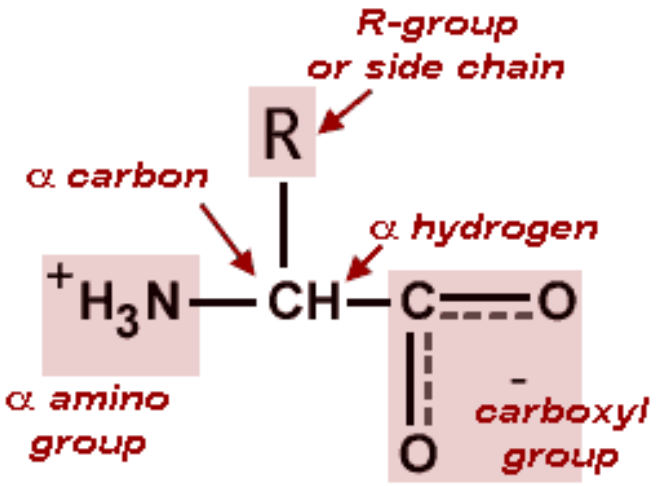
Biochemistry & Clinical Pathology
(2114)

Unit 2
Brief Chemistry of Amino Acids

DEFINITION OF AMINO ACIDS

- *Amino acids are the unsymmetrical organic molecules containing a carboxylic group , amino group and hydrogen atom at the same carbon atom and remaining valancy of carbon is satisfied with a side chain denoted by 'R'.*
- *This side chain 'R' is responsible for chemical and biological behavior of amino acid.*
- *Since Amino acids form proteins (Polypeptides) by condensation reaction involving peptide/amide bonds so also called monopeptide.*



CLASSIFICATION OF AMINO ACIDS

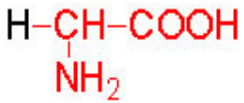
- *Amino acids are generally divided into groups on the basis of their side chains (R'' groups).*
- *Amino Acids are classified into:*

1. *Nonpolar Amino Acids*
2. *Polar (Neutral) Amino Acids*
3. *Charged (+ve & -ve) Amino Acids*

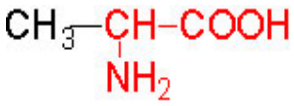
NON-POLAR AMINO ACIDS

- *Only carbon and hydrogen in their side chains.*
- *Generally unreactive but hydrophobic.*
- *Determining the 3-D structure of proteins (they tend to cluster on the inside of the molecule).*

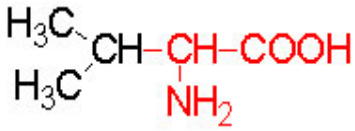
Glycine (Gly)



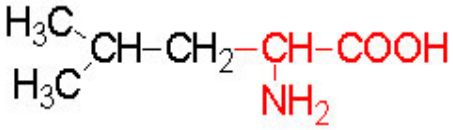
Alanine (Ala)



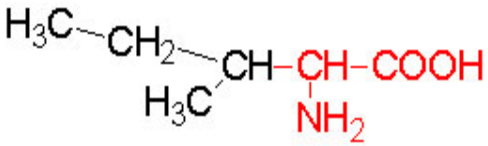
Valine (Val)



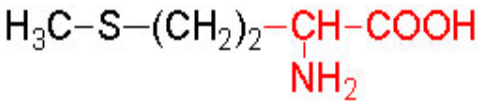
Leucine (Leu)



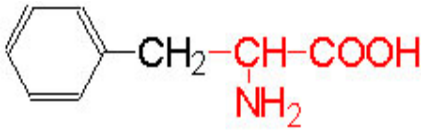
Isoleucine (Ile)



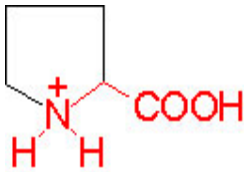
Methionine (Met)



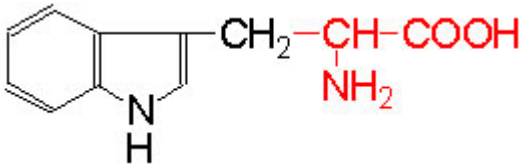
Phenylalanine (Phe)



Proline (Pro)



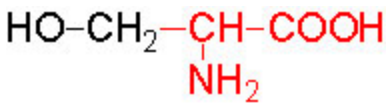
Tryptophan (Trp)



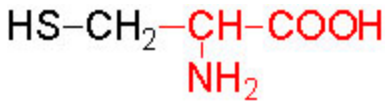
- *The simplest amino acid is Glycine, which has a single hydrogen atom as its side chain. Glycine is the only chiral amino acid.*
- *Alanine, Valine, Leucine and Isoleucine have saturated hydrocarbon 'R' groups (i.e. they only have hydrogen and carbon linked by single covalent bonds). Leucine and Isoleucine are isomers.*
- *The side chain of Methionine includes a sulfur atom but remains hydrophobic in nature.*
- *Phenylalanine is similar to Alanine with an additional benzene group on the end. Phenylalanine is highly hydrophobic and is found buried within globular proteins.*
- *Tryptophan is highly hydrophobic and tends to be found immersed inside globular proteins.*
- *Proline is unique amongst the amino acids – its side chain is bonded to the backbone nitrogen as well as to the a-carbon.*
- *Because of this proline is technically an imino rather than an amino acid.*
- *The ring is not reactive, but it does restrict the geometry of the backbone chain in any protein where it is present.*

POLAR AMINO ACIDS

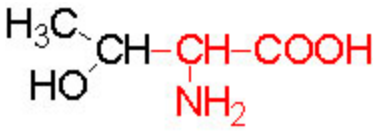
Serine (Ser)



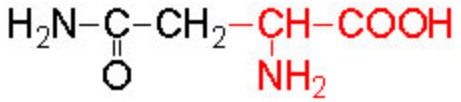
Cysteine (cys)



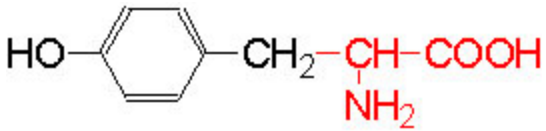
Threonine (Thr)



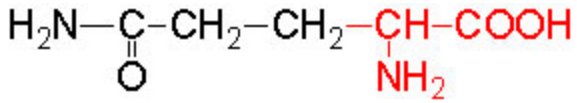
Asparagine (Asn)



Tyrosine (Tyr)



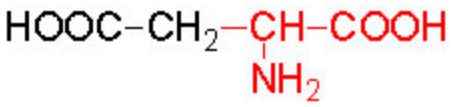
Glutamine (Gln)



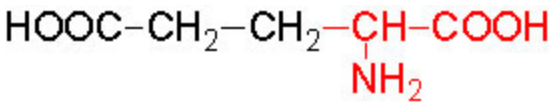
- ***Tyrosine*** *is similar to Phenylalanine with an additional hydroxyl (-OH) group.*
- *It is polar and very weakly acidic. Tyrosine plays an important catalytic role in the active site of some enzymes. (Reversible phosphorylation of –OH group in some enzymes in the regulation of metabolic pathways).*
- ***Serine*** *and* ***Threonine*** *play important role in enzymes which regulate phosphorylation and energy metabolism.*
- ***Cysteine*** *has sulfur-containing side group.The group has the potential to be more reactive.It is not very polar.*
- ***Cysteine*** *is most important for its ability to link to another cysteine via the sulfur atoms to form a covalent disulfide bridge, important in the formation and maintenance of the tertiary (folded) structure in many proteins.*
- ***Asparagine and Glutamine*** *are the amide derivatives of Aspartate (Aspartic acid) and Glutamate (Glutamic acid). They cannot be ionised and are therefore uncharged.*

NEGATIVELY CHARGED NON-POLAR AMINO ACIDS

Aspartic acid (Asp)



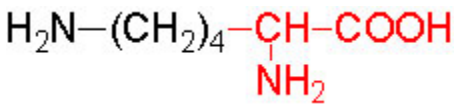
Glutamic acid (Glu)



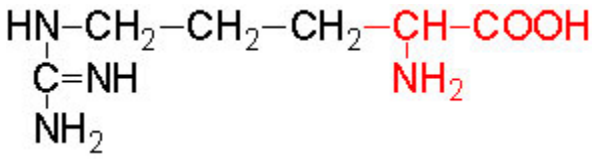
- *Two amino acids with negatively charged (i.e. acidic) side chains - Aspartate (Aspartic acid) and Glutamate (Glutamic acid).*
- *These amino acids confer a negative charge on the proteins of which they are part.*

POSITIVELY CHARGED NON-POLAR AMINO ACIDS

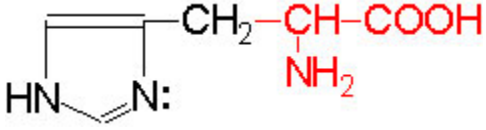
Lysine (Lys)



Arginine (Arg)



Histidine (His)



- ***Lysine*** *and* ***Arginine*** *both have pKs around 10.0 and are therefore always positively charged at neutral pH.*
- *With a pK of 6.5,* ***Histidine*** *can be uncharged or positively charged depending upon its local environment.*
- *Histidine has an important role in the catalytic mechanism of enzymes and explains why it is often found in the active site.*

CLASSIFICATION BASED ON CHEMICAL CONSTITUTION

- ***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*

ESSENTIAL AMINO ACIDS

- *Essentially required in diet since humans are incapable of forming requisite carbon skeleton.*

Arginine⭐	*Lysine*
Histidine⭐	*Methionine*
Isoleucine	*Threonine*
Leucine	*Phenylalanine*
Valine	*Tryptophan*

⭐ *Essential in children, not in adults.*

NON-ESSENTIAL AMINO ACIDS

- *These are not required in diet since these can an be synthesized from α-keto acids by transamination and subsequent reactions.*

Alanine
Asparagine
Aspartate
Glutamate
Glutamine

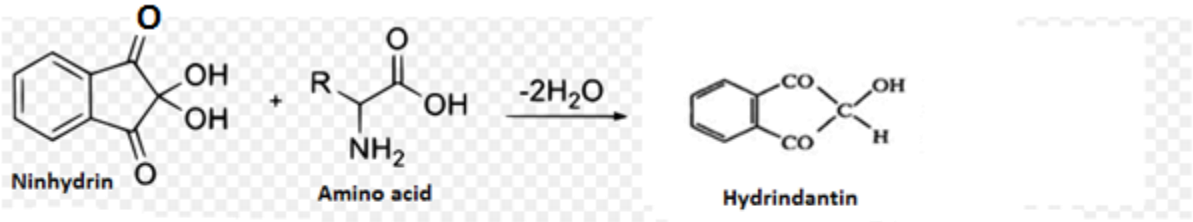
Glycine
Proline
Serine
Cysteine (from Met⋆)
Tyrosine (from Phe⋆)

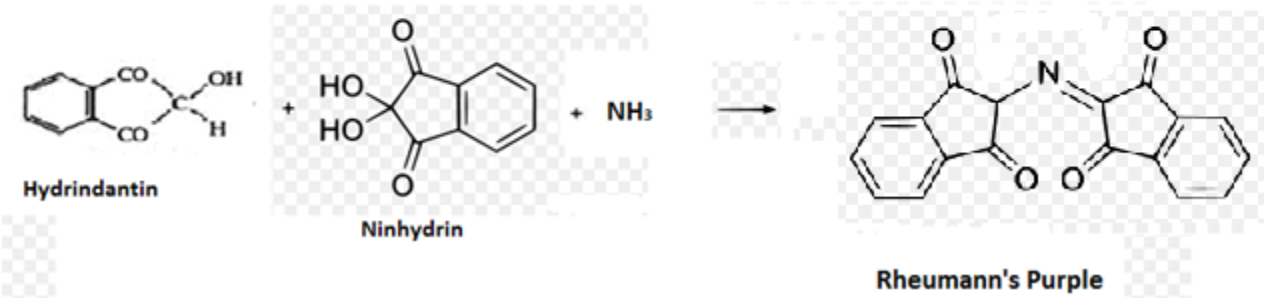
⋆ *Essential amino acids*

QUALITATIVE TESTS FOR AMINO ACIDS

1. *Ninhydrin test*

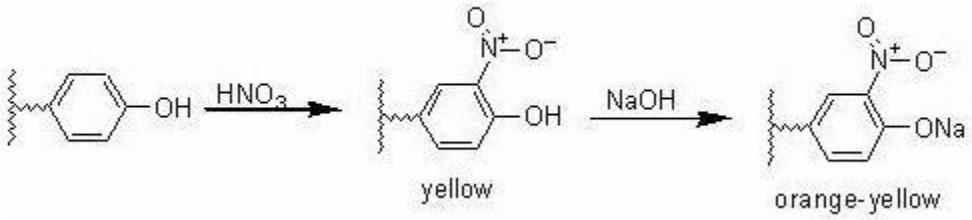
In the pH range of 4-8, all α- amino acids react with ninhydrin (triketohydrindene hydrate), a powerful oxidizing agent to give a purple colored product (diketohydrin) termed Rhuemann's purple.





2. Xanthoproteic acid test

Aromatic amino acids, such as Phenyl alanine, tyrosine and tryptophan, respond to this test. In the presence of concentrated nitric acid, the aromatic phenyl ring is nitrated to give yellow colored nitro-derivatives. At alkaline pH, the color changes to orange due to the ionization of the phenolic group.



- ***Pauly's diazo Test***

This test is specific for the detection of Tryptophan or Histidine. The reagent used for this test contains sulphanilic acid dissolved in hydrochloric acid. Sulphanilic acid upon diazotization in the presence of sodium nitrite and hydrochloric acid results in the formation a diazonium salt. The diazonium salt formed couples with either tyrosine or histidine in alkaline medium to give a red coloured chromogen (azo dye).

- ***Millon's test***

Phenolic amino acids such as Tyrosine and its derivatives respond to this test. Compounds with a hydroxybenzene radical react with Millon's reagent to form a red colored complex. Millon's reagent is a solution of mercuric sulphate in sulphuric acid.

- ***Histidine test***

This test was discovered by Knoop. This reaction involves bromination of histidine in acid solution, followed by neutralization of the acid with excess of ammonia. Heating of alkaline solution develops a blue or violet coloration.

- ***Hopkins cole test***

This test is specific test for detecting tryptophan. The indole moiety of tryptophan reacts with glyoxilic acid in the presence of concentrated sulphuric acid to give a purple colored product. Glyoxilic acid is prepared from glacial acetic acid by being exposed to sunlight.

- ***Sakaguchi test***

Under alkaline condition, α- naphthol (1-hydroxy naphthalene) reacts with a mono-substituted guanidine compound like arginine, which upon treatment with hypobromite or hypochlorite, produces a characteristic red color.

- ***Lead sulphide test***

Sulphur containing amino acids, such as cysteine and cystine. upon boiling with sodium hydroxide (hot alkali), yield sodium sulphide. This reaction is due to partial conversion of the organic sulphur to inorganic sulphide, which can detected by precipitating it to lead sulphide, using lead acetate solution.

- ***Folin's McCarthy Sullivan Test***

Imino acids such as Proline and hydroxyproline condense with isatin reagent under alkaline condition to yield blue colored adduct. Addition to sodium nitroprusside[Na2Fe(CN)5NO] to an alkaline solution of methionine followed by the acidification of the reaction yields a red colour. This reaction also forms the basis for the quantitative determination of methionine.

- ***Isatin test***

Imino acids such as Proline and hydroxyproline condense with isatin reagent under alkaline condition to yield blue colored adduct.

BIOLOGICAL VALUES OF AMINO ACIDS

Essential amino acids (mg/ g protein)	Milk	Eggs	Beef	Wheat	Rice	Soy
Phe + Tyr	102	93	80	80	91	88
His	27	22	34	25	26	28
Ile	47	54	48	35	40	50
Leu	95	86	81	72	86	85
Lys	78	70	89	31	40	70
Met+Cys	33	57	40	43	36	28
Thr	44	47	46	31	41	42
Valine	64	66	50	47	58	53
Trp	14	17	11	12	13	14
All essential amino acids	477	**490** ↑	445	351	405	430

CLINICAL SIGNIFICANCE OF AMINO ACIDS

- ***L-tyrosine*** *is sometimes recommended by practitioners as helpful for weight loss, clinical depression.*
- ***Dopamine*** *derived from* ***tyrosine*** *is a neurotransmitter.*
- *Thyroxine is an important thyroid hormone from* ***tyrosine****.*
- *Gamma aminobutyric acid (GABA) derived from* ***glutamic acid****; which is a neurotransmitter.*
- *Cycloserine derived from serine is an anti-tuberculous drug.*
- ***Arginine*** *stimulates the healing of burning wound and other wounds.*
- ***Arginine*** *together with lysine can limit herpes attacks (bladders in the mouth) for people that carry this virus.*
- ***Cysteine*** *can help to recover the damage by smoking and alcohol.*
- ***Glutamine*** *can give the mind new energy and can help to prevent and treat inflammations of the large intestine.*

DISEASES RELATED TO AMINO ACIDS

Medical condition	Approximate incidence (per 100,000 births)	Defective process	Defective enzyme	Symptoms and effects
Albinism	<3	Melanin synthesis from tyrosine	Tyrosine 3-monooxygenase (tyrosinase)	Lack of pigmentation; white hair, pink skin
Alkaptonuria	<0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine; late-developing arthritis
Argininemia	<0.5	Urea synthesis	Arginase	Mental retardation
Argininosuccinic acidemia	<1.5	Urea synthesis	Argininosuccinase	Vomiting; convulsions
Carbamoyl phosphate synthetase 1 deficiency	<0.5	Urea synthesis	Carbamoyl phosphate synthetase 1	Lethargy; convulsions; early death
Homocystinuria	<0.5	Methionine degradation	Cystathionine β-synthase	Faulty bone development; mental retardation
Maple syrup urine disease (branched-chain ketoaciduria)	<0.4	Isoleucine, leucine, and valine degradation	Branched-chain α-keto acid dehydrogenase complex	Vomiting; convulsions; mental retardation; early death
Methylmalonic acidemia	<0.5	Conversion of propionyl-CoA to succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting; convulsions; mental retardation; early death
Phenylketonuria	<8	Conversion of phenylalanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; mental retardation


THANK YOU