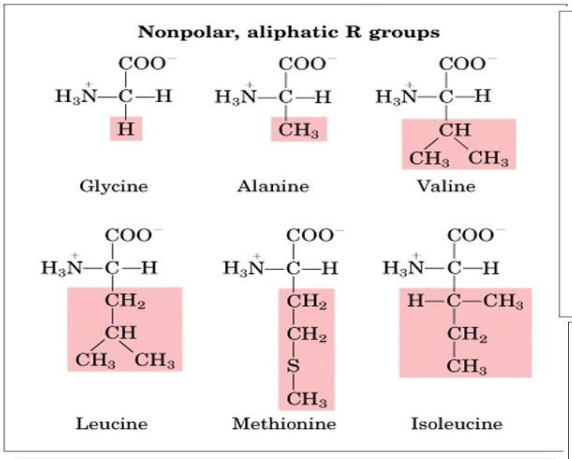
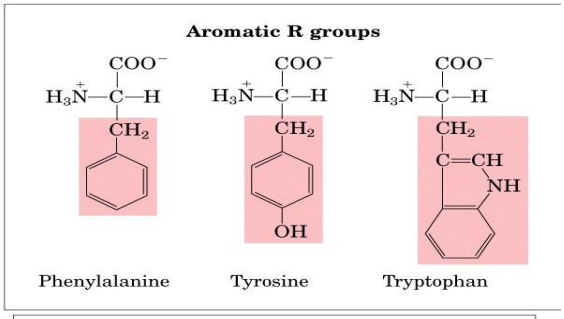
Amino Acid- Qualitative tests

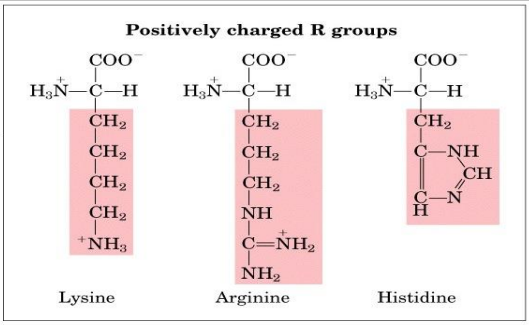
Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. There are 20 natural amino acids that are found within proteins convey a vast array of chemicals versatility. All of them are L-α amino acids.

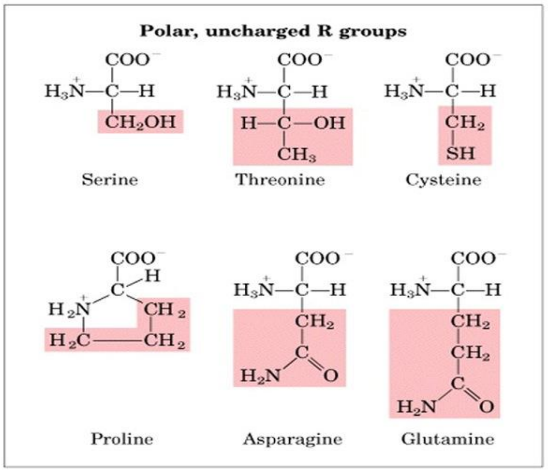
All amino acids found in proteins have this basic structure, differing only in the structure of the R-group or the side chain. The simplest, and smallest, amino acid found in proteins is **glycine** for which the R-group is hydrogen (H). All these amino acids are found in solutions in their ionized form. They are polarized and their ionization depends on the pH of the medium where they are located. According to their ionization (polarity) in water, they are classified into 4 categories: 1- Non-polar. 2- Uncharged polar. 3- Basic polar (positively charged). 4- Acidic polar (negatively charged).

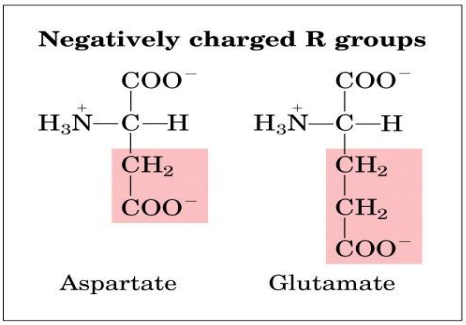
Polar amino acids are more soluble in water than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution, and therefore, to act as acids and bases. A molecule that functions as such is known as an **amphoteric**. - The pH value at which concentration of anionic and cationic groups are equal (i.e the net charge of this molecule equals zero) is known as isoelectric point (pI), a point at which the molecule does not move to either cathode or anode if it is put in electric field and its solubility is minimum. - Amino acids are able to rotate polarized light either to the left (livo) l. or to the right (dextro) d, since they have an asymmetric C atom (a carbon atom linked to 4 different groups), glycine which lacks asymmetric C atom (has 2 H+ on α-C) is an exception(can not rotate light).











Amino Acid- Qualitative tests

2.1. Qualitative tests of amino acids:

Solubility test:

Amino acids are generally soluble in water and insoluble in non-polar organic solvents such as hydrocarbons. This is because the presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution, and therefore, to act as acids and bases

Objective: to test the solubility of amino acids in different solvent.

Materials:

- Different amino acid solutions: glycine , lysine, Arginine Solvents:
- HCL
- NaOH
- chloroform
- Test tubes

Method:

1. Add 4ml of different solvents in 3 clean test tubes then place 1 ml ofglycine, then repeat the experiment with Arginine and lysine
2. Shake the tubes thoroughly, then leave the solution for about one minute,
3. Notice what happened to the solution
4. Record your result

2.1.2 Ninhydrin test

Objective: to detect α-L-amino acids.

Caution: Ninhydrin is a strong oxidizing agent, it should be handled with care, and applied apart from contact with skin or eyes, gloves and mask is a must, using hood is required, if accidently get in touch with the skin, the resulting stains is a temporarily one, that will be eliminated within 24 hours.

Principle: Ninhydrin (triketohydrindene hydrate) degrades amino acids into aldehydes (on pH range 4-8), ammonia and CO2 though a series of reactions. The net result is ninhydrin in a partially reduced from hydrindantin. Ninhydrinthen condenses with ammonia and hydrindantin to produce an intensely blue or purple pigment, sometimes called ruhemann's purple: Proline and hydroxyproline (amino acids) give yellow color.

The blueish-purple result is usually associated with primary amino acids. In these amino acids, the N is free to react with ninhydrin. However, in proline, the N is not available for reaction as it is locked in the ring structure. Therefore no ammonia is produced, so no blue color is presented.

1-Place 1 ml of each of the solutions in a test tube and add 1 ml of ninhydrin solution.

2- Boil the mixture over a water bath for 2 min. (be carful!!!)

3- Allow to cool and observe the blue color formed

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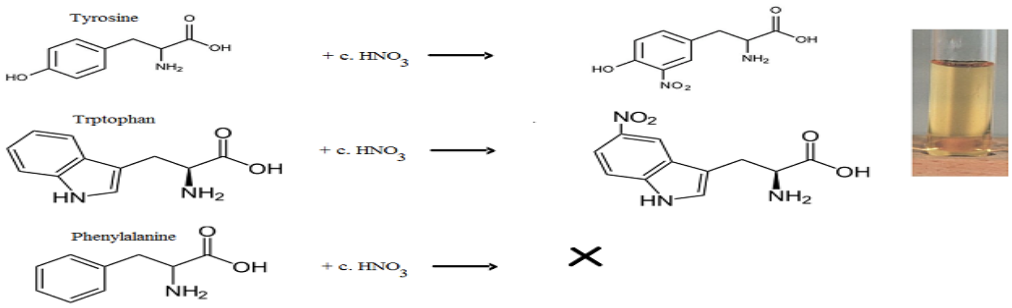
2.1.3. Xanthoproteic test

Objective: to differentiate between aromatic amino acids which give positive results and other amino acids. Amino acids containing an aromatic nucleus form yellow nitro derivatives on heating with concentrated HNO 3.

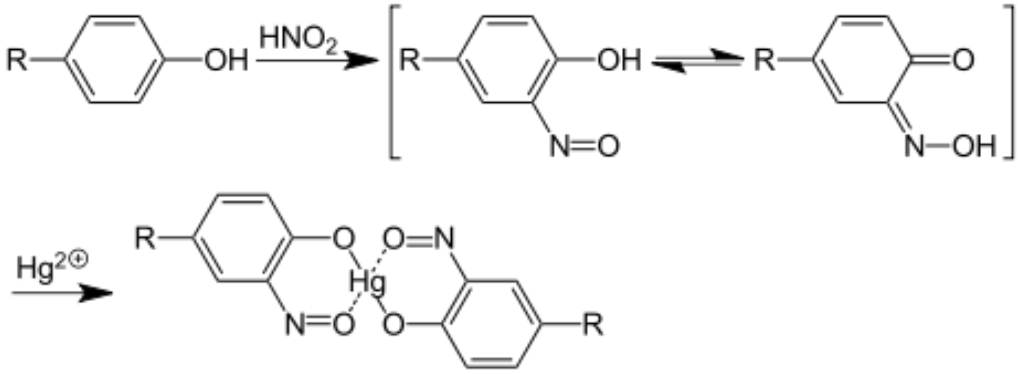
Principle: - Concentrated nitric acid reacts with the aromatic rings that are derivatives of benzene giving the characteristic nitration reaction. Amino acids tyrosine and tryptophan contain activated benzene rings which are easily nitrated to yellow colored compounds. The aromatic ring of phenyl alanine dose not react with nitric acid despite it contains a benzene ring, but it is not activated, therefore it will not react.



2.1.4. Millon's test

This test is specific for tyrosine, the only amino acid containing a phenol group, a hydroxyl group attached to benzene ring. Objective: to detect the presence of tyrosine in the sample.

Principle: - In Milon's test, the phenol group of tyrosine is first nitrated by nitric acid in the test solution. Then the nitrated tyrosine complexes mercury ions in the solution to form a brick-red solution or precipitate of nitrated tyrosine, in all cases, appearance of red color is positive test. Note: all phenols (compound having benzene ring and OH attached to it) give positive results in Millon's test.



2.1.5. Sakaguchi Test

Objective: Sakaguchi test is a specific qualitative test for the detection of amino acid containing gauanidium group [R-NH-C= (NH2)2+-NH2]. In other words it's a test for guanidines, i.e arginine.

Principle: In alkaline solution, arginine react with α-naphthol and sodium hypobromite /chlorite as an oxidize agent, to form red complexes as a positive result

Material:

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glycine,

arginine

10%NaOH

α-naphthol in 10% ethanol

5%sodium hypobromate (or sodium hypochlorite)

Method:

Label 3 test tube and put in each one 2 ml of the amino acid solution .

Add to each tube 2ml of NaOH solution. Mix well

Add to each tube 2ml of α-naphthol solution. Mix well

Add to each tube 3 drops of sodium hypobromite solution, and record your result

2.1.6. Detection of amino acids containing sulfhydral group (- SH)- Lead Sulfite Test

This test is specific for –SH containing amino acid (Cystein).

Principle: - Some of sulfur in cystine, is converted to sodium sulfide by boiling with 40% NaOH. - The Na 2 S can be detected by the precipitation of PbS from an alkaline solution. - The amino acids containing sulfhydryl group when heated with base, the sulfhydryl group and disulfhydryl are directly converted to inorganic sulfur. Which is confirmed by the black precipitate of PbS (lead sulfide) when adding lead acetate Pb (CH3COO)2.

Questions:

1. Why proline gives a yellow color with ninhydrin test?
2. Discuss the reasons of which aromatic amino acids give positive result but not aliphatic ones in Xanthoproteic test.
3. Would phenol give positive results with Xanthoproteic test , explain your answer?
4. Which of the amino acids contain (-SH) group?
5. What is the difference between cystein and cystin ?