INTRODUCTION

Biopolymers present in nature are Polysaccharides [Carbohydrates], Proteins, and Nucleic Acids

➢ *Proteins are made up of hundreds of smaller units called amino acids that are attached to one another by peptide bonds, forming a long chain.*

➢ *Amino acids are monomers of peptide and protein biopolymers*

Amino Acids $\xrightarrow{\text{Peptide/ amide linkage}}$ *Peptides {Di-. Tri- , Tetra-, Oligo-}* $\xrightarrow{\text{covalent/ H-bond/ van der Waal \& 3D folding}}$ *Proteins*

➢ *The sequence and quantity of amino acid arrangement determine the final protein structure*

➢ *Side chain of an amino acid determines*

- *protein folding*
- *binding to specific ligand*
- *interaction with its environment*

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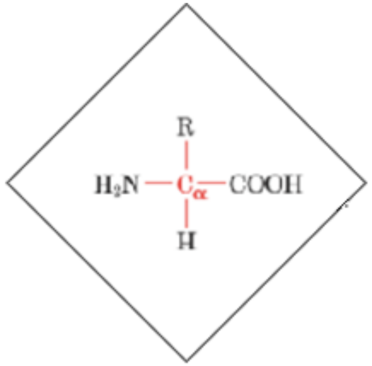
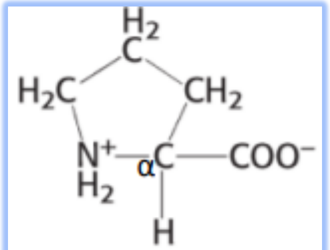
Amino Acids

Amino acid: *is defined as a carboxylic acid having a protonated amino gp on the α-C atom.*
For all the common amino acids except glycine, the α-carbon is bonded to four different gps: a carboxyl group, an amino group, an R group, and a hydrogen atom. In case of glycine, the R group is replaced by another H-atom. They have a 1º amino group & a carboxylic acid substituent on the same carbon atom, with the exception of proline, (which has a 2º amino group).



Proline classified as an imino acid, its α-amine is a 2º amine with its a nitrogen having two covalent bonds to carbon (to the α-carbon and side chain carbon), rather than 1º amine.
Incorporation of amino nitrogen into a five membered ring constrains rotational freedom around $-N_\alpha$ - C_α-bond in proline to specific rotational angle, reduces structural flexibility of polypeptide regions containing proline.



Proline
(Pro, P)

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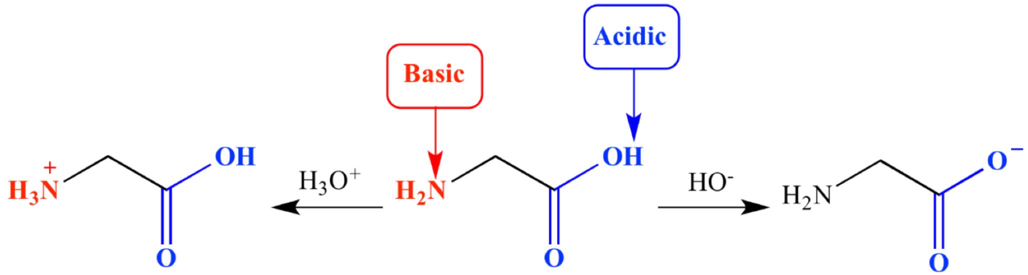
<u>Characteristic features of Amino Acids</u>:

1. *Amphoteric/ amphiprotic in nature:*



They have acidic and basic tendencies. The carboxyl group is able to lose a proton and the amine group is able to accept a proton. Amino acids are also ionic in character, and behave as ampholytes, meaning they move to their isoelectric points [pI] when placed in a pH gradient under an electric field.

2. Distinct R – gp determines the structure and thus unique biochemical functions of a protein:

As we shall study next, all naturally occurring sugars belong to the D series. It is interesting that nearly all known plant and animal proteins are composed entirely of L-amino acids. However, certain bacteria contain D-amino acids in their cell walls, and several antibiotics (e.g., actinomycin D and the gramicidins) contain varying amounts of D-leucine, D-phenylalanine, and D-valine.

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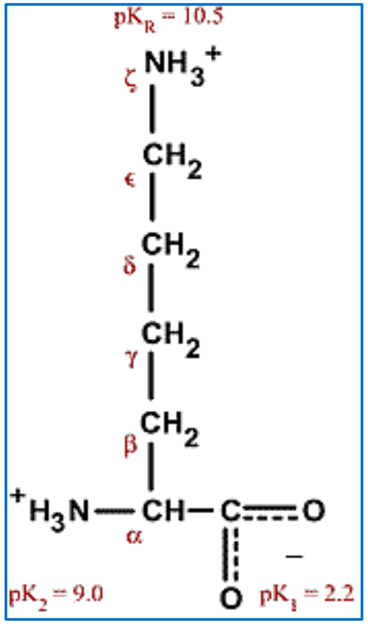
The side chain or R group attached to the α-carbon of each amino acid has unique characteristics arising from the size, shape, solubility, charge and ionization properties of its R group

For all the common amino acids except glycine, the α-carbon is bonded to four different groups: an amino, a carboxyl, an R group, and a hydrogen atom. In case of glycine, the R group is replaced by another hydrogen atom. The additional carbons in an R group are commonly designated β , γ, δ, ε, and so forth, proceeding out from the α-carbon.



Solubility is decided by polar/ non-polar nature of R group [hydrophilic/ hydrophobic R]

Classification according to the functional group on the side chain at neutral pH is

- *Nonpolar [hydrophobic]*
- *Polar but uncharged [hydrophilic]*
- *Polar negatively charged [hydrophilic]*
- *Polar positively charged [hydrophilic]*
- *Aromatic [hydrophilic/ hydrophobic]*

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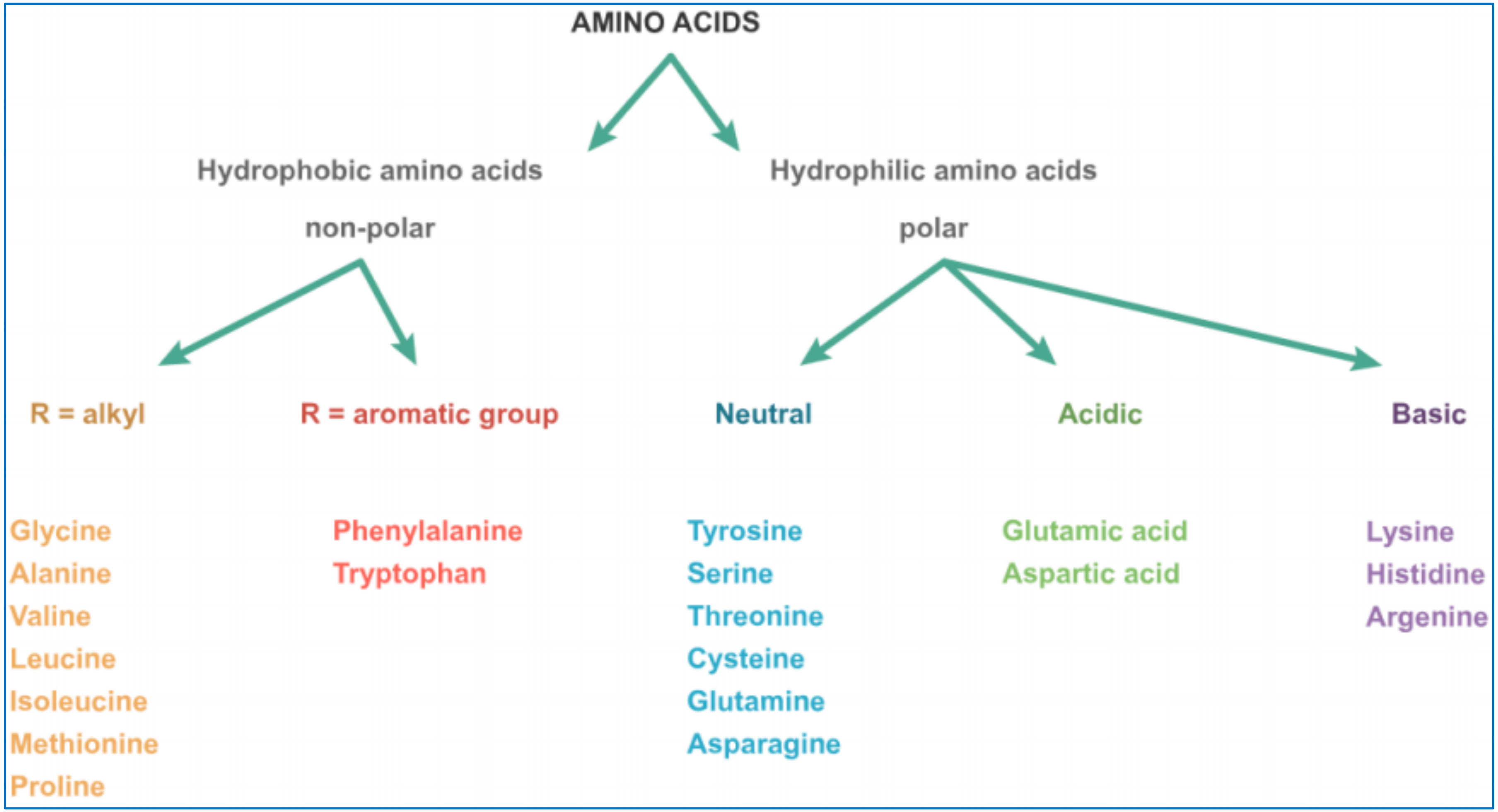
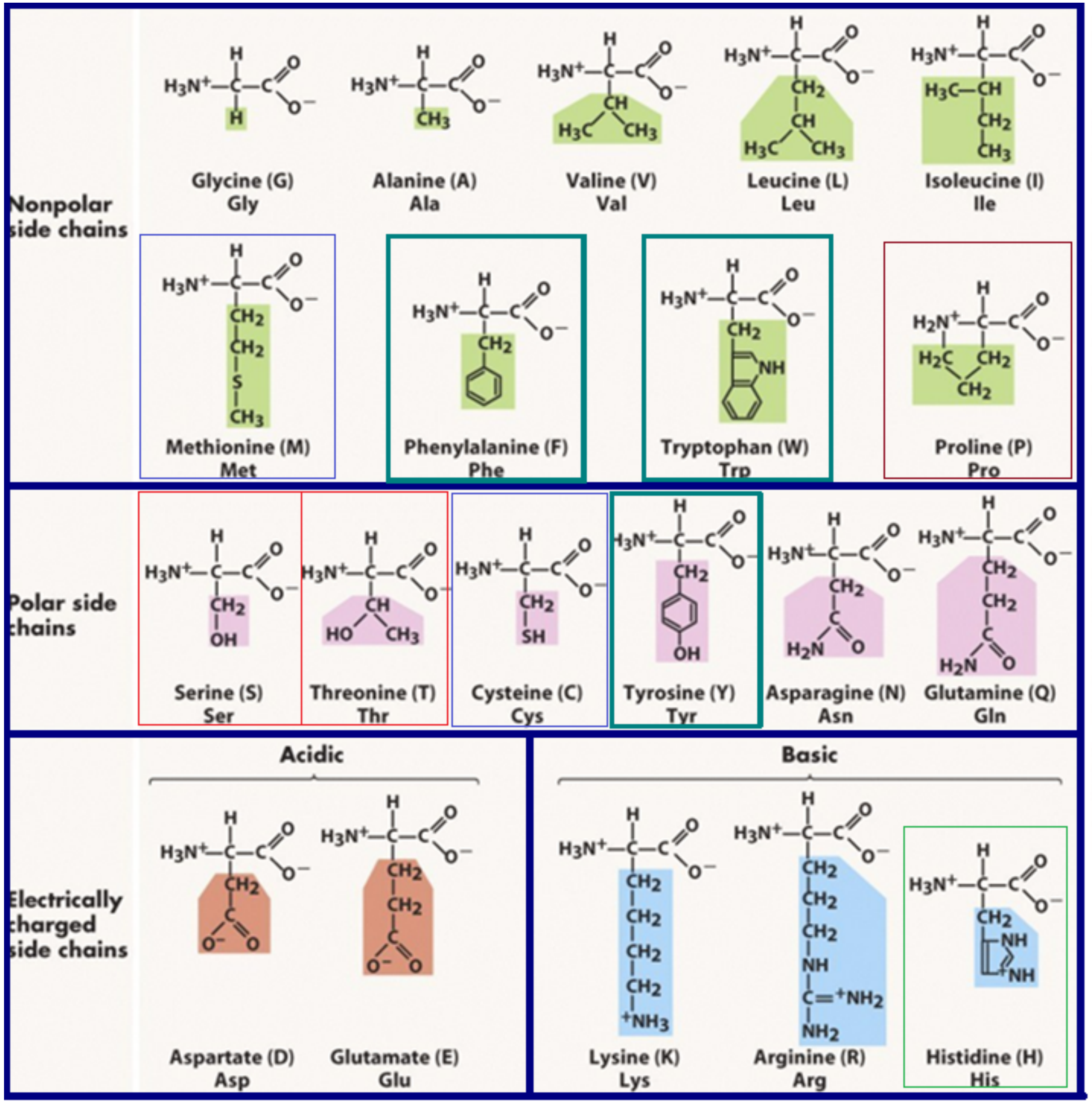

AMINO ACIDS
Hydrophobic amino acids
non-polar
Hydrophilic amino acids
polar
R = alkyl
R = aromatic group
Neutral
Acidic
Basic
Glycine
Alanine
Valine
Leucine
Isoleucine
Methionine
Proline
Phenylalanine
Tryptophan
Tyrosine
Serine
Threonine
Cysteine
Glutamine
Asparagine
Glutamic acid
Aspartic acid
Lysine
Histidine
Argenine

Classification of Amino acids by their R group



Aromatic

S- containing R:
Met, Cys

OH containing R:
Ser, Thr

Imino containing R:
Pro

Imidazole containing R:
His

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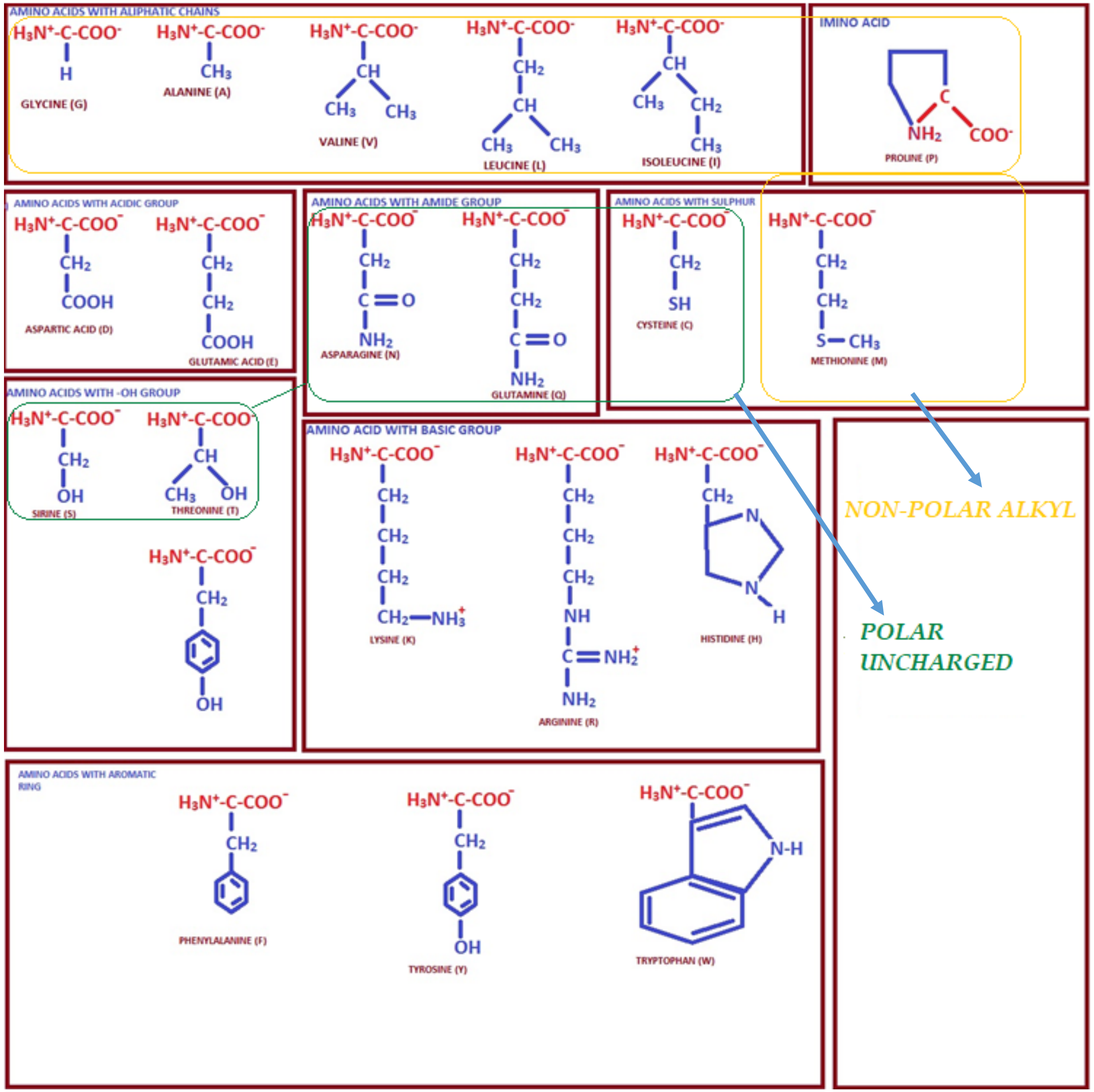

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The amino acids differ only in the substituent R attached to the α-carbon atom. This variation in R group gives proteins their structural and therefore functional diversity (as seen in enzymes/co-factors/ hormones).

Amino acids are classified based on:

- *Standard and Non-standard amino acids (aa)*
- *Essential and non-essential aa*
- *Side chain functional group*

1. *Standard amino-acids: Those 20 amino acids are encoded by universal genetic code*
2. *Non-Standard amino-acids: Two amino acids incorporated into proteins by unique synthetic mechanism*

•*Selenocysteine: Incorporated when mRNA translated included SECIS (selenocysteine insertion seq) element, causes the UGA codon to encode selenocysteine instead of stop codon)*

•*Pyrrolysine: used by methanogenic archaea in enzyme that they use to produce methane. It is coded for UAG stop codon.*

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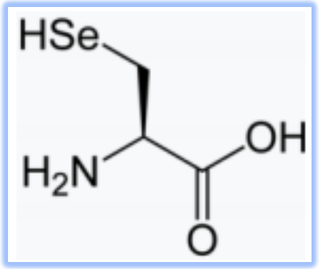
Non-standard Amino Acids

- *Selenocysteine, 21st protein L-α amino acids*
- *Selenium atom replaces the sulfur of its elemental analog, cysteine*
- *Selenocysteine is not the product of a posttranslational modification, but is inserted directly into a growing polypeptide during translation.*
- *Selenocysteine is charged on a special tRNA called tRNASec specific for UGA (STOP) codon inserted into growing polypeptide during translation*



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*There are 20 common amino acids occurring in nature and most proteins comprise of these. Of these 20, 10 amino acids are **essential** amino acids and must be obtained from our diets as the human body cannot synthesize them in adequate amounts. They are:*

Essential	Non-essential
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Hydroxyproline
Tryptophan	Hydroxylysine
Valine	Proline
	Serine
	Tyrosine

derived

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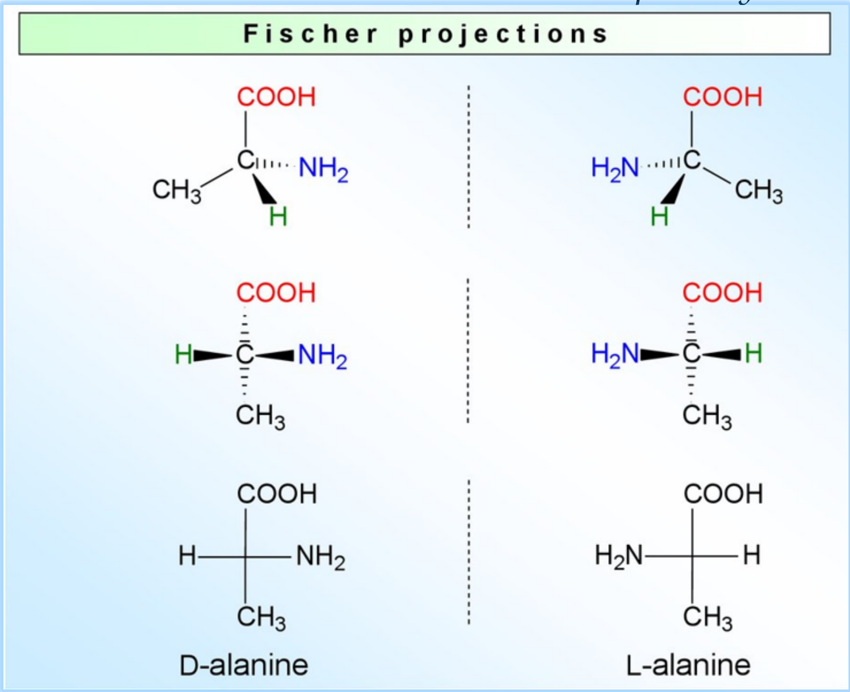
Configuration of Amino Acids

*The α-carbon atom is thus a chiral centre. Because of the tetrahedral arrangement of the bonding orbitals around the α-carbon atom, the four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible stereoisomers, non-superimposable mirror images of each other, called **enantiomers**. All molecules with a chiral centre are also optically active-that is, they rotate the plane of plane polarized light.*



Only the L-stereoisomers with a configuration related to the absolute configuration of the reference molecule L –glyceraldehyde are found in proteins.

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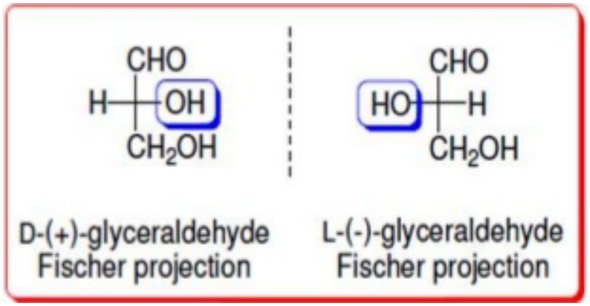
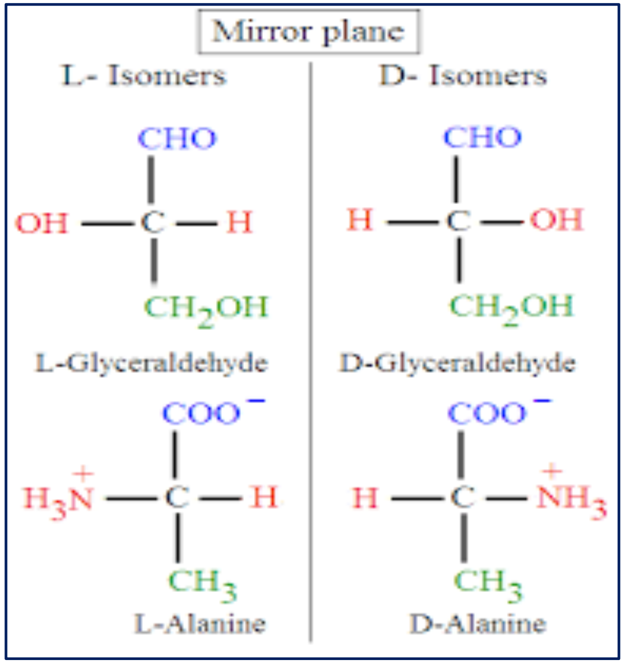
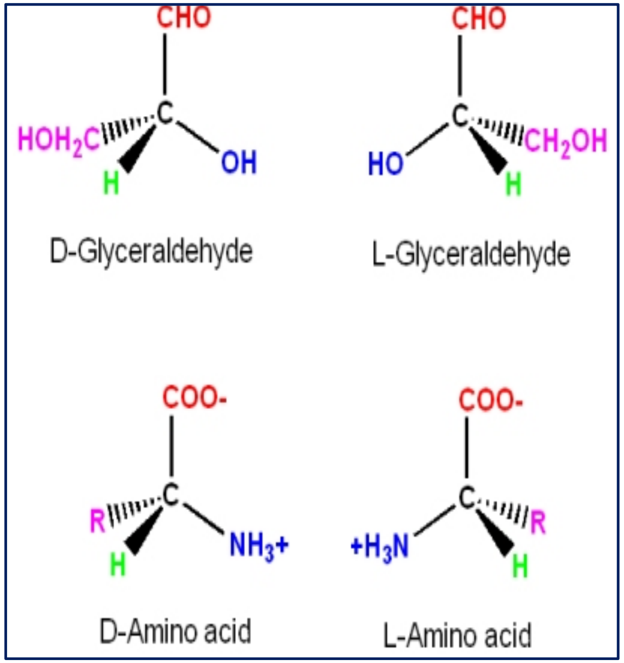
A D-amino acid is one where, in its Fischer projection formula, with carboxyl group on the top and R-group at the bottom of the vertical axis, amino group is on the right. Similarly, an L-amino acid has amino group on the left.







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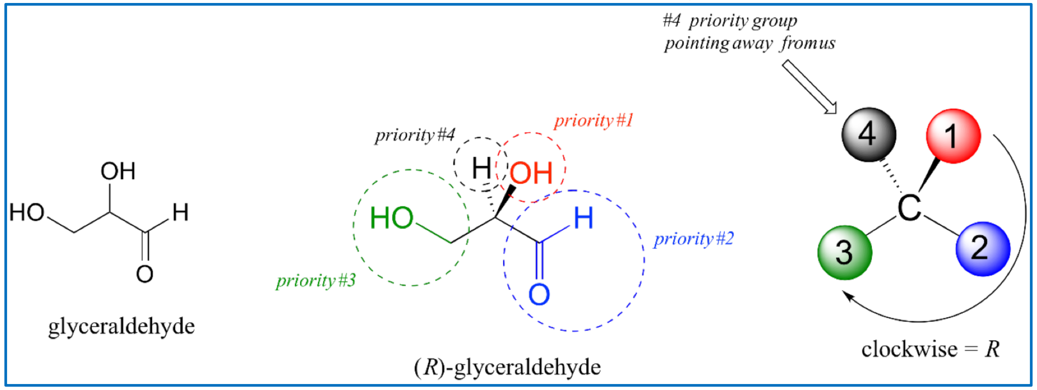
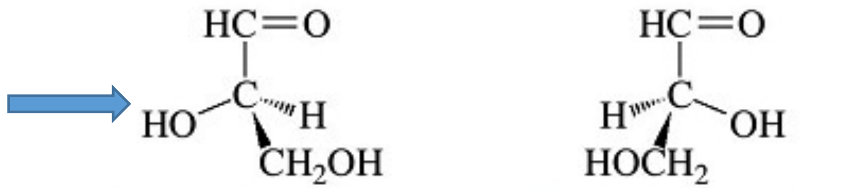
Counterclockwise is R (as H is on a horizontal bond)

For the configuration of a Fischer projection formula, ***only if the lowest priority substituent is on a <u>vertical</u> bond****, will a clockwise arrow from the highest priority to the next highest priority substituent denote R-configuration & counterclockwise denote S. On the other hand,* ***if the lowest priority substituent is on a <u>horizontal</u> bond****, counterclockwise specifies R and clockwise specifies S configuration.*



Anticlockwise is S



(R)-(+)-glyceraldehyde **(S)-(–)-glyceraldehyde**

Clockwise is R

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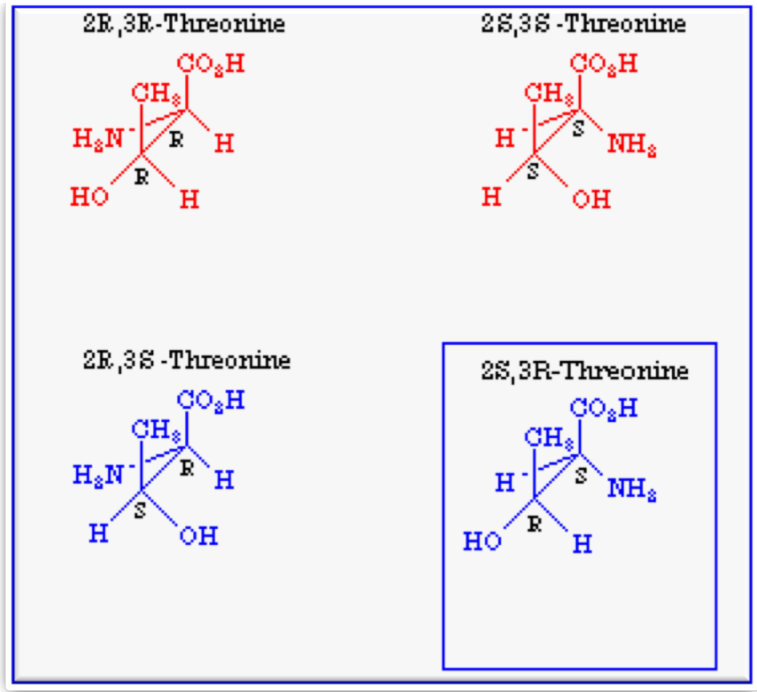
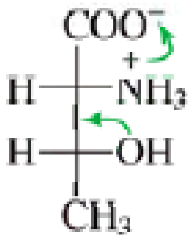
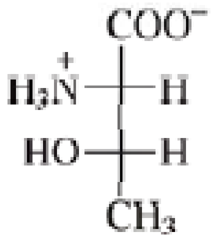
Threonine has two chiral centers: C-2, C-3; so it has $2^n = 2^2 = 4$ stereoisomers.

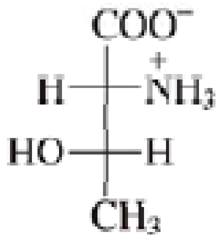




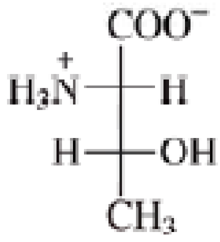
A



B



C



D

Naturally occurring L-threonine is (2S, 3R)-threonine. Thus, since H is on a horizontal bond, (2S, 3R) for C-2 & C-3 is denoted by stereoisomer D, where $^+NH_3$ group is on the left.

The common amino acids of proteins have been assigned three-letter abbreviations and one-letter symbols.

The three-letter code comprises of the first three letters of the amino acid name. e.g. Glycine: Gly

The one letter code indicates the composition and sequence of amino acids in proteins. e.g. Valine: V

Abbreviations for Amino Acids

- Each amino acid has standard 3-letter and 1-letter abbreviations (shown in the table below)

Name	3-Let	1-Let	Name	3-Let	1-Let
Glycine	Gly	G	Serine	Ser	S
Alanine	Ala	A	Threonine	Thr	T
Valine	Val	V	Asparagine	Asn	N
Leucine	Leu	L	Glutamine	Gln	Q
Isoleucine	Ile	I	Tyrosine	Tyr	Y
Phenylalanine	Phe	F	Aspartic Acid	Asp	D
Methionine	Met	M	Glutamic Acid	Glu	E
Proline	Pro	P	Lysine	Lys	K
Tryptophan	Trp	W	Arginine	Arg	R
Cysteine	Cys	C	Histidine	His	H

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In addition to the 20 common amino acids, proteins may contain residues created by modification of common residues already incorporated into a polypeptide.

Among these uncommon amino acids are 4-hydroxyproline, a derivative of proline, and 5-hydroxylysine, derived from lysine. The former is found in plant cell wall proteins, and both are found in collagen, a fibrous protein of connective tissues.

6-N-Methyllysine is a constituent of myosin, a contractile protein of muscle. Another important uncommon amino acid is 1-carboxyglutamate, found in the blood-clotting protein prothrombin and in certain other proteins that bind Ca^{2+} as part of their biological function. More complex is desmosine, a derivative of four Lys residues, which is found in the fibrous protein elastin. Selenocysteine is a special case. This rare amino acid residue is introduced during protein synthesis rather than created through a postsynthetic modification. It contains selenium rather than the sulfur of cysteine. Actually derived from serine, selenocysteine is a constituent of just a few known proteins.

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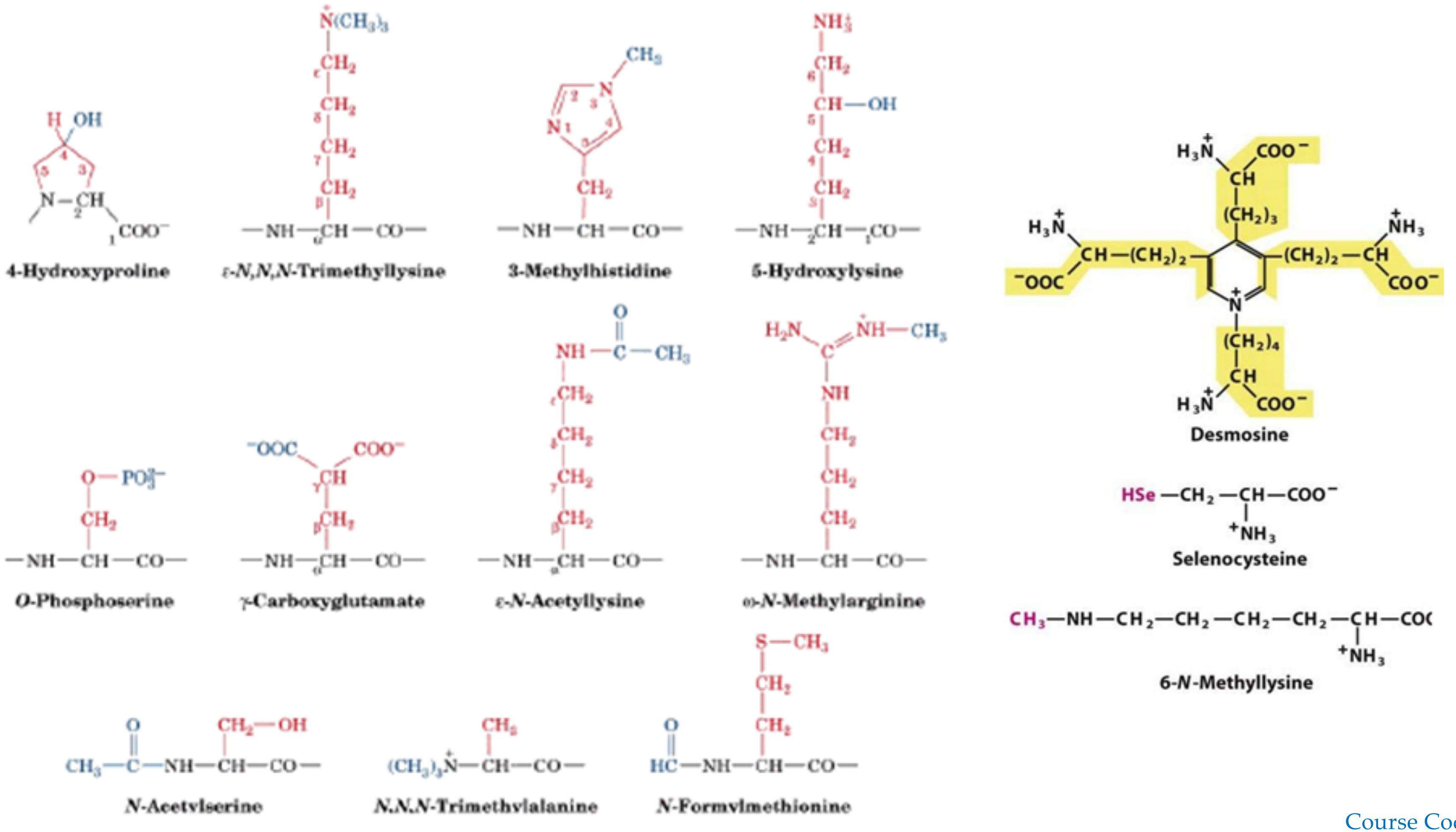
Uncommon amino acids that are components of certain proteins



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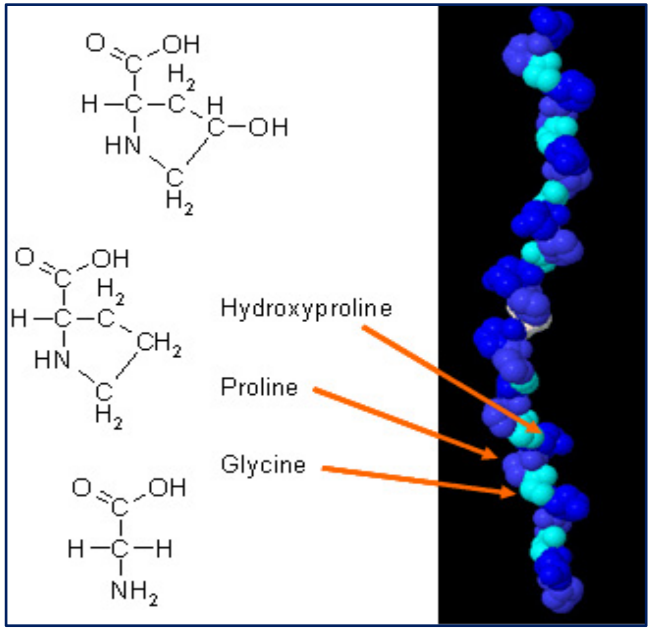
Collagen is a tough protein found in tendons and connective tissue, in skin, bone, and teeth. It contains large amounts of glycine, proline, and hydroxyproline.

When the collagen molecule is assembled, it incorporates Pro [Proline] where Hyp [4-Hydroxyproline] is required. Once the protein is complete, some of the proline residues are oxidized to 4-hydroxyproline. This oxidation requires vitamin C to catalyze it. Thus vitamin C deficiency causes scurvy—symptoms of scurvy suffered by sailors (loose teeth, sores, and blisters) is caused by the inability to make collagen.



α-Amino acids	Functions
1.Ornithine	Intermediate in the biosynthesis of urea
2.Citrulline	Intermediate in the biosynthesis of urea
3.Arginosuccinic acid	Intermediate in the biosynthesis of urea
4.Thyroxine	Thyroid hormone derived from tyrosine.
5.Triiodothyronine	Thyroid hormone derived from tyrosine
6.SAM	Methyl donor in biological system.
7.Homocysteine	Intermediate in methionine metabolism. A risk factor for coronary heart diseases.
8. 3,4-Dihydroxy phenyl alanine (DOPA)	A neurotransmitter, precursor for melanin
9.Creatinine	Derived from muscle and excreted in urine.

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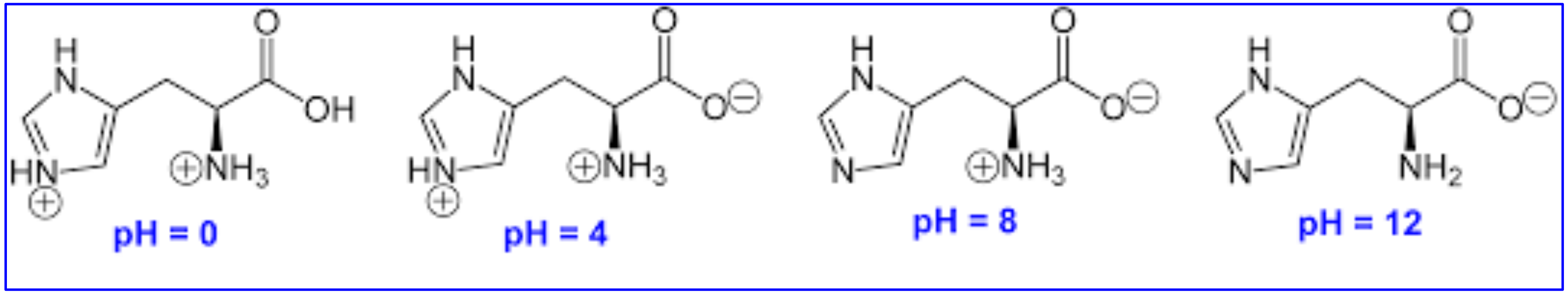
When an amino acid lacking an ionizable R group is dissolved in water at neutral pH, it exists in solution as the dipolar ion, or zwitterion ("hybrid ion"), which can act as either an acid or a base therefore amphoteric and are often called ampholytes.

The pK_a is a measure of the tendency of a group to give up a proton, and it decreases ten times as the pK_a increases by one unit.

The pK_a of any functional group is greatly affected by its chemical environment, a phenomenon sometimes exploited in the active sites of enzymes to promote enzyme-catalyzed reaction mechanisms.

When an amino acid having side chain with ionizable hydrogens R group is dissolved in water it can exist in different forms or tautomers depending on the pH of the solution.

e.g. Histidine has 4 different forms



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The characteristic pH at which the net electric charge is nil is called the ***isoelectric point*** *or* ***isoelectric pH****, designated as* ***pI****.*

pI = pH at which there is no net electric charge

For glycine, which has no ionizable group in its side chain, the isoelectric point is simply the arithmetic mean of the two pK_a values:

$$\boxed{pI = \frac{2.34 + 9.69}{2} = 6.02}$$

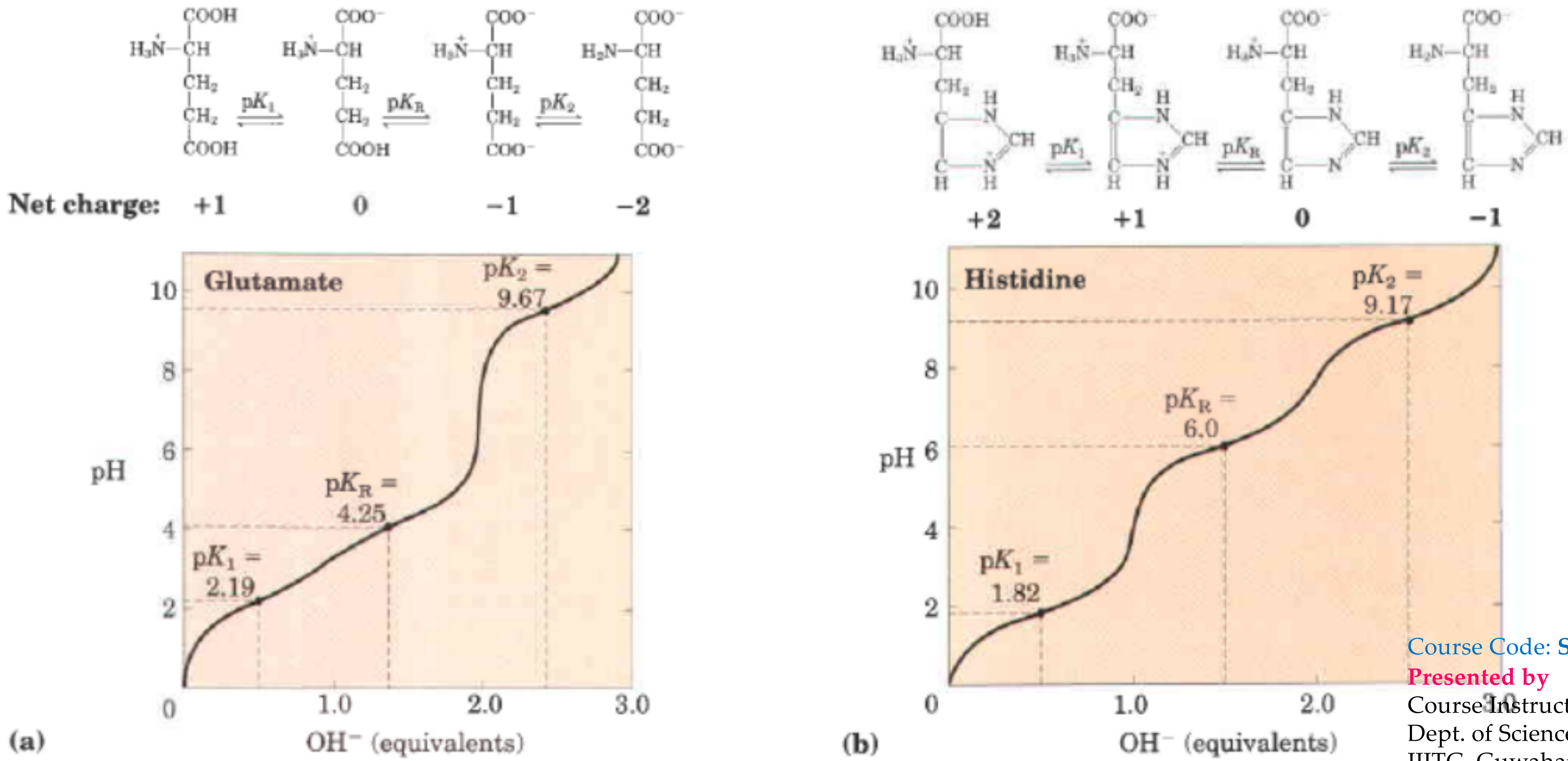
The pI of an amino acid whose side chain is not ionizable, is an average of its two pK_a values.

The pI of an amino acid which has an ionizable side chain, is the average of the pK_a values of similarly ionizing groups (either positively charged to uncharged groups or uncharged groups to negatively charged groups).

pK_a values of Amino acids

Amino acid	**pK_a α-COOH**	**pK_a α–NH3**	**pK_a side chain**
Alanine	2.34	9.69	-
Arginine	2.17	9.04	12.48
Asparagine	2.02	8.84	-
Aspartic acid	2.09	9.82	3.86
Cysteine	1.92	10.46	8.35
Glutamic acid	2.19	9.67	4.25
Glutamine	2.17	9.13	-
Glycine	2.34	9.60	-
Histidine	1.82	9.17	6.04
Isoleucine	2.36	9.68	-
Leucine	2.36	9.60	-
Lysine	2.18	8.95	10.79
Methionine	2.28	9.21	-
Phenylalanine	2.16	9.18	-
Proline	1.99	10.60	-
Serine	2.21	9.15	-
Threonine	2.63	9.10	-
Tryptophan	2.38	9.39	-
Tyrosine	2.20	9.11	10.07
Valine	2.32	9.62	-

Amino acids vary in their acid-base properties and have characteristic titration curves. All amino acids with a single α-amino group, a single α-carboxyl group, and an R group that does not ionize have titration curves resembling that of glycine. However, amino acids with an ionizable R group have more complex titration curves, with three stages corresponding to the three possible ionization steps; thus they have three pK_a values.



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Separation of Amino Acids

The various techniques by which the amino acids can be separated are:

Electrophoresis: *separates amino acids on basis of their pI values. Paper/gel containing amino acid mixture is placed in a buffered solution between two electrodes & an electric field is applied. Amino acid with pI< pH of buffer has overall negative charge & migrates towards anode. After separation, paper is dipped into ninhydrin solution & dried in an oven.*

Paper/ Thin Layer Chromatography: *separates amino acids on basis of their polarity. Least polar amino acid travels farthest, most polar amino acid travels least. Then paper is developed with ninhydrin to reveal the spots.*

Ion-Exchange Chromatography: *can both identify & determine relative amounts of amino acids in the mixture. A solution of mixture of amino acids is loaded onto the top of a column packed with an insoluble resin. The amino acids move through the column at different rates and are separated differentially, collected in fractions, ninhydrin added to each fraction and relative amounts of amino acids found using visible spectroscopy.*

An instrument that automates ion-exchange chromatography is called an amino acid analyzer.

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