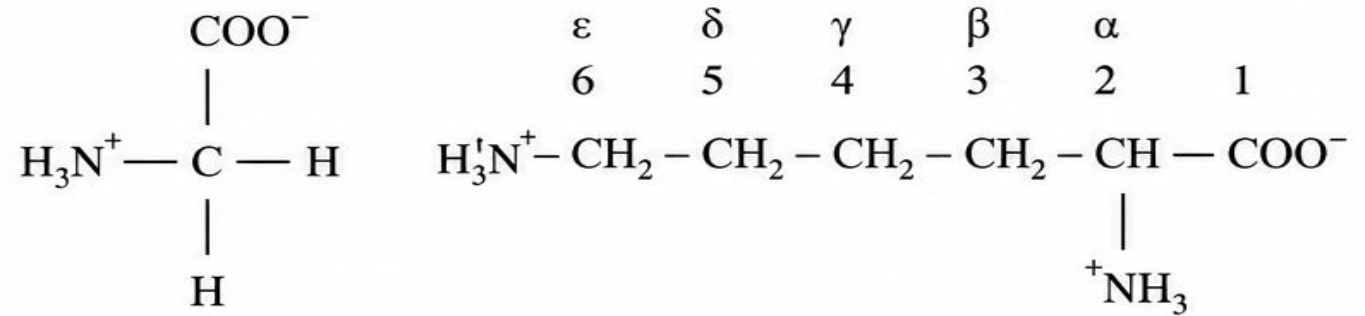


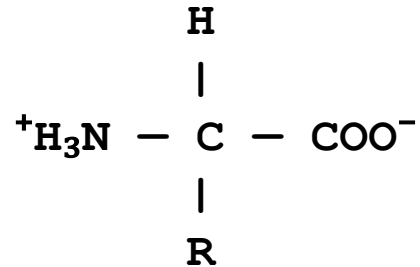
Figure: Structure of glycine and lysine.

In α -amino acids both the amino group and the carboxyl group are attached to the same carbon atom. However, many naturally occurring amino acids not found in protein, have structures that differ from the α -amino acids.

In these compounds the amino group is attached to a carbon atom other than the α -carbon atom and they are called β , γ , δ , or ϵ amino acids depending upon the location of the C-atom to which amino group is attached.

Basic structure of amino acids

Most amino acids found in proteins have the same basic structure.

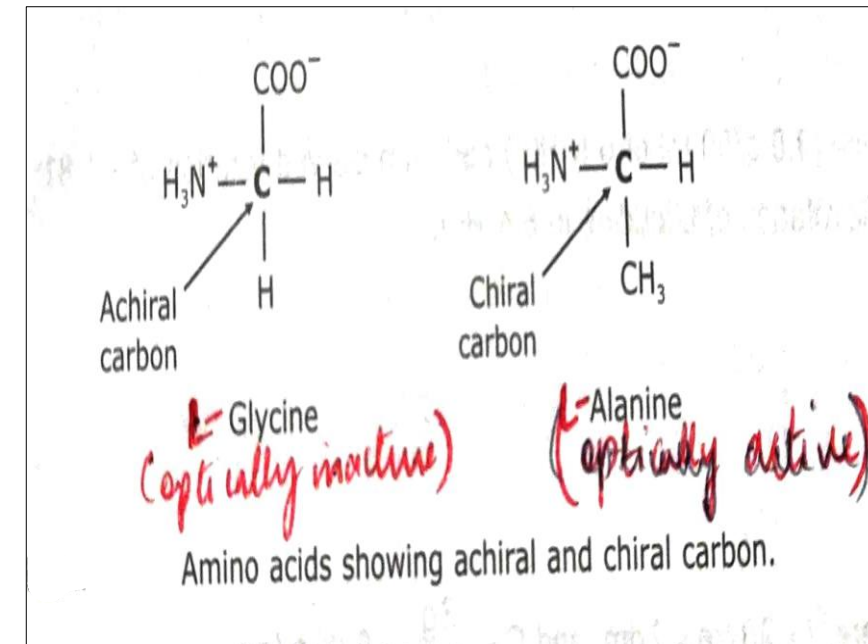
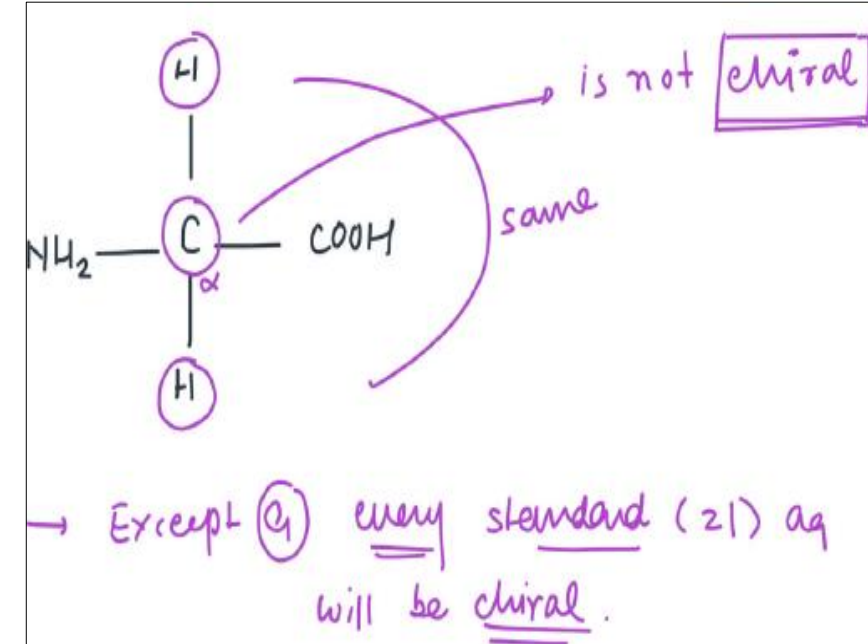


R group is referred to as the **side chain**.

The only major difference among the standard amino acids is the **R group**.

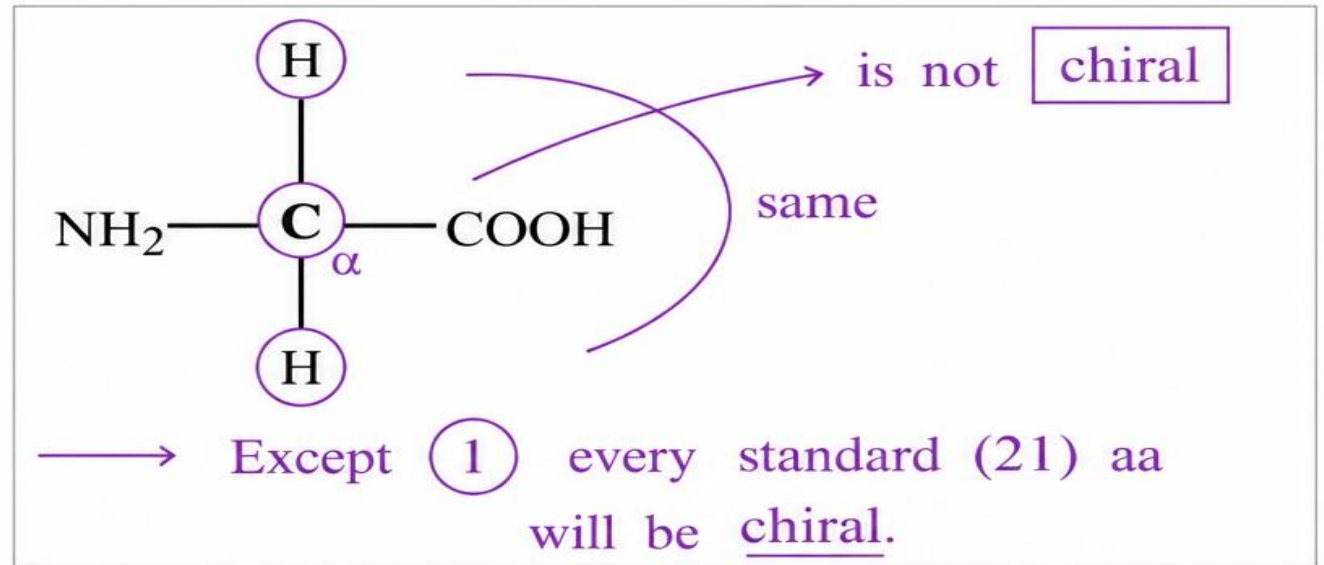
The R group determines: Size, Shape, Charge, Polarity, Hydrophobicity & Chemical reactivity

Since 4 different groups are attached to the α -carbon atom (which is C-atom next to COOH) – These AMINO ACIDS ARE called **CHIRAL AMINO ACIDS**. Except one standard amino acid--- **GLYCINE**

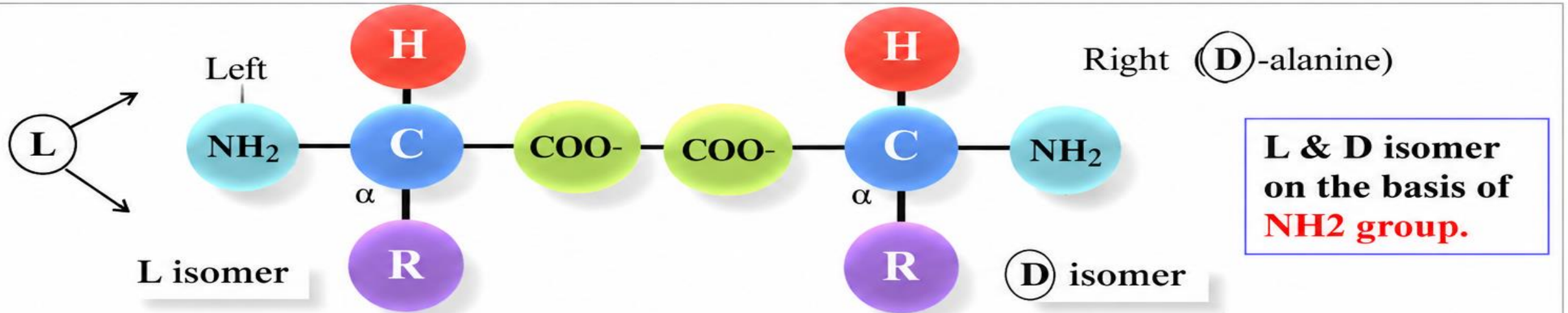


Amino acids are chiral

Except for glycine, amino acids have a chiral α -carbon.



They therefore exist as stereoisomers (L & D isomer)



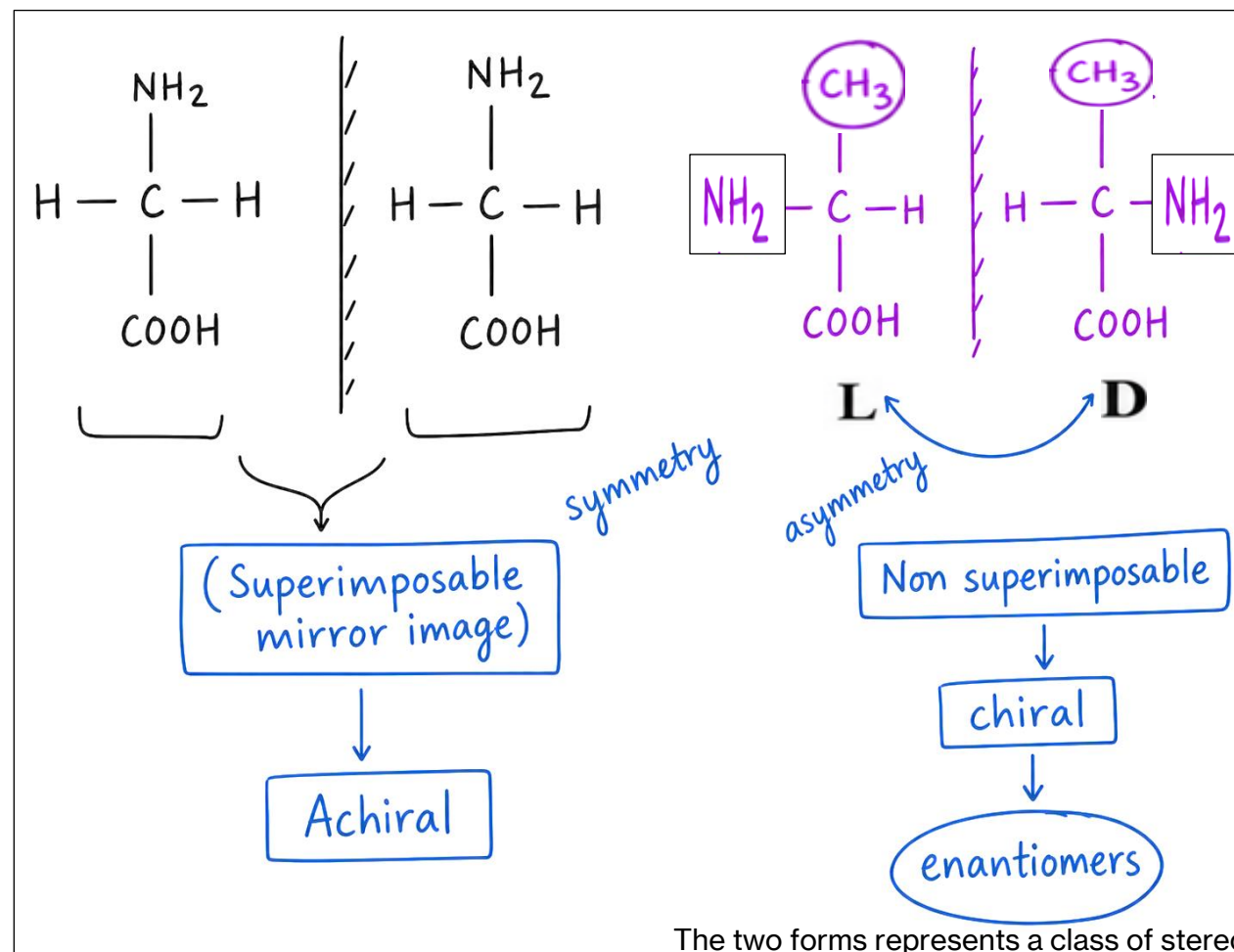
➤ Only L isomer amino acids are constituents of proteins.

⇒ D – aa → through post translational modification

CHIRAL CENTRES GIVE RISE TO ENANTIOMERS

Optically active molecules are asymmetric; that is, they are not superimposable on their mirror image in the same way that a left hand is not superimposable on its mirror image, a right hand

Molecules that are nonsuperimposable mirror images are known as enantiomers of one another



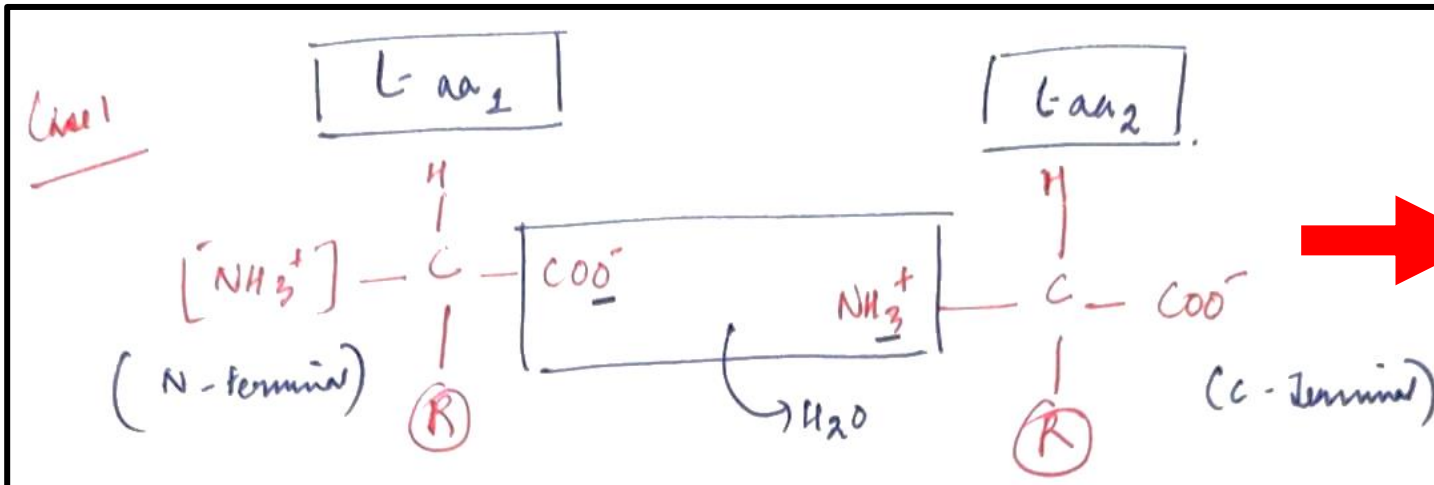
The two forms represents a class of stereoisomers called enantiomers

Ex: Glycine

Ex: Alanine

Biological proteins almost exclusively contain: **L-amino acids**

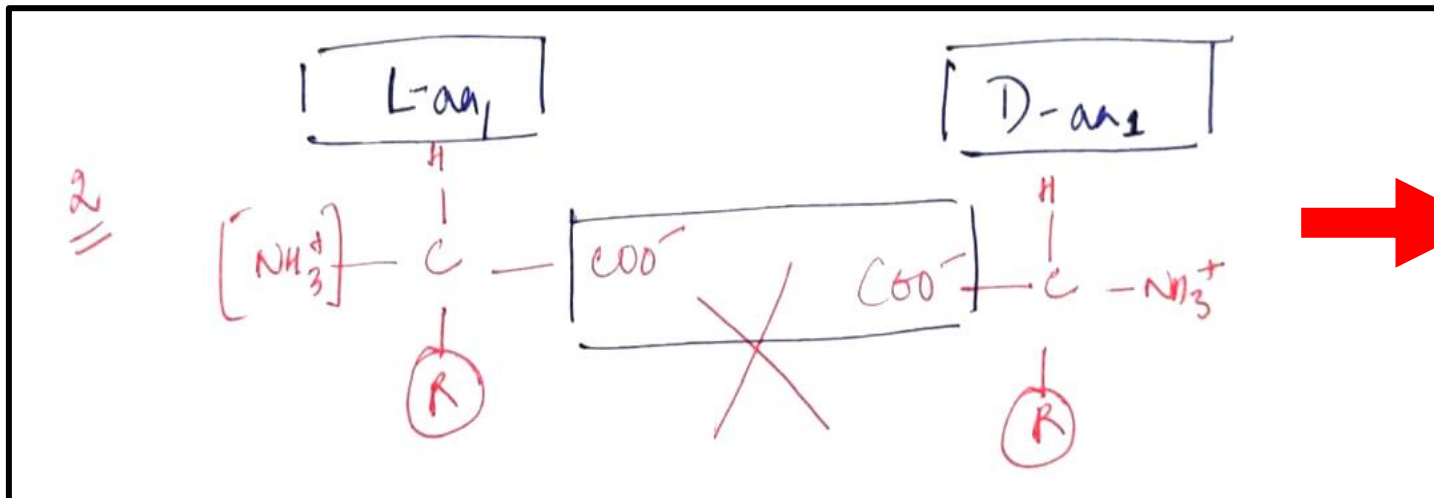
Although both D- and L-amino acids can exist chemically, the ribosomal protein-synthesis machinery incorporates essentially **L-amino acids** into proteins.



1) The **carboxyl group** ($-COO^-$) of the first L-amino acid reacts with the **amino group** ($-NH_3^+$) of the second. A molecule of water is removed: $-COOH + -NH_2 \rightarrow -CO-NH- + H_2O$

The resulting **$-CO-NH-$ linkage is the peptide bond**. This is the normal arrangement used by ribosomes to build proteins.

Direction of the chain:
N-terminus $\rightarrow$ C-terminus



2) A normal ribosome is **stereospecific**. Its **aminoacyl-tRNA synthetases** and **ribosomal machinery** are designed to recognize and incorporate **L-amino acids**.

Therefore, a D-amino acid is not normally charged onto the appropriate tRNA and incorporated into a ribosomal synthesized protein.

L-amino acid + D-amino acid $\rightarrow$ peptide bond can form chemically
but
L-amino acid + D-amino acid $\rightarrow$ not the normal pathway of ribosomal protein synthesis.

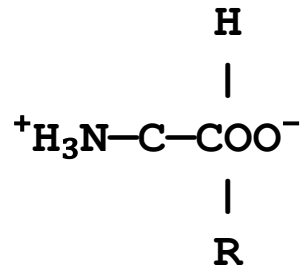
Amino acids as zwitterions

An amino acid contains both:

- acidic group $\rightarrow$ $-\text{COOH}$
- basic group $\rightarrow$ $-\text{NH}_2$

They can transfer protons internally.

At approximately neutral pH:



This molecule has:

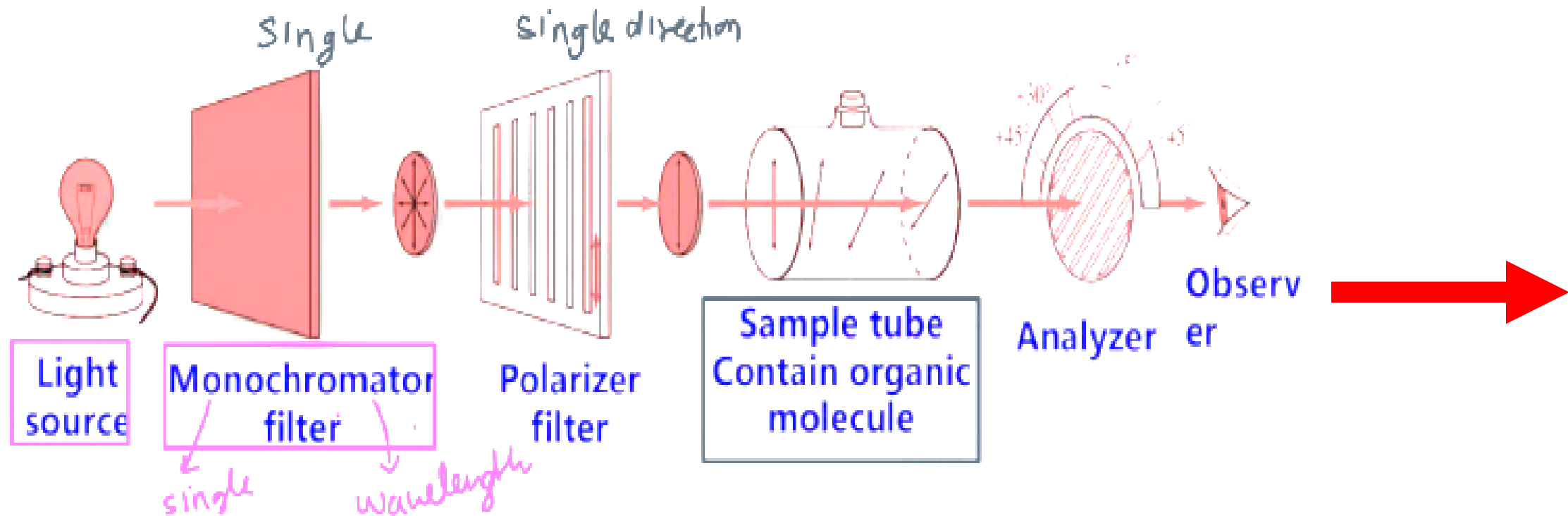
- Positive charge on amino group
- Negative charge on carboxyl group

But overall:
Net charge = 0

Such a molecule is called a:
Zwitterion

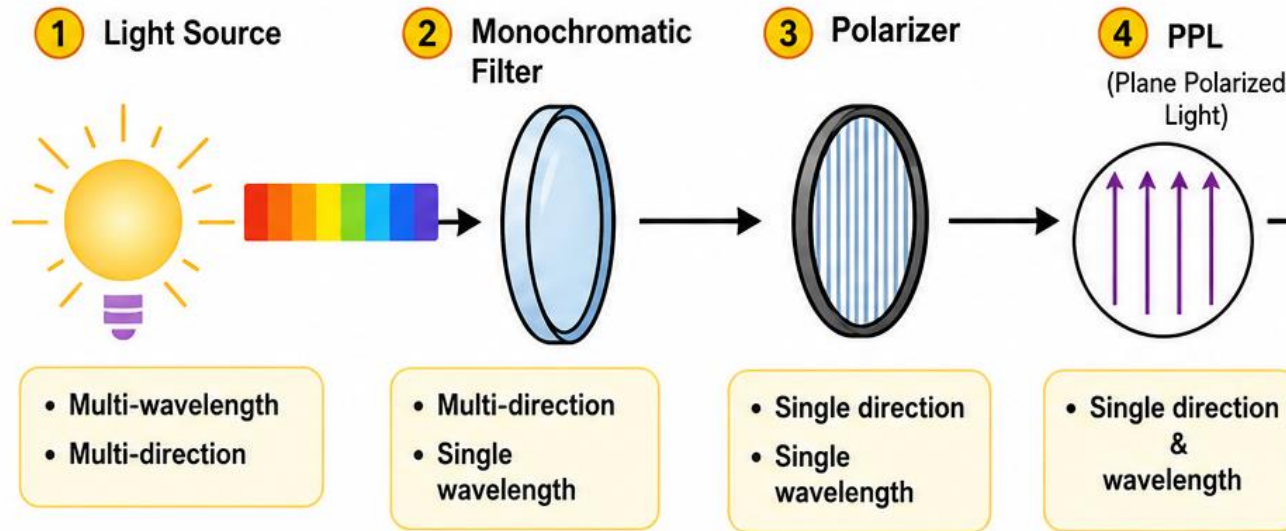
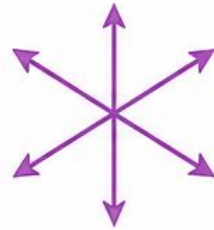
Optical Property of amino acid

- When a **plane polarized** light passes through a solution containing a single chiral compound, The chiral compound causes the plane of vibration to rotation .

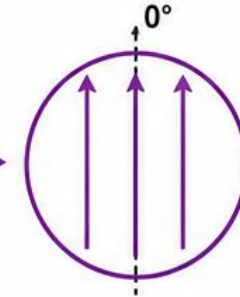


- Compound that rotate the plane of polarized light to the **right** (clockwise) are called **Dextrorotatory. (d)**
- Compound that rotate the plane of polarized light to the **left** (counter clockwise) are Called **levorotatory. (l)**

- 1 D-isomer → clockwise = d-isomer
 - 2 L-isomer → anticlockwise = l-isomer
- } Rotation

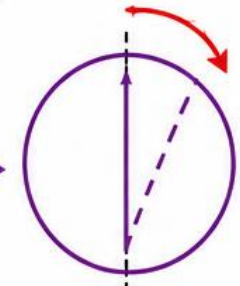


5A Achiral (Any) Sample



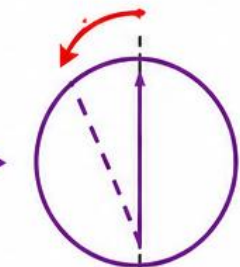
No Rotation

5B D-isomer (d) Sample



Clockwise Rotation
(d-isomer)

5C L-isomer (l) Sample



Anticlockwise Rotation
(l-isomer)

- 1 Light Source
Produces light of multiple wavelengths in multiple directions.
- 2 Monochromatic Filter
Selects a single wavelength of light; still in multiple directions.
- 3 Polarizer
Allows light in a single direction (single wavelength).
- 4 PPL (Plane Polarized Light)
Plane-polarized light travels in a single direction and single wavelength.

- 5A Achiral sample → No rotation of plane-polarized light.
- 5B D-isomer (d) → Clockwise rotation.
- 5C L-isomer (l) → Anticlockwise rotation.

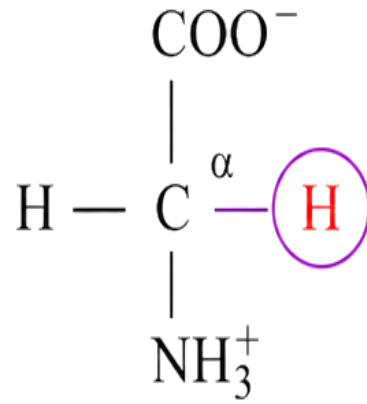
Amino acids

SINGLE LETTER CODE

Amino acids	Three-letter code	Single letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I

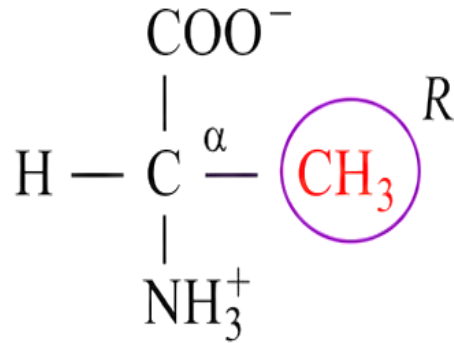
Amino acids	Three-letter code	Single letter code
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Glycine
Gly
G



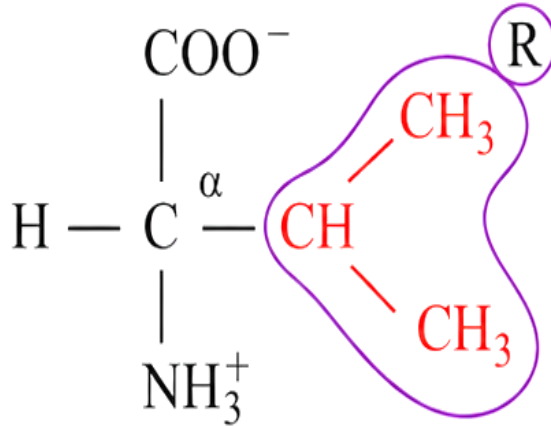
- simplest
- H (R)
- Achiral
- most flexible
- smallest
- simplest
- R- Nonpolar
- Hydrophobic

Alanine
Ala
A



- small aa.
- R – (CH₃) methyl → Nonpolar
- chiral
- Hydrophobic aa.

Valine
Val
V

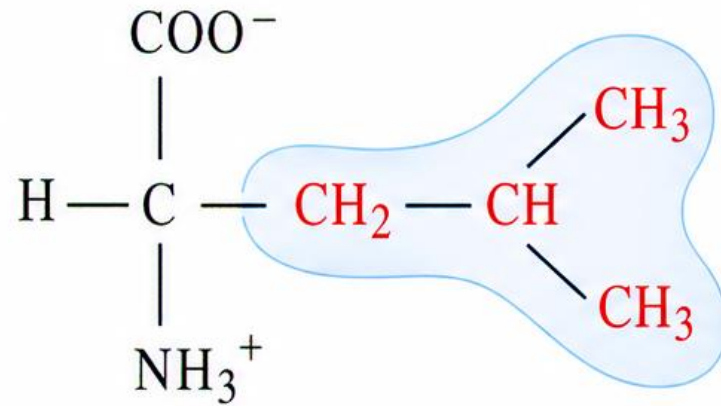


- small aa
- branched aa
- V- shaped (R)
- R → isopropyl
- chiral
- Hydrophobic

Leucine

Leu

L

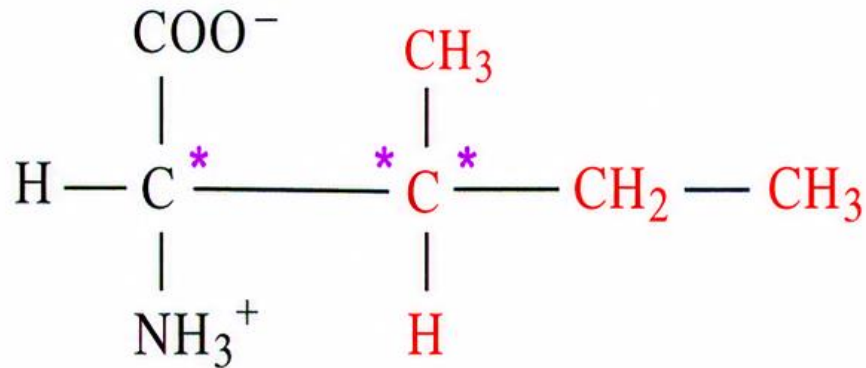


- R-group (γ -shaped)
- Chiral C (1 C*)
- Hydrophobic
- Branched aa.

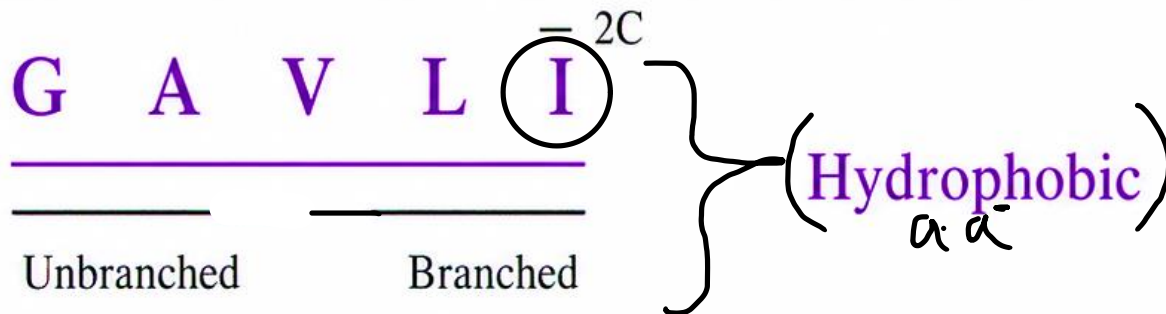
Isoleucine

Ile

I



- R-group: (L-shaped)
- Two chiral centre
- R-isobutyl group
- Hydrophobic
- Branched

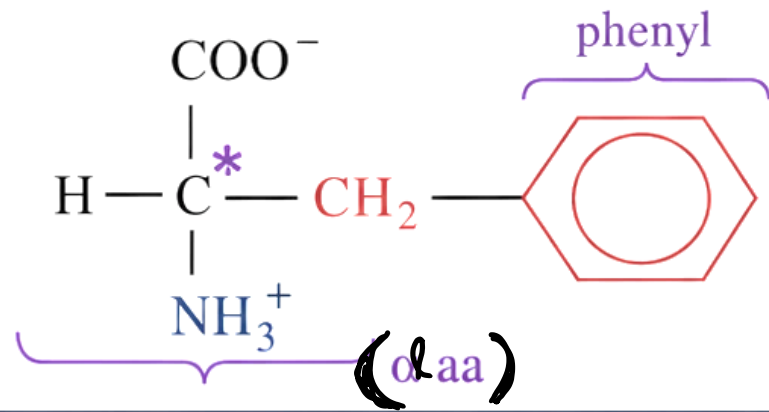


- C - 1 $\rightarrow$ methane
- C - 2 $\rightarrow$ ethane
- C - 3 $\rightarrow$ propane
- C - 4 $\rightarrow$ butane

Phenylalanine

Phe

F



- R → methyl benzene (phenyl)

- Aromatic aa.

- R → Non polar

- Hydrophobic

- Chiral centre = 1 (< C)

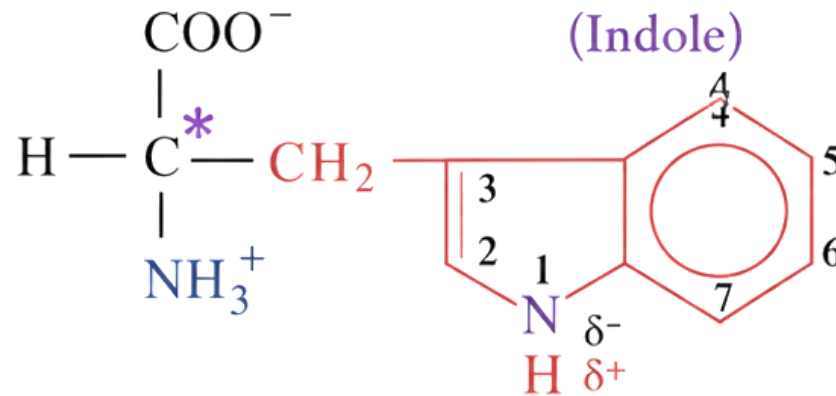
- Absorbance

$\lambda = 252 \text{ nm}$

Tryptophan

Trp

W



- R – Indole

- R → polar

- Hydrophilic

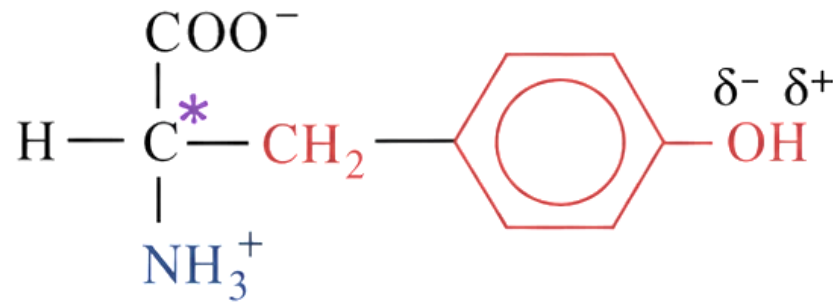
- Absorbance → $\lambda = 280 \text{ (max)}$

- 1 chiral centre

Tyrosine

Tyr

Y



- Phenol alanine (Tyrosine)

- R – Phenol (pK_a = 10.46)

- R – polar

- Hydrophilic

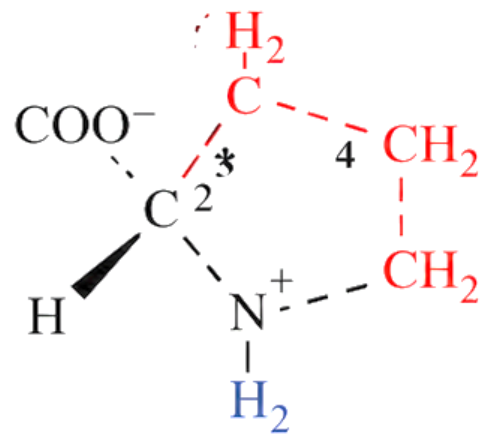
- Absorbance → $\lambda = 274 \text{ nm}$

- 1 chiral centre

Aromatic aa.

Proline

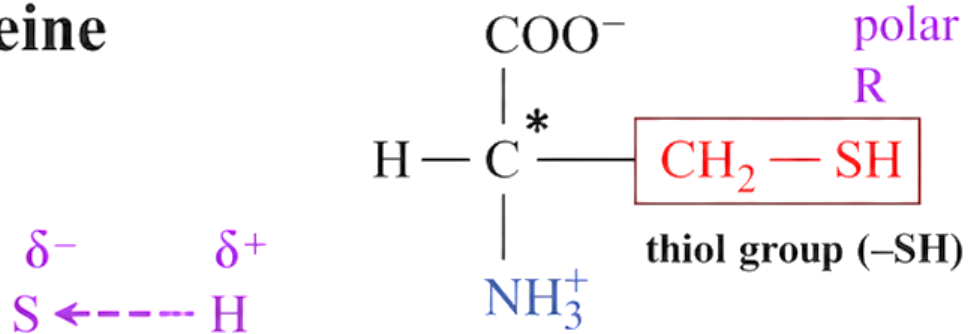
Pro
P



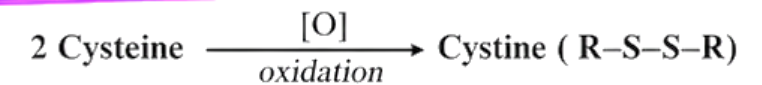
- 2' amino (imino group)
- Imino acid
- Cyclic
- R- No polar str. (Hydrophobic)
- Most rigid aa.
- Chiral centre = 1

Cysteine

Cys
C

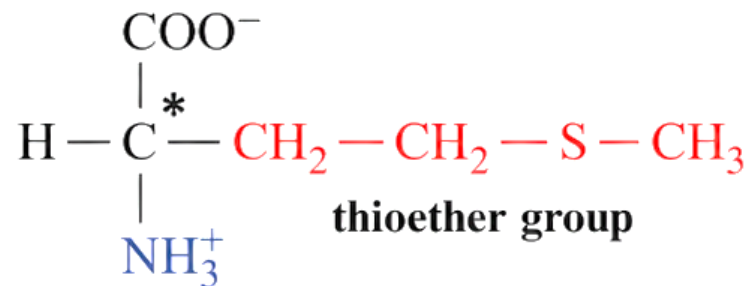


- sulphur containing aa. ★ 1c*
- polar R group
- Hydrophilic
 - undergo oxidation
 - Disulphide bond



Methionine

Met
M

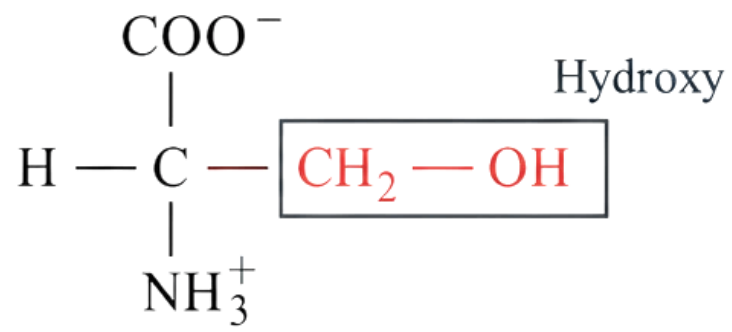


- sulfur containing ★ 1c*
- R- Non polar
- Hydrophobic
- undergo oxidation
- No disulphide bond

Serine

Ser

S

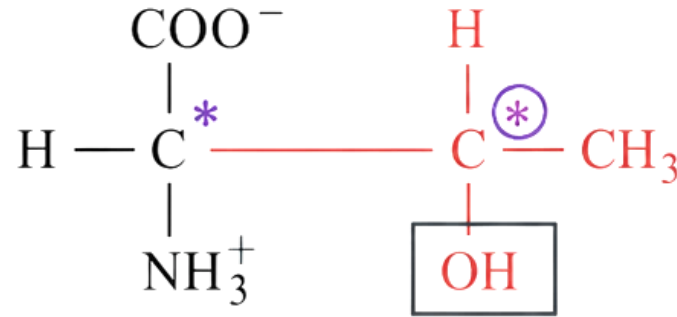


- Hydroxyl group (R)
- Polar $\rightarrow$ Hydrophilic
- 1 chiral centre
- undergo phosphorylation

Threonine

Thr

T

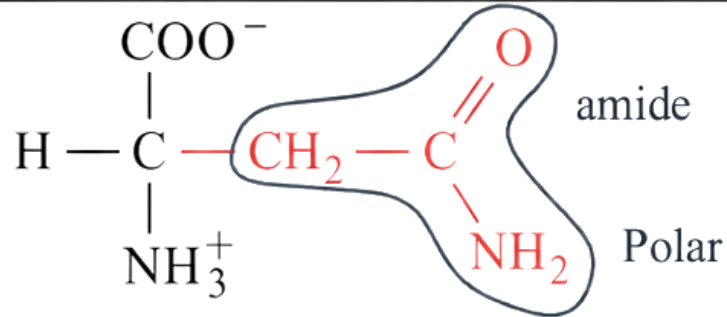


- Hydroxyl containing aa
- Polar $\rightarrow$ Hydrophilic
- chiral $\rightarrow$ 2 centre
- undergo phosphorylation.

Asparagine^e

Asn

N

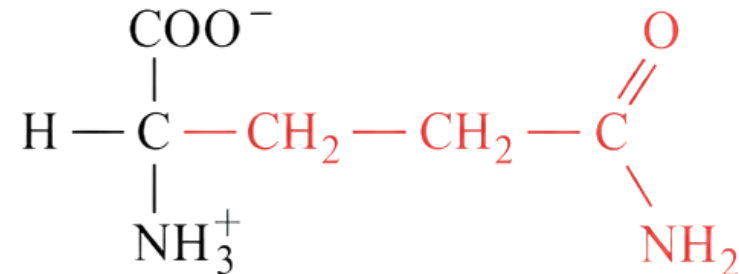


- amide group -R.
- R'- polar $\rightarrow$ hydrophilic
- derived from $\rightarrow$ aspartate
- 1 chiral centre

Glutamine^e

Gln

Q

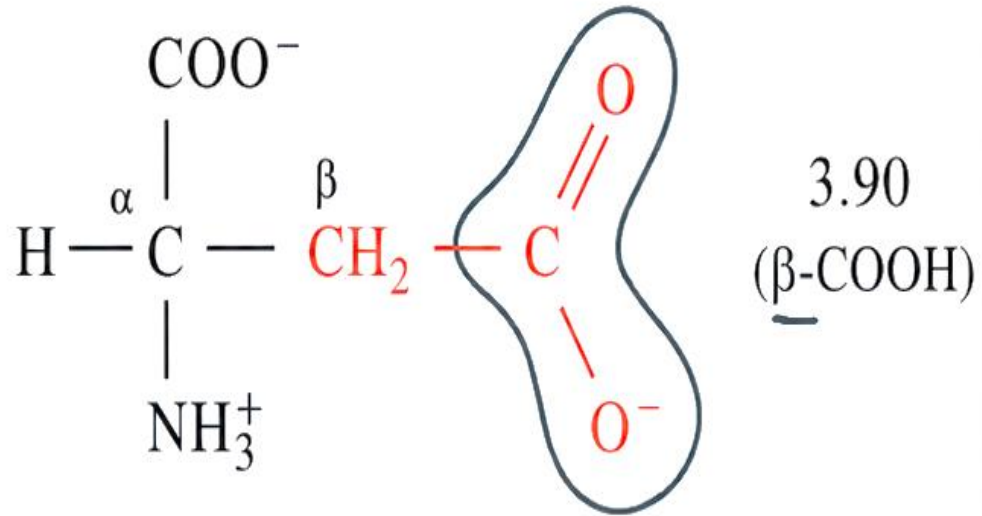


- amide group.
- R- polar
- 1 - chiral centre
- derived from $\rightarrow$ glutamate

Aspartic acid^e

Asp

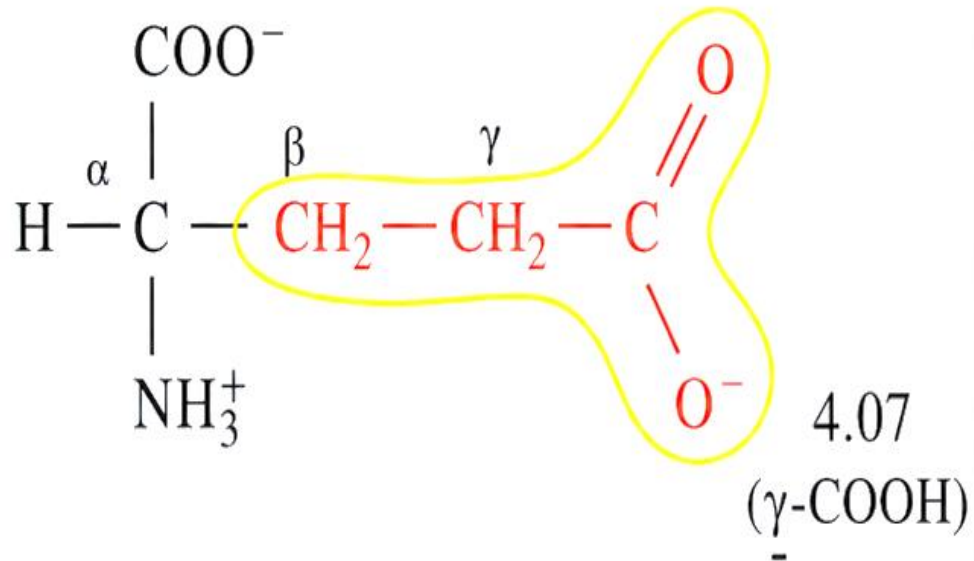
D



Glutamic acid^e

Glu

E



Acidic amino acid

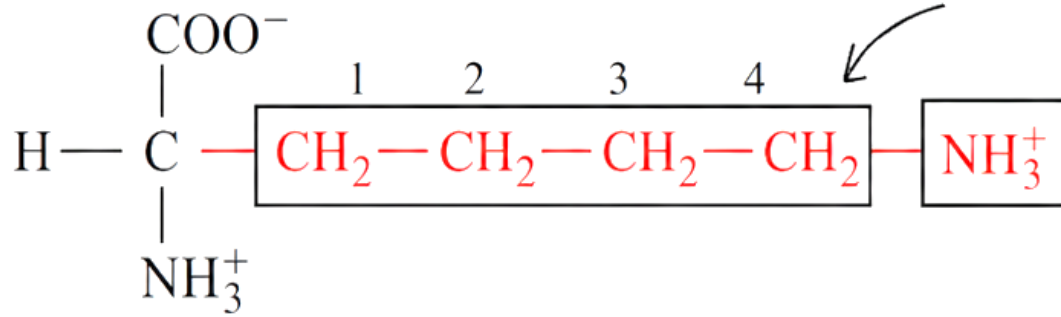
- β-COOH (pKa = 3.9)
 - -ve charged
 - 1 chiral centre
 - Polar - Hydrophilic
- pI min.

- γ-COOH (pKa = 4.07)
- -ve charged
- 1 chiral
- polar
- hydrophilic.

Basic amino acid

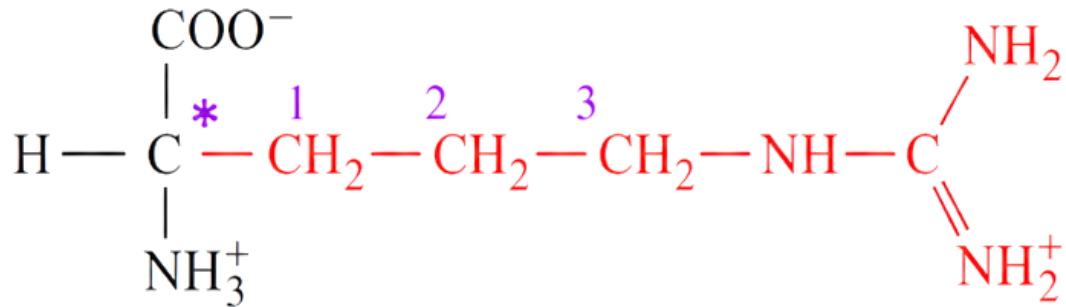
R- Butyl amino

Lysine
Lys
K



- R- butyl amino.
- polar
- + ve Charged
- most important
- histone (DNA)
- transcription(DNA)

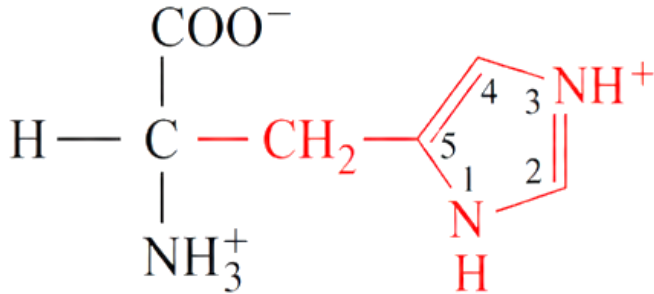
Arginine
Arg
R



(guanidino)

- R- **Guanidino**
- Highest pka
- pI = Highest
- 1 -Chiral Carbon

Histidine
His
H



(imidazole)

- R- Imidazole
- polar → hydrophobic
- pI ⇒ Nearly ≈ 7 (Neutral)
- act as buffer.

Two other α -amino acids

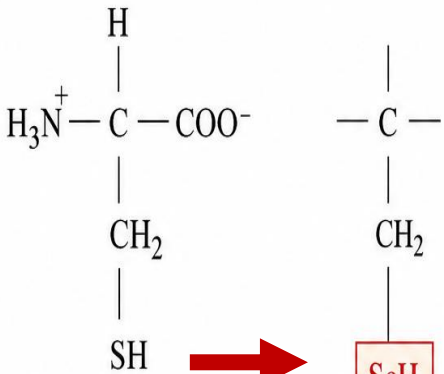
(uncommon)

Incorporation

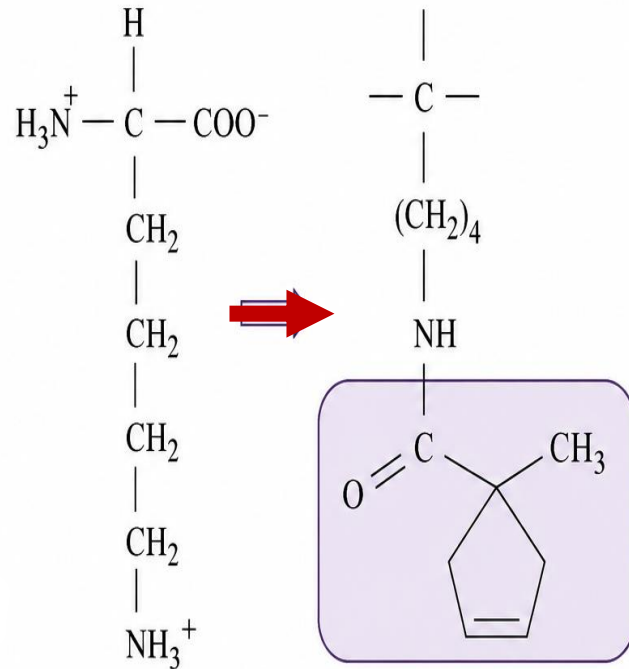
Selenocysteine

(selenium)

Pyrrolysine



Cysteine



Lysine

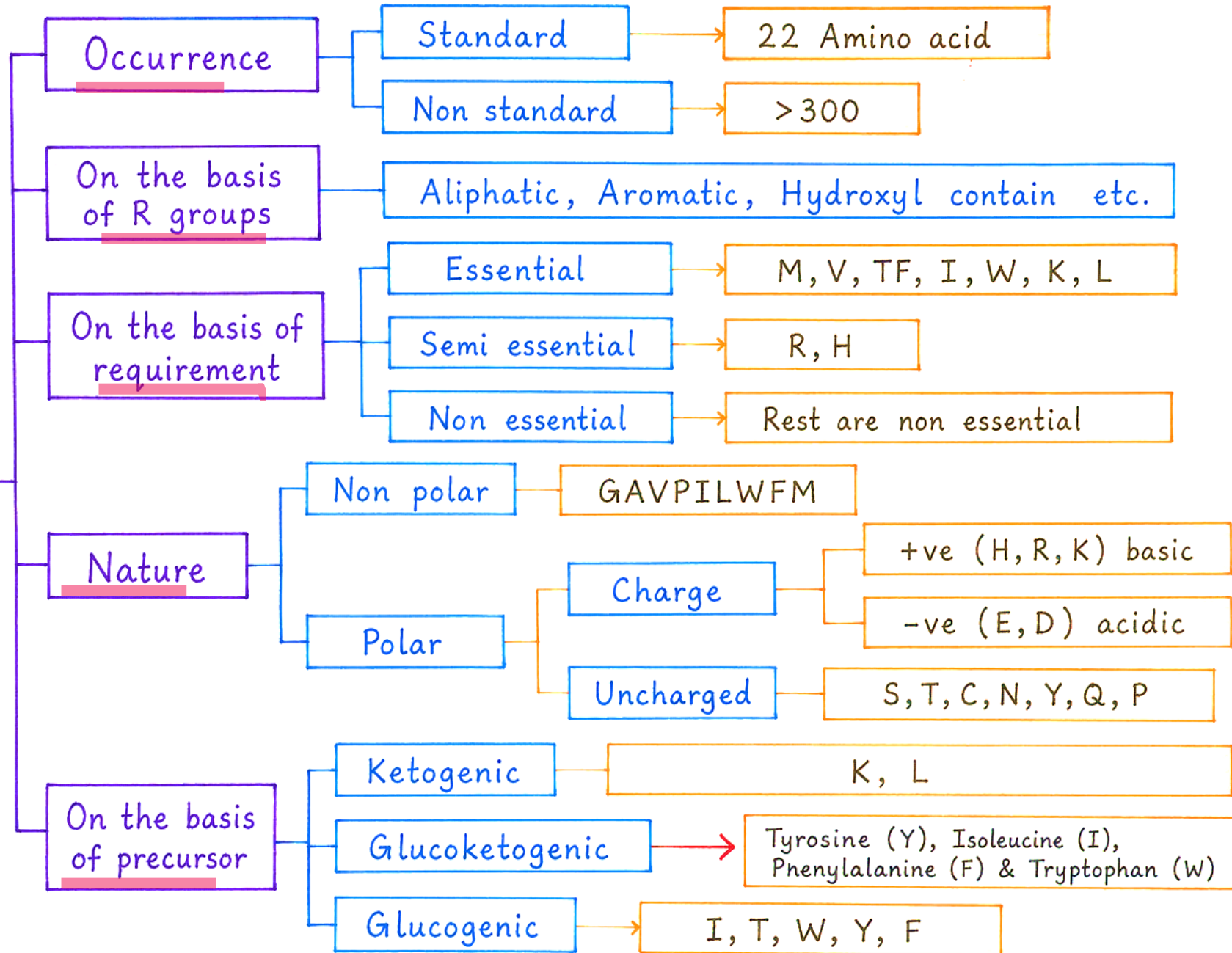
It was originally thought that all unconventional amino acids were made by modifying one of the standard amino acids after it was incorporated into protein, a process called a post-translational modification. But amino acids like selenocysteine, pyrrolysine are inserted into proteins by the translational machinery.

Selenocysteine (Sec or U) is introduced during protein synthesis rather than created through a postsynthetic modification. It contains selenium rather than sulphur of its structural analog, cysteine. Since selenocysteine is incorporated into polypeptides during translation, it is referred to as 21st amino acid. However, it is specified by a triplet codon, UGA (a stop codon). Selenocysteine has its own tRNA containing the anticodon UCA and it is formed by modifying a serine that has been attached to the selenocysteine tRNA. Enzymes like glutathione peroxidase and formate dehydrogenase contain selenocysteine in their catalytic center. Pyrrolysine (Pyl or O) is similar to lysine and is present in some bacterial proteins. It is coded by UAG codon.



CLASSIFICATION Of AMINO ACIDS

AMINO ACID



Three-letter code	Single letter code
Ala	A
Arg	R
Asn	N
Asp	D
Cys	C
Gln	Q
Glu	E
Gly	G
His	H
Ile	I

Three-letter code	Single letter code
Leu	L
Lys	K
Met	M
Phe	F
Pro	P
Ser	S
Thr	T
Trp	W
Tyr	Y
Val	V

Classification of Amino acids

01

Based on
Structure
&
Chemical
nature

- 1. Aliphatic side chain
- 2. Side chain with OH group
- 3. Side chain with "S"
- 4. Side chain with acidic group
- 5. Side chain with basic group
- 6. Aromatic amino acids
- 7. Imino acids

02

Based
On
Polarity

- Hydrophilic (polar)
- Hydrophobic (Non-polar)

03

Based
On
Metabolic fate

- Glucogenic
- Ketogenic
- Both

04

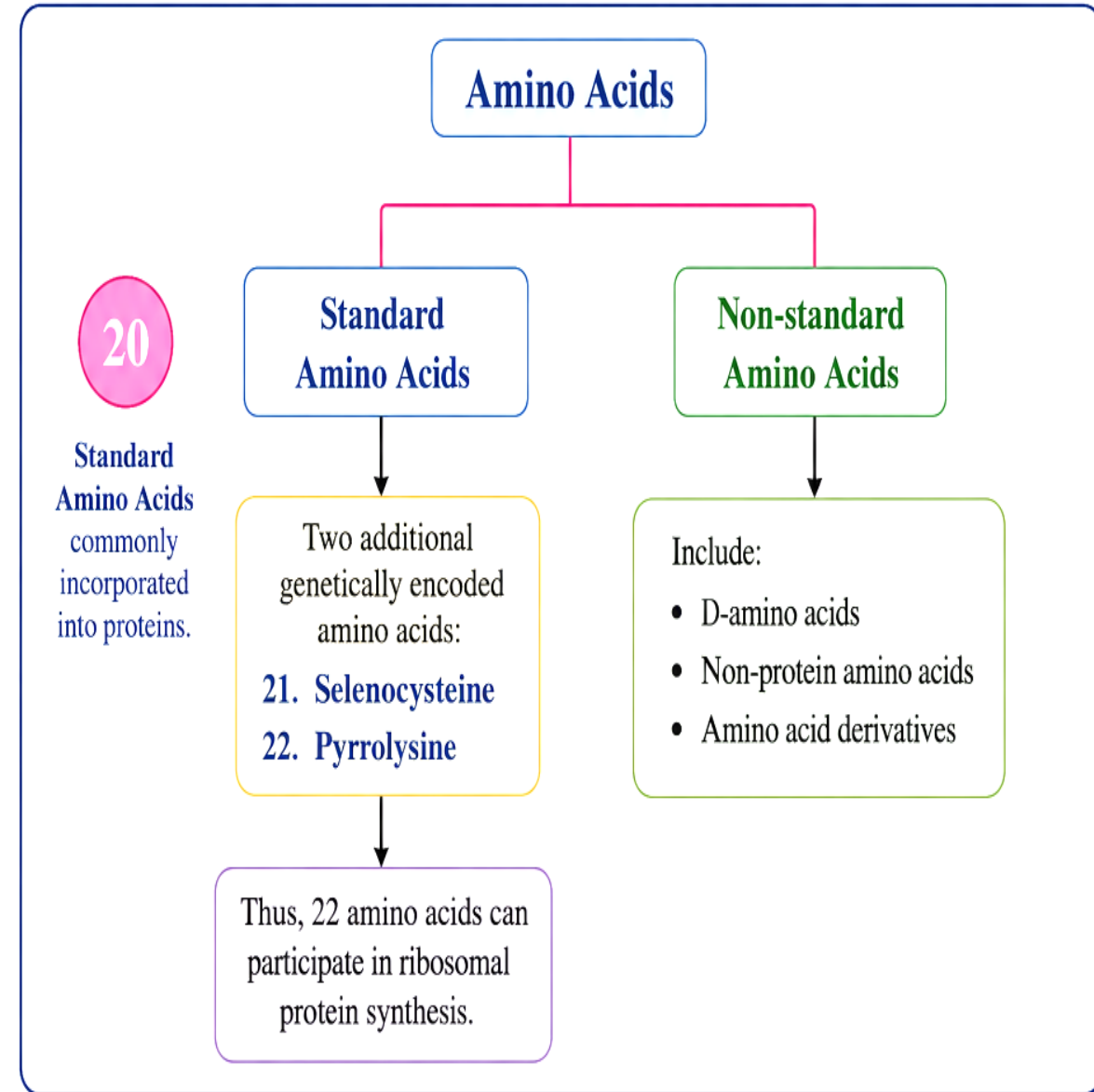
Nutritional
Classification

- Essential
- Nonessential
- Semi essential

standard Amino Acids

More than 300 amino acids are present in cells; however, only 22 amino acids participate in protein synthesis ribosomically. Such amino acids are called standard or proteinogenic amino acids.

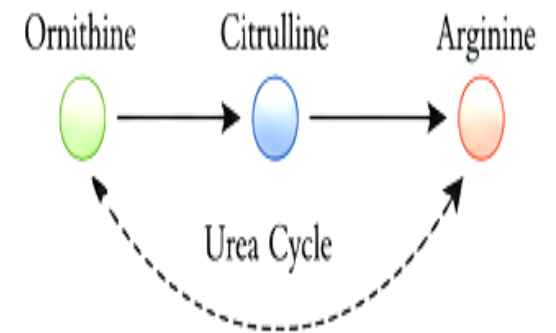
Some amino acids are more abundant in proteins than other amino acids. Four amino acids—**leucine**, **serine**, **lysine**, and **glutamic acid**—are the most abundant amino acid residues in a typical protein. **Tryptophan** and **methionine** are **rare** amino acids in a protein.



Nonstandard Amino Acids

More than three hundred amino acids have been found in cells. Twenty-two amino acids are ribosomically incorporated into proteins and are called proteinogenic or standard amino acids. Apart from the 22 standard amino acids, all other amino acids are not ribosomically incorporated into proteins are called non-standard. In addition to the standard amino acids, some proteins may contain non-standard amino acid residues formed by post-translational modification of standard amino acid residues already incorporated into a polypeptide. These modifications are often essential for the function or regulation of a protein. Examples of some of these amino acids are 4-Hydroxyproline (derivative of proline), 5-Hydroxylysine (derivative of lysine), desmosine (derivative of lysine), N-acetylserine, N-formylmethionine and γ -carboxyglutamate (found in the blood clotting protein prothrombin).

Besides their role in proteins, amino acids and their derivatives have many other biologically important functions. Many nonstandard amino acids are not found in proteins. These amino acids often occur as intermediates in the metabolic pathways for standard amino acids. For example, ornithine and citrulline are key intermediates in the biosynthesis of arginine and in the urea cycle. Similarly, azaserine, a nonstandard amino acid, acts as an antibiotic.



Classification On The Basis Of R Group

AMINO ACID CLASSIFICATION

Small amino acids – Glycine, Alanine

Branched amino acids – Valine, Leucine, Isoleucine

Hydroxy amino acids (-OH group) – Serine, Threonine

Sulfur amino acids – Cysteine, Methionine

Aromatic amino acids – Phenylalanine, Tyrosine, Tryptophan

Acidic amino acids and their derivatives – Aspartate, Asparagine, Glutamate, Glutamine

Basic amino acids – Lysine, Arginine, Histidine

Imino acid - Proline

Classification based on Polarity

Codes

Non Polar

- **GAV** ke log **PILloW**
lekar **F M** sunte hai



- **G-GLY**
- **A-ALANINE**
- **V-VALINE**
- **P-PROLINE**
- **I-ILE**
- **L-LEU**
- **W-TRP**
- **F-PHE**
- **M-MET**

Polar, Uncharged

- **S**mall **T**own **C**hangu
New **Q**ues puchta hai



- **S-SER**
- **T-THR**
- **C-CYS**
- **A-ASN**
- **Q-GLN**

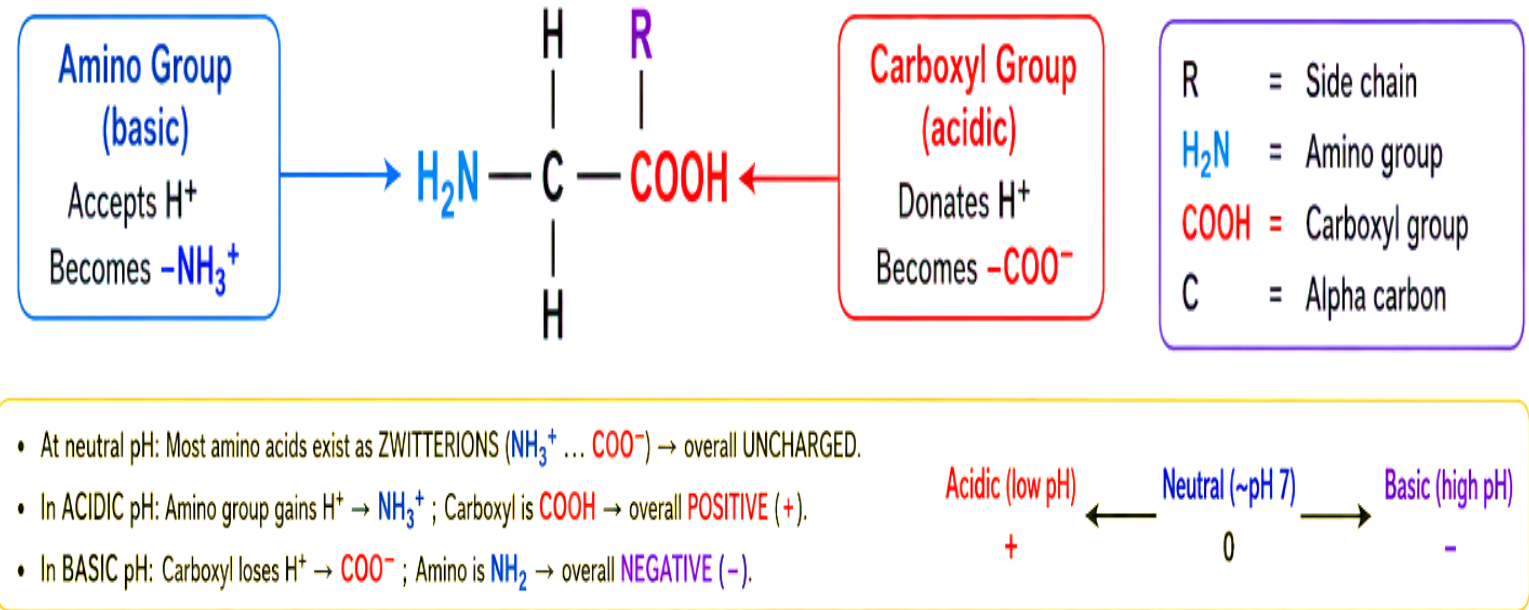
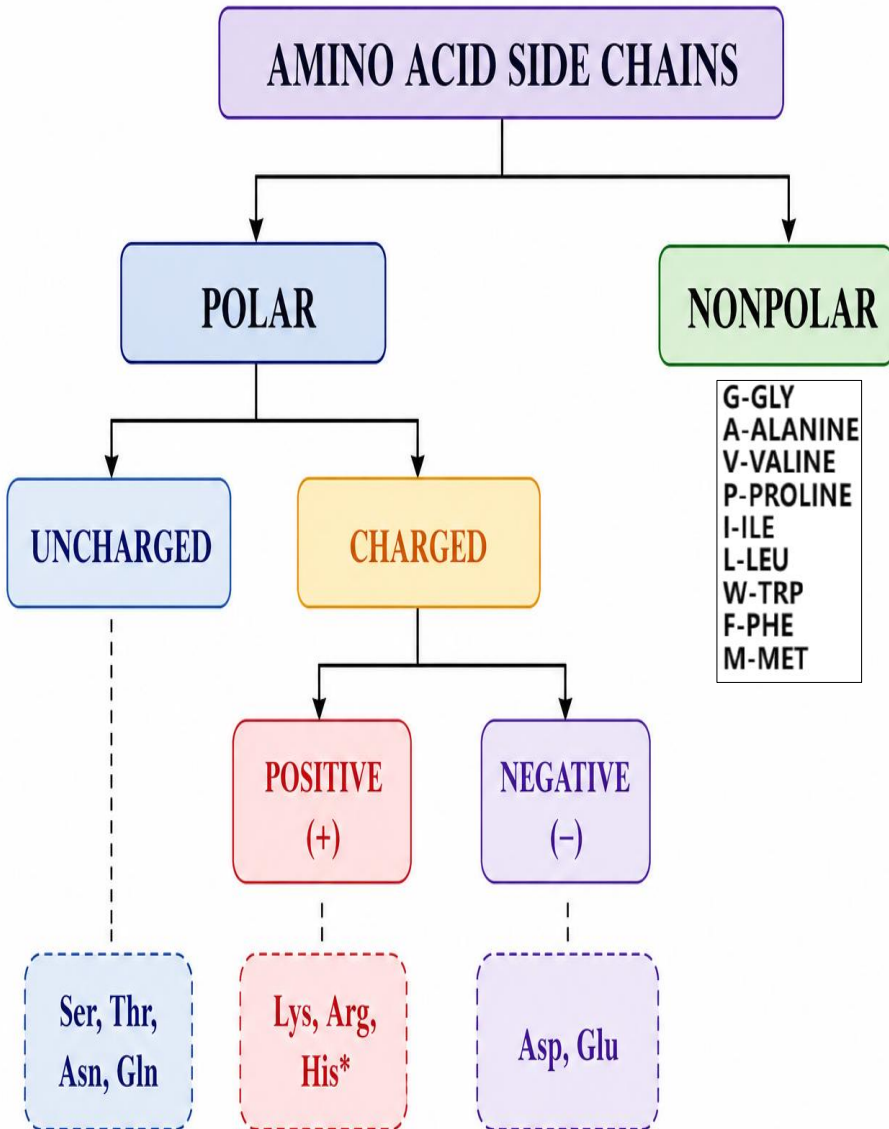
Polar, charged

- **+VE- H, R K** → Histidine, Arginine & Lysine
- **-VE, E, D** →

Aspartic acid^e
Asp
D

Glutamic acid^e
Glu
E

Classification based on Polarity



AMINO ACID SIDE CHAINS

POLAR

UNCHARGED

Ser, Thr,
Asn, Gln

CHARGED

POSITIVE (+)

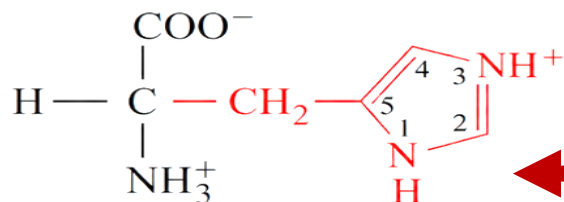
Lys, Arg,
His*

NEGATIVE (-)

Asp, Glu

NONPOLAR

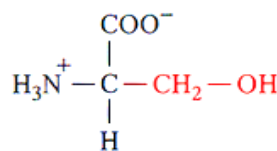
Gly, Ala, Val,
Pro, Ile, Leu,
Trp, Phe, Met



UNCHARGED POLAR

Side chains contain polar groups but no ionizable groups at physiological pH ~ 7.4. They can form hydrogen bonds with water → Hydrophilic but remain neutral (uncharged).

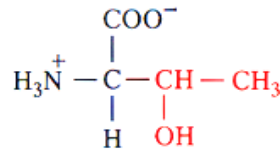
Serine (Ser, S)



Reason:

–OH is polar but not ionized at pH 7.4 → uncharged.

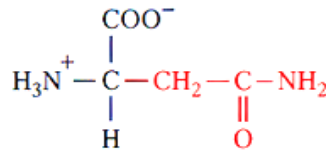
Threonine (Thr, T)



Reason:

–OH is polar but not ionized at pH 7.4 → uncharged.

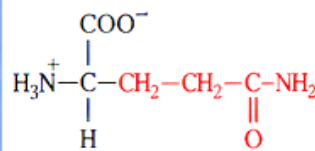
Asparagine (Asn, N)



Reason:

Amide (–CONH₂) is polar but not ionized at pH 7.4 → uncharged.

Glutamine (Gln, Q)



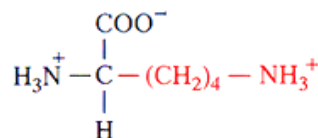
Reason:

Amide (–CONH₂) is polar but not ionized at pH 7.4 → uncharged.

CHARGED POSITIVE (Remain + at pH 7.4)

Side chains contain basic groups (amines or imidazole) with pK_a > 7. At physiological pH (7.4), these groups are protonated → carry + charge.

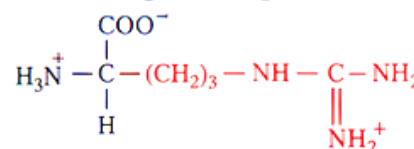
Lysine (Lys, K)



Reason:

ε-NH₃⁺ (pK_a ~ 10.5) remains protonated at pH 7.4 → + charge.

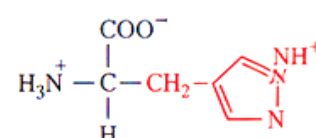
Arginine (Arg, R)



Reason:

Guanidinium group (pK_a ~ 12.5) is strongly basic → fully protonated at pH 7.4 → + charge.

Histidine (His, H)



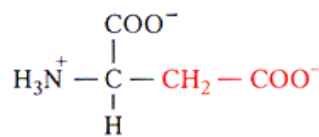
Reason:

Imidazole (pK_a ~ 6.0) is partially protonated near pH 7.4 → can be positively charged.

CHARGED NEGATIVE (Remain – at pH 7.4)

Side chains contain acidic groups (carboxyl) with pK_a < 7. At physiological pH (7.4), these groups are deprotonated → carry – charge.

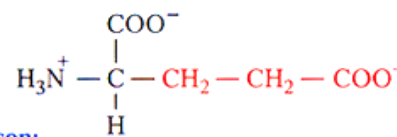
Aspartic acid (Asp, D)



Reason:

β-COOH (pK_a ~ 3.9) loses H⁺ at pH 7.4 → becomes COO[–] → – charge.

Glutamic acid (Glu, E)



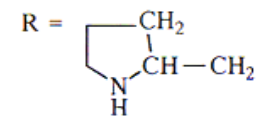
Reason:

γ-COOH (pK_a ~ 4.1) loses H⁺ at pH 7.4 → becomes COO[–] → – charge.

NONPOLAR

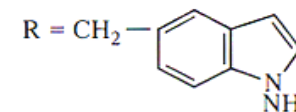
Side chains are hydrocarbon in nature → no ionizable groups → hydrophobic.

- Glycine (Gly, G) R = H
- Alanine (Ala, A) R = CH₃
- Valine (Val, V) R = CH(CH₃)₂
- Proline (Pro, P)

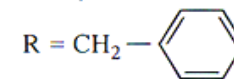


- Isoleucine (Ile, I) R = CH(CH₃)–CH₂–CH₃
- Leucine (Leu, L) R = CH₂–CH(CH₃)₂

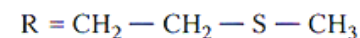
- Tryptophan (Trp, W)



- Phenylalanine (Phe, F)



- Methionine (Met, M)



* Histidine is called a basic amino acid because its side chain can be positively charged near physiological pH.

Classification based on Nutritional requirement

Essential Amino Acids

Amino acids that the human body **cannot synthesize or produce** in sufficient amounts.

They **must be obtained from diet**.

There are **9 essential amino acids** in adults:

Example: Essential amino acids --(PVT TIM HaLL)

Phenylalanine: P, Valine: V, Threonine: T, Tryptophan: T, Isoleucine: I, Methionine: M, Histidine: H, Leucine: L and Lysine: L

(In children, Arginine is also considered essential because their body cannot make enough).

Non-Essential Amino Acids

Amino acids that the body **can synthesize from its own from other compounds endogenously through metabolic pathways** (like glucose, pyruvate, or other amino acids).

They don't necessarily need to come from diet, but dietary intake may still be beneficial.

Examples: Alanine, asparagine, aspartic acid, glutamic acid, serine, and tyrosine.

P.v.t. T.I.M. H.A.L.L.

Phe = Phenylalanine

Val = Valine

Thr = Threonine

Trp = Tryptophan

Ile = Isoleucine

Met = Methionine

His = Histidine

Arg = Arginine*

Leu = Leucine

Lys = Lysine

Conditionally essential amino acids

(Semi-essential Amino Acids)

Amino acids that are **not usually essential** but **become essential** during **specific circumstances** like illness, stress, or injury.

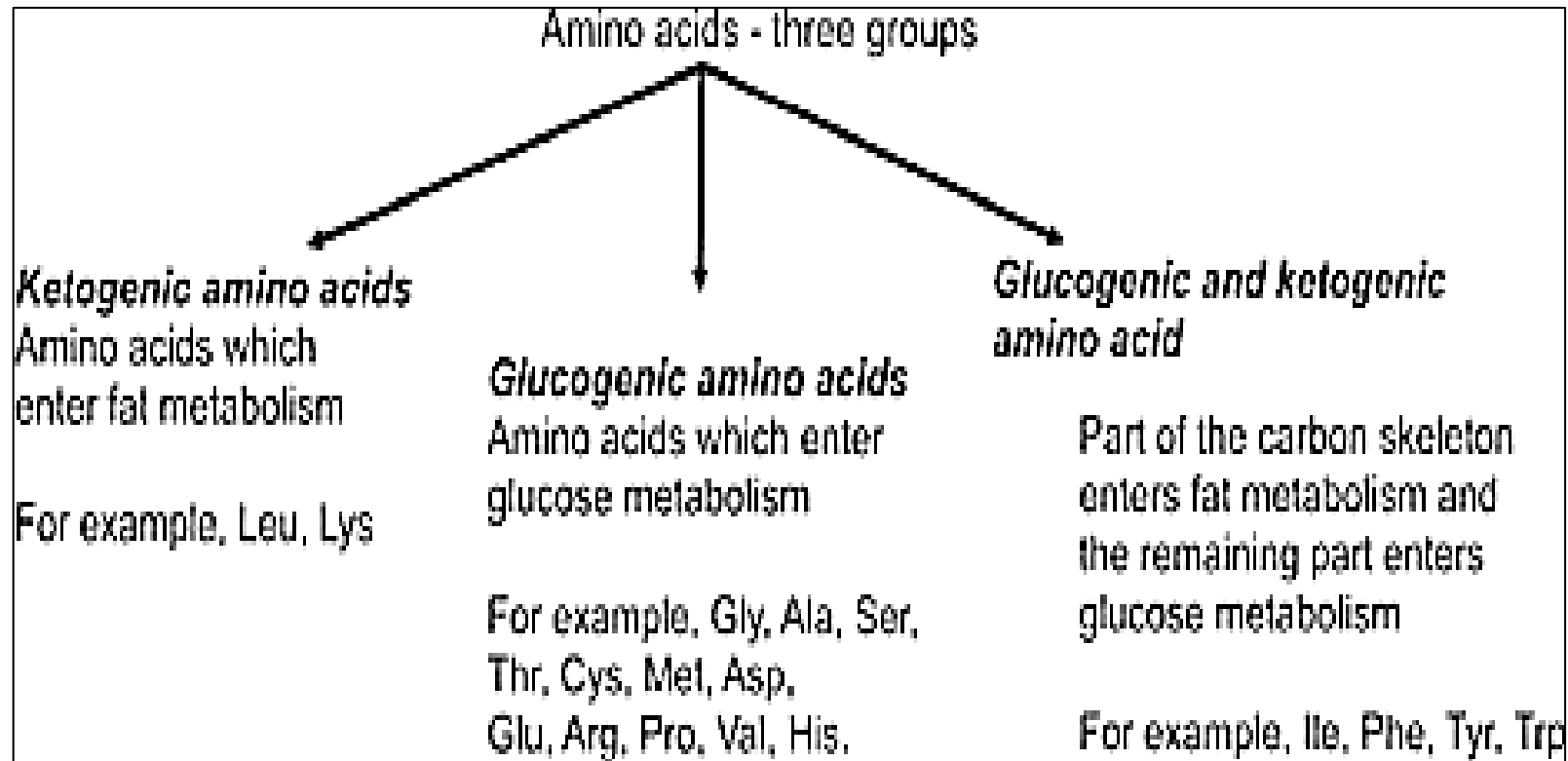
Examples: **Arginine**

The body's ability to synthesize these may be limited during times of stress, making dietary intake necessary.

Nonessential	Alanine Arginine [*] Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
Essential	Histidine Methionine Threonine Valine	Isoleucine Phenyl- alanine Tryptophan	Leucine Lysine

Arg is semiessential, depending on developmental stage

Classification based on Metabolic fate



These amino acids are broken down into **ketone bodies or fatty acids.**

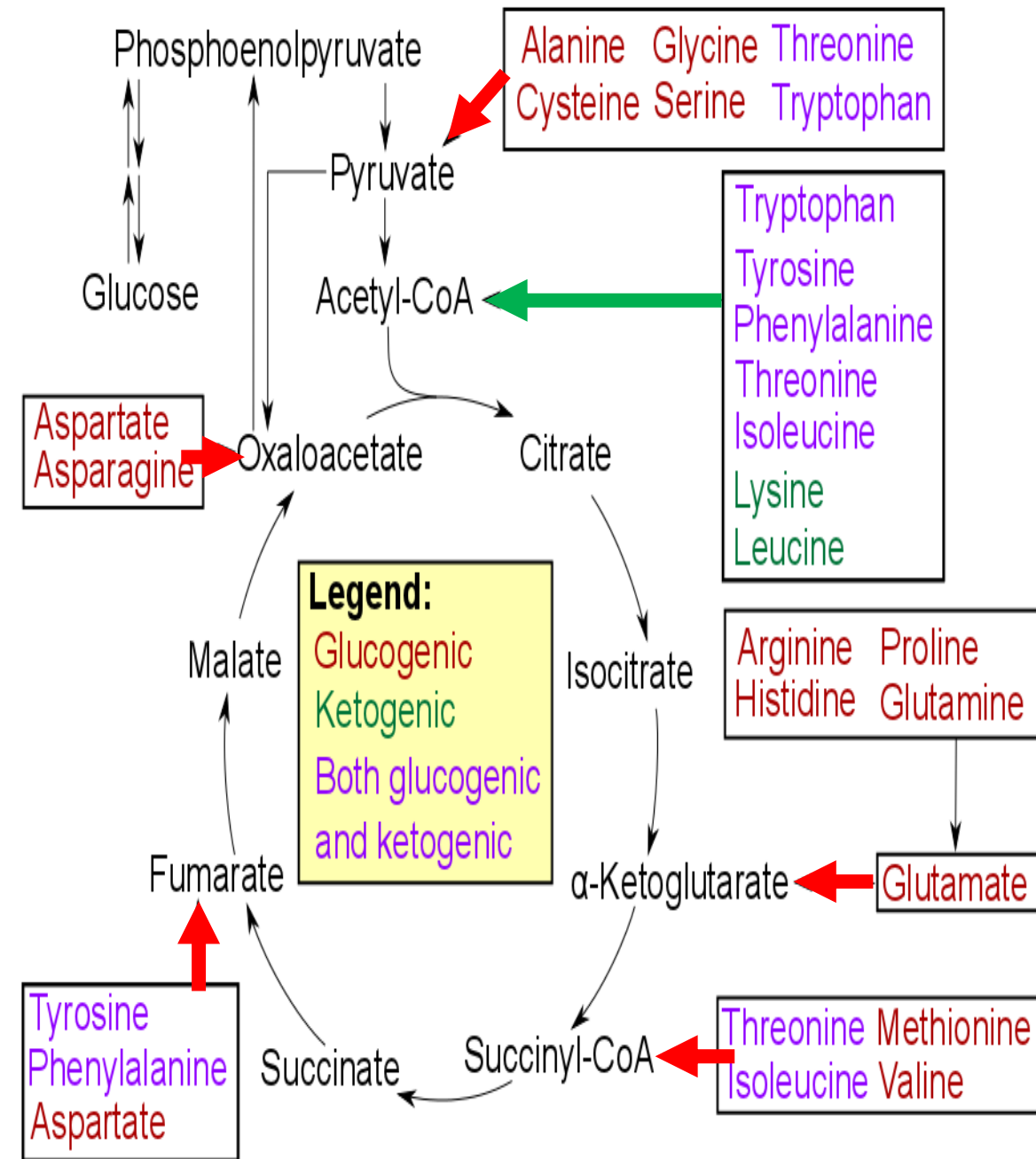
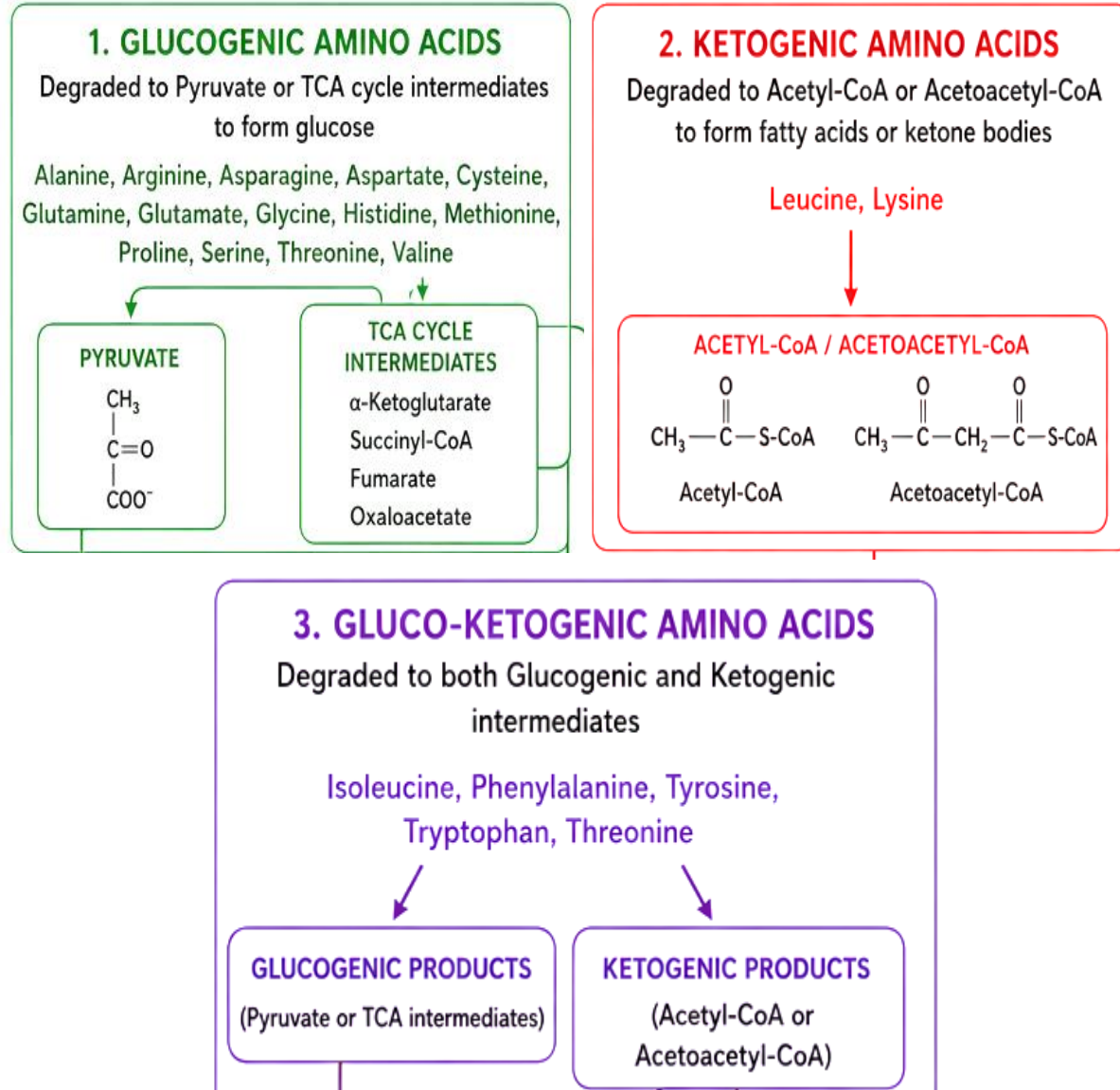
These amino acids can be **converted into glucose** to be used as an energy source

These amino acids break down to form **precursors for both ketone bodies and glucose**

Glucogenic Amino acids	Glucogenic and Ketogenic	Ketogenic Amino acids
Alanine Arginine* Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
Histidine Methionine Threonine Valine	Isoleucine Phenylalanine Tryptophan	Leucine Lysine

FATES OF AMINO ACIDS IN METABOLIC PATHWAYS

Glucogenic → Glycolysis / Krebs Cycle • Ketogenic → Fatty Acid Synthesis / Ketone Bodies • Glucogenic & Ketogenic → Both



ABSORBANCE

Absorbance refers to **how much light** amino acids can absorb at a particular wavelength of light- often measure this using a **UV-visible spectrophotometer**.

- **Not all amino acids absorb light equally.** Their ability depends on whether **their structure** contains **chromophores** (groups in the molecule that can absorb UV light).

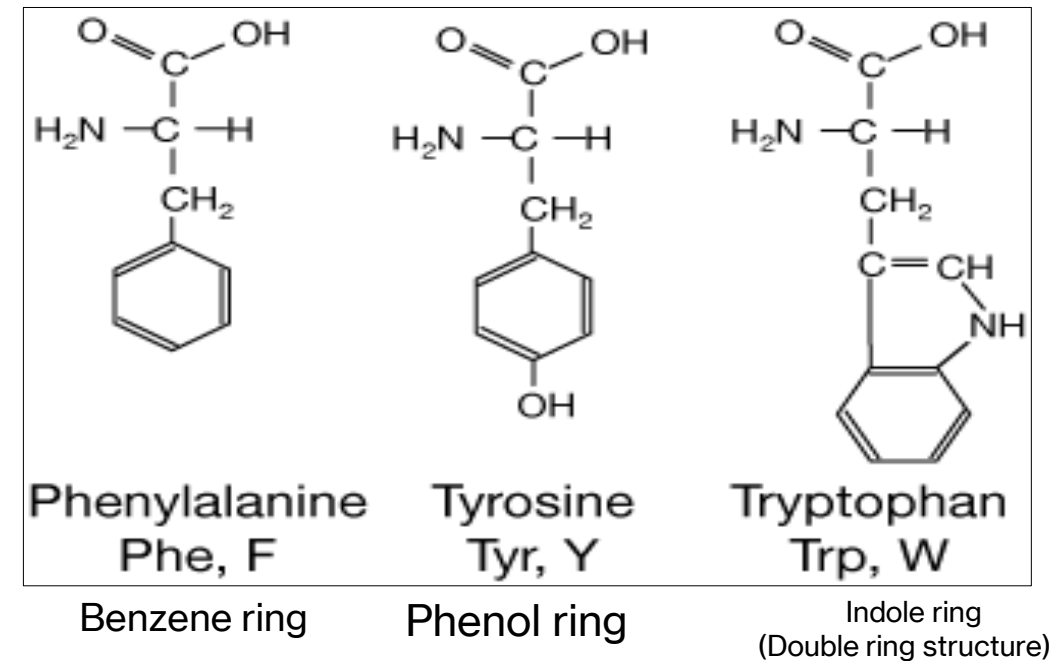
Aromatic amino acids are the main ones that **absorb strongly in the UV range**:

Tryptophan → strongest absorbance at **280 nm** (due to its indole ring).

Tyrosine → absorbs at **274–280 nm** (phenolic ring).

Phenylalanine → weaker absorbance at **257–260 nm** (benzyl ring).

These are important because proteins contain them, so protein concentration is often measured at **280 nm**



Even if side chains do not absorb strongly, the **peptide bond (-CO-NH-)** itself absorbs strongly at **190–220 nm** in the **far UV region**.

The **absorbance** comes from **electrons in certain bonds** (like double bonds or lone pairs) that can **jump to higher energy levels** when UV light provides the right amount of energy-- called an **electronic transition**.

Properties of Amino acids

Physical Properties

1. Amino acids are **colorless, crystalline solid**.
2. Many amino acids are **sweet** (glycine, alanine, serine). Some are **bitter** (valine, leucine, isoleucine) and Some are **tasteless**.
2. All amino acids have a high **melting point greater than 200°**
3. They are soluble in water, slightly soluble in alcohol, and dissolve with difficulty in methanol, ethanol, and propanol. R-group of amino acids and pH of the solvent play important role in solubility.
4. On heating to high temperatures, **they decompose**.
5. All amino acids (except glycine) are **optically active**.
6. Amino acids can connect with a peptide bond involving their amino and carboxylate groups. A covalent bond formed between the alpha-amino group of one amino acid and an alpha-carboxyl group of other forming -CO-NH-linkage. Peptide bonds are planar and partially ionic.

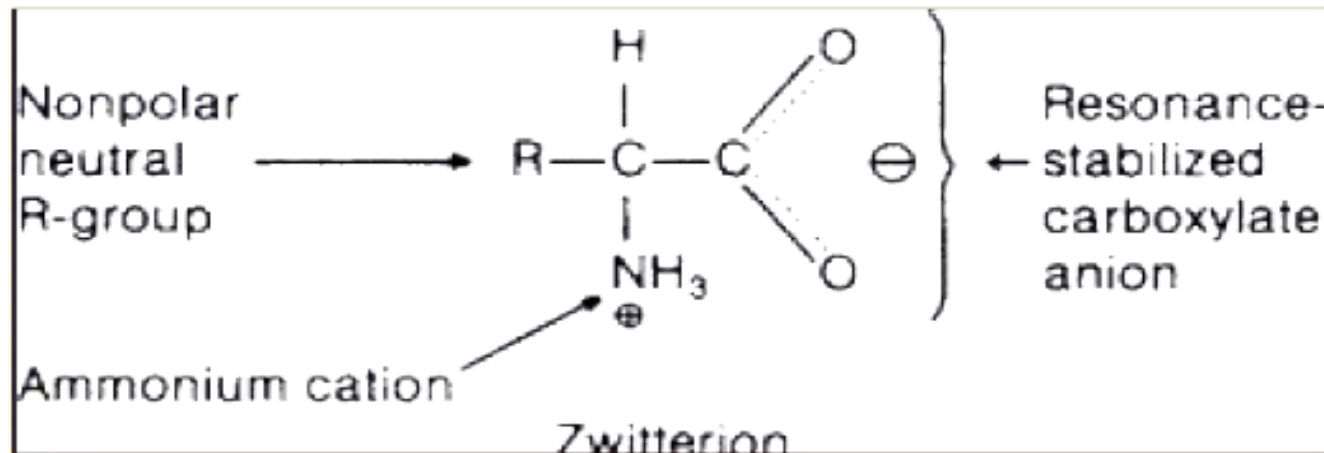
Chemical Properties

1. Zwitterionic property or ionization of Amino acids

Zwitter-ionic property

A zwitterion is a molecule with functional groups, of which at least one has a positive and one has a negative electrical charge. Net charge of the molecule is zero. Amino acids are the best-known examples of zwitterions.

They contain an amine group (basic) and a carboxylic group (acidic). The (neutral) zwitterion is the usual form amino acids exist in solution.

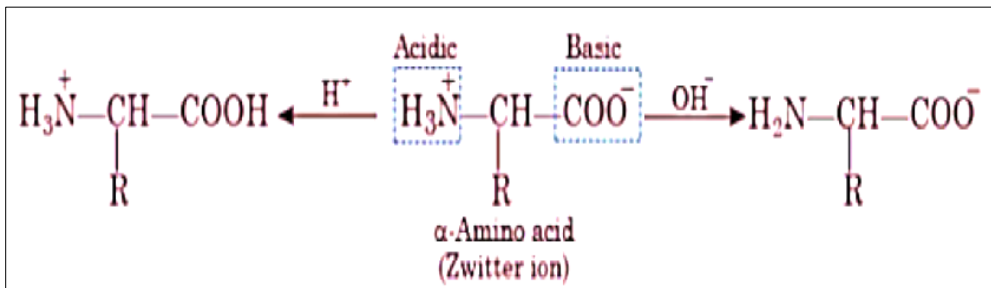


Isoelectric point

- The isoelectric point (pI) is the pH at which the **number of positive charges equals the number of negative charges**, and the overall charge on the amino acid is zero.
- At this point, when subjected to an electric field the proteins do not move either towards anode or cathode, hence this property is used to isolate proteins.

2. Amphoteric property

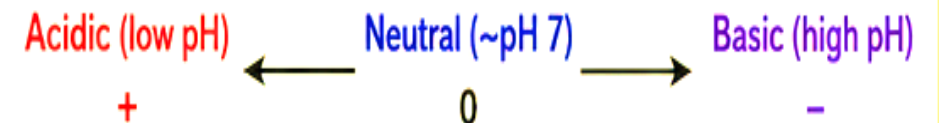
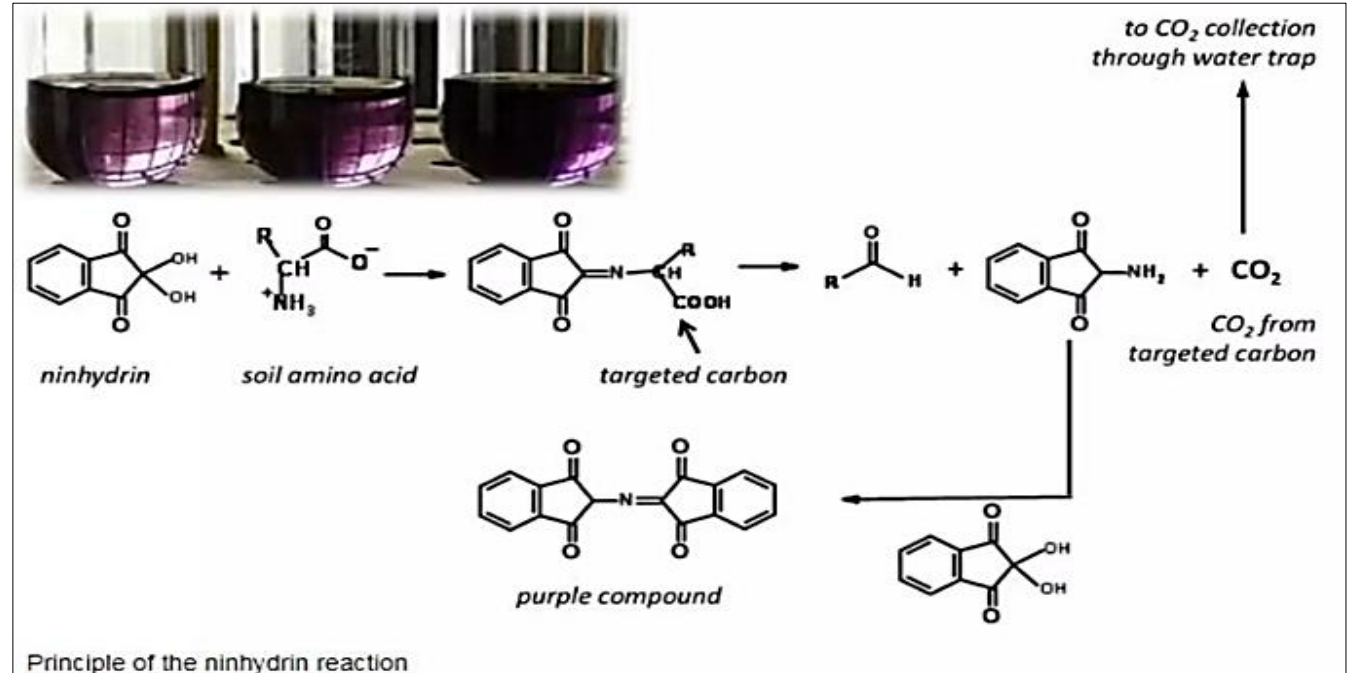
- Amino acids are amphoteric in nature that is **they act as both acids and base** due to the two amine and carboxylic groups present.
- They carry both positive and negative charges depending on the surrounding pH.



- At neutral pH: Most amino acids exist as ZWITTERIONS ($\text{NH}_3^+ \dots \text{COO}^-$) → overall UNCHARGED.
- In ACIDIC pH: Amino group gains H^+ → NH_3^+ ; Carboxyl is COOH → overall POSITIVE (+).
- In BASIC pH: Carboxyl loses H^+ → COO^- ; Amino is NH_2 → overall NEGATIVE (-).

3. Ninhydrin test

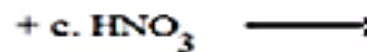
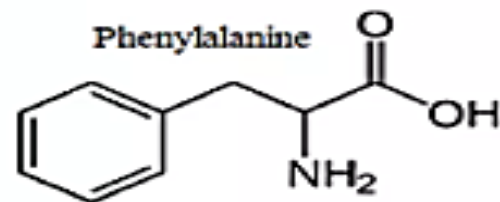
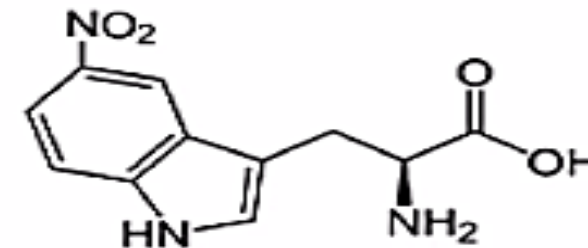
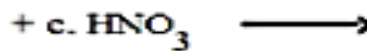
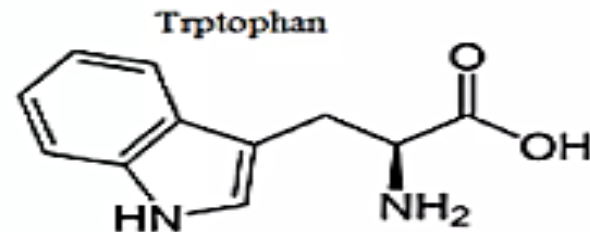
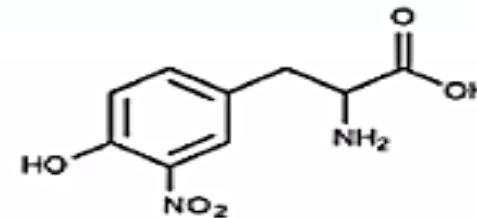
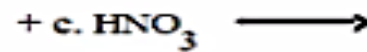
When 1 ml of Ninhydrin solution is added to a 1 ml protein solution and heated, the formation of a violet color indicates the presence of α -amino acids.



4. Xanthoproteic test

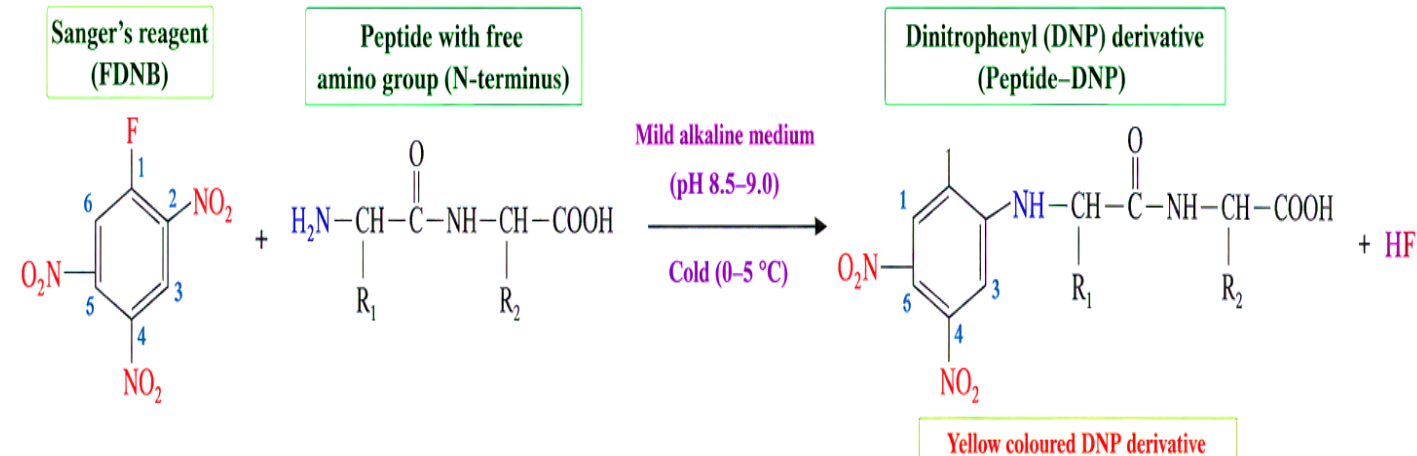
The xanthoproteic test is performed for the **detection of aromatic amino acids** (tyrosine, tryptophan, and phenylalanine) in a protein solution.

The nitration of benzoid radicals present in the amino acid chain occurs due to a reaction with nitric acid, giving the solution yellow coloration.



5. REACTION WITH SANGER'S REAGENT

Sanger's reagent (1-fluoro-2, 4-dinitrobenzene; FDNB) reacts with a **free amino group** in the peptide chain in a **mild alkaline** medium under **cold conditions** to form a stable **dinitrophenyl (DNP)** derivative.

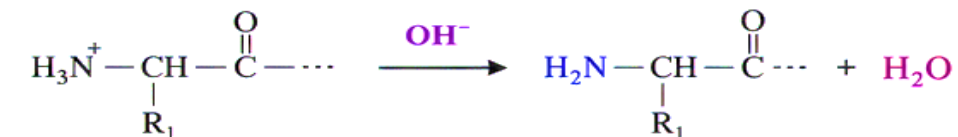


DETECTION

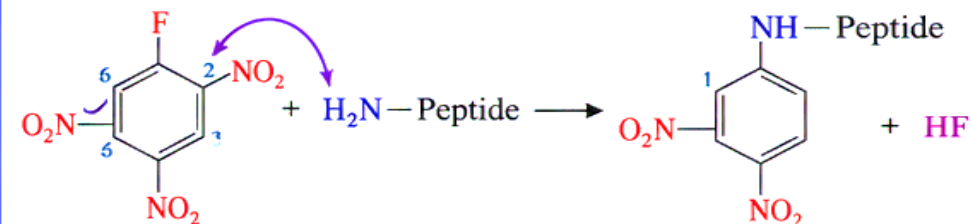
- DNP derivatives are **yellow** coloured.
- They show strong absorbance at 360 nm ($\epsilon \approx 17,000 \text{ M}^{-1} \text{ cm}^{-1}$).
- Used for identification and estimation of free amino groups and N-terminal amino acids.

MECHANISM

- In mild alkaline medium, the free amino group is deprotonated to give a nucleophilic -NH_2 .



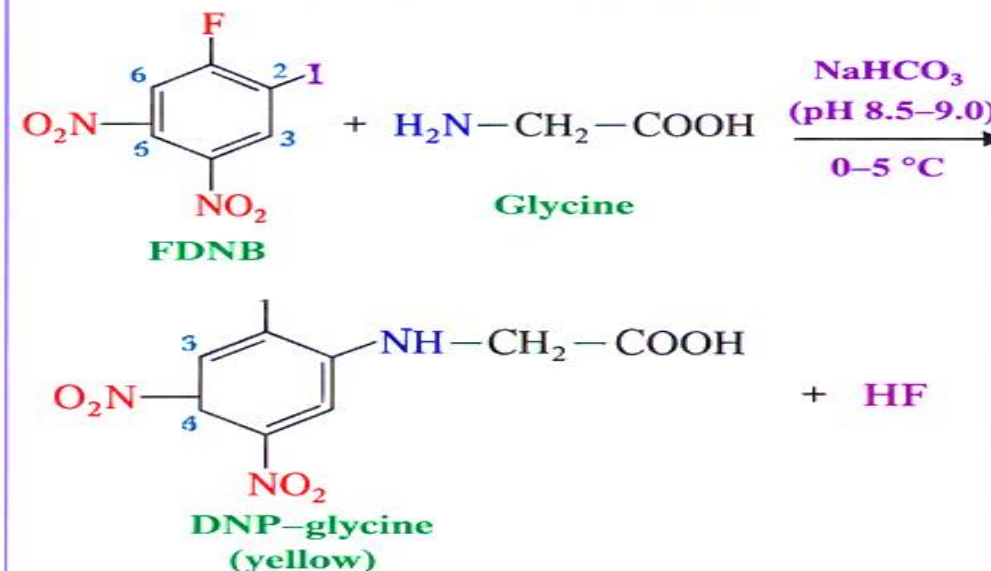
- The -NH_2 attacks the electron deficient aromatic carbon bearing fluorine ($\text{S}_{\text{N}}\text{Ar}$ mechanism).



- HF is eliminated and a stable DNP derivative is formed.

EXAMPLE

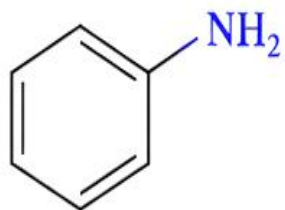
Reaction of glycine with FDNB



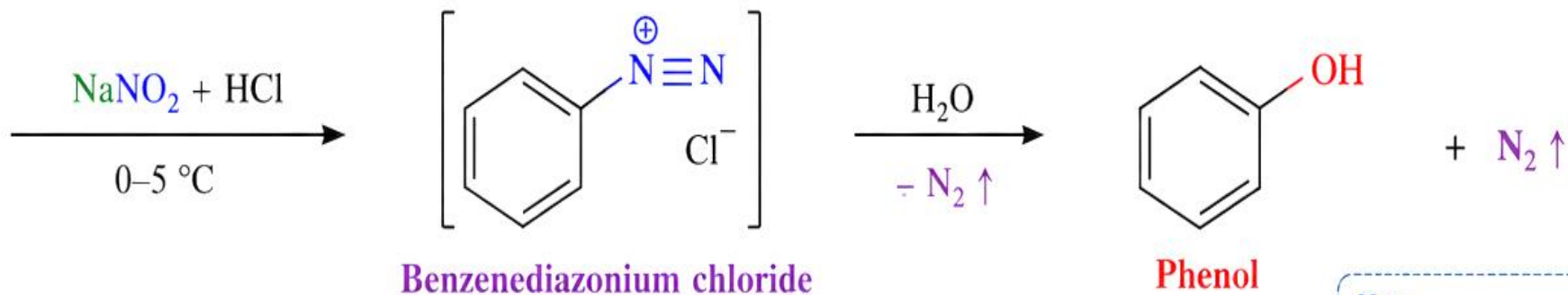
6. REACTION WITH NITROUS ACID

Nitrous acid (HNO_2), generated *in situ* from $\text{NaNO}_2 + \text{HCl}$, reacts with 1° aliphatic or aromatic amines to liberate **nitrogen gas** and form the corresponding **alcohol** ($-\text{OH}$).

A 1° aromatic amine



Aniline



Note:

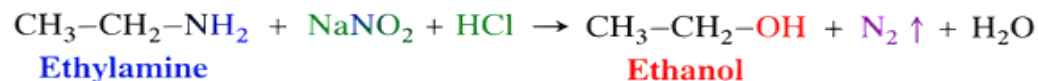
This reaction is specific for 1° amines.

CONDITIONS : Cold ($0-5^\circ\text{C}$) is essential to prevent decomposition of HNO_2 and side reactions.

KEY POINTS

- $\text{NaNO}_2 + \text{HCl}$ generates HNO_2 *in situ*.
- HNO_2 converts 1° amines into diazonium salts.
- Diazonium salts are unstable in water and lose N_2 to give phenols (in case of aromatic amines).
- For aliphatic 1° amines $\rightarrow$ corresponding **alcohols** are formed.

EXAMPLE (ALIPHATIC 1° AMINE)



General Properties of α -Amino Acids

Amphoteric Nature

Amino acids act as both acid and base due to the presence of $-\text{COOH}$ and $-\text{NH}_2$ groups, allowing them to act as buffers.

Isoelectric Point (pI)

The pH at which an amino acid carries no net charge and exists predominantly as a zwitterion; it does not migrate in an electric field at this pH.

High Melting Point

Amino acids have relatively high melting points (usually $>200^\circ\text{C}$) due to strong electrostatic (ionic) interactions in the crystal lattice.

UV Absorption

Aromatic amino acids (Trp, Tyr, Phe) absorb UV light around 280 nm — used to estimate protein concentration.

Optical Activity

All amino acids except glycine possess a chiral α -carbon and are optically active, rotating plane-polarised light.

Solubility

Generally soluble in water and insoluble in non-polar organic solvents, owing to their ionic (zwitterionic) nature.

Ninhydrin Reaction

Amino acids react with ninhydrin to produce a purple (Ruhemann's purple) colour, used for their qualitative and quantitative detection (proline gives yellow).

Peptide Bond Formation

The α -carboxyl group of one amino acid condenses with the α -amino group of another, releasing water and forming a peptide bond.

Physiological
importance
of
essential and non-
essential α - amino acids.

Physiological importance of essential α - amino acids.

Essential Amino Acids

Essential amino acids cannot be synthesised by animal tissues (owing to the absence of the requisite enzymatic pathways) and must be supplied through diet. Their physiological roles include:

Amino Acid	Physiological Importance
Leucine, Isoleucine, Valine (BCAAs) → → →	Muscle protein synthesis; energy source during exercise; regulate blood glucose
Lysine →	Collagen formation, calcium absorption, carnitine synthesis; growth in young animals
Methionine →	Methyl group donor (via SAM); synthesis of cysteine, creatine, and choline; antioxidant defence
Threonine →	Component of mucin/collagen; supports gut and immune function

Phenylalanine



Precursor of tyrosine, catecholamines (dopamine, adrenaline) and thyroid hormones

Tryptophan



Precursor of serotonin, melatonin and niacin (vitamin B3)

Histidine (semi-essential)



Precursor of histamine; important in growth and tissue repair

Arginine (semi-essential)



Nitric oxide synthesis, urea cycle, wound healing, immune function

Mnemonic for Essential Amino Acids: "PVT TIM HALL" — Phenylalanine, Valine, Threonine, Tryptophan, Isoleucine, Methionine, Histidine (semi-essential), Arginine (semi-essential), Leucine, Lysine.

Physiological importance of non-essential α -amino acids

Non-Essential Amino Acids

These are synthesised endogenously from metabolic intermediates (glycolysis, TCA cycle) or from essential amino acids, yet remain physiologically vital:

Amino Acid	Physiological Importance
Glycine	Component of collagen, glutathione, haem and purine synthesis; inhibitory neurotransmitter
Alanine	Glucose-alanine cycle for inter-organ nitrogen and energy transport
Glutamic acid / Glutamine	Excitatory neurotransmitter (Glu); ammonia detoxification and nitrogen transport (Gln); fuel for rapidly dividing cells
Aspartic acid	Urea cycle intermediate; involved in purine and pyrimidine biosynthesis

Serine	Phospholipid synthesis; precursor of glycine and cysteine
Cysteine	Disulphide bond formation in proteins; glutathione and taurine synthesis
Tyrosine	Precursor of melanin, thyroid hormones and catecholamines
Proline	Structural rigidity in collagen triple helix

Clinical/comparative note: Deficiency of essential amino acids in diet leads to conditions such as kwashiorkor (protein-energy malnutrition), while inherited defects in amino acid metabolism (e.g. phenylketonuria, due to inability to convert phenylalanine to tyrosine) illustrate their physiological significance.

References:

Kumar, P. & Mina, U. (2024). *Life Sciences: Fundamentals and Practice – Part I*. 9th ed. Pathfinder Publication, New Delhi.

Nelson, D. L. & Cox, M. M. *Lehninger Principles of Biochemistry*. W. H. Freeman.