Lab (1): Qualitative tests of amino acids

Introduction:

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. There are 20 natural amino acids found within proteins convey a vast array of chemicals versatility. All of them are L-α amino acids.(1)

All amino acids found in proteins consist of a basic amino group (—NH2), an acidic carboxyl group (—COOH), a hydrogen atom (—H) and a distinctive side chain (—R). Amino acids 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).

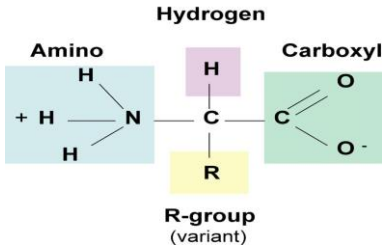
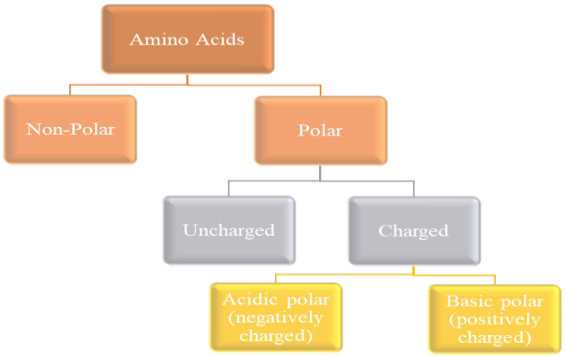


Figure 1. Basic structure of amino acid. (2)

All amino acids in the solutions 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:

1. Non-polar.
2. Uncharged polar.
3. Charged polar amino acids:
   i. Basic polar (positively charged).
   ii. Acidic polar (negatively charged).



Amino acids have different physical and chemical properties including: Amphoteric property, isoelectric point (pI), optical activity and light absorption.

1. Amphoteric Property:

Amphoteric compound is a molecule that can act as acids (donate a proton) and bases (accept a proton). Amphoteric properties of amino acids due to the presence of their ionizable α-amino and α-carboxylic group. They can act sometimes as acids and sometimes as bases depending on the pH of their media. Presence of carboxyl group

COOH that able to donate proton (H+) and converted to COO^- providing an acidic behaviour, whereas presence of amino group NH_2 that able to accept proton (H+) and converted to NH_3^+ providing a basic behaviour (Figure 2). (3)

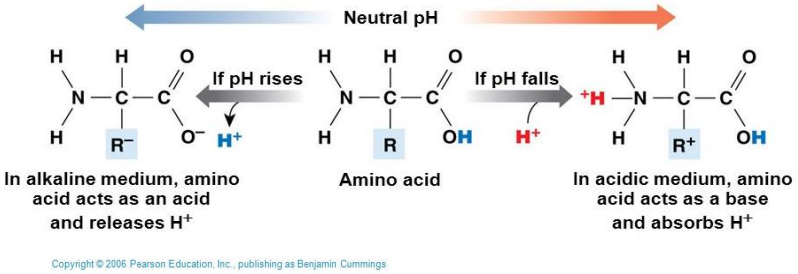


Figure 2. Acid-base behaviour of amino acids.

2. Isoelectric point:

It may be noted that at a particular pH, the amino acid molecules are in the dipolar form (Zwitterion) and the net charge of the molecule is zero (i.e. the positive charge equals the negative charge); this is the isoionic or isoelectric point of the amino acid. Each amino acid has a different pI, and at this point, its solubility is minimal and it does not migrate when placed in an electric field (unlike the cation and the anion). (4)

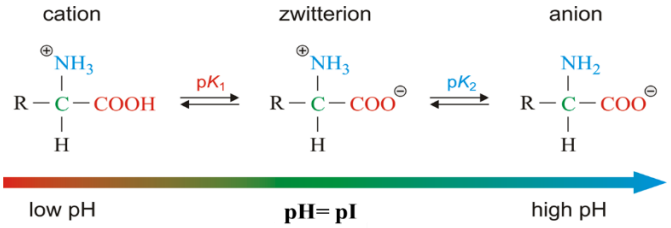


Figure 3. Zwitterion form of amino acids.

3. Optical activity:

Amino acids are able to rotate polarized light either to the left (Levorotatory) L isomer, or to the right (Dextrorotatory) D isomer, since they have an asymmetric C atom (a carbon atom linked to 4 different groups). (4)

PAUSE AND THINK ➔ What about glycine?

4. Absorption in the ultraviolet region:

The aromatic amino acids tryptophan, tyrosine, and phenylalanine absorb ultraviolet light at 280 nm, which explains the absorption of proteins at 280nm. (4)

Experiment (1). Solubility test:

Aim:

- Investigate the solubility of selected amino acid in various solutions.

Principle:

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.[5]

Materials:

Chemical

Glycine, arginine, glutamine, distal water, NaOH, HCl and chloroform.

Equipment and Glassware

Test tubes, rack, pipette, pipette pump, water bath.

Protocol:

1. Add 2 ml of different solvents in 4 clean test tubes then place 0.5 ml of glycine.
2. Shake the tubes thoroughly, then leave the solution for about one minute.
3. Notice what happened to the solution.
4. Repeat steps 1-3 for arginine and glutamine.
5. Record your results.

Results:

Amino acid	Solvent	Degree of solubility
Glycine	Water	
	NaOH	
	HCl	
	Chloroform	
Arginine	Water	
	NaOH	
	HCl	
	Chloroform	
Glutamine	Water	
	NaOH	
	HCl	
	Chloroform	

Experiment (2). Ninhydrin test:

Aim:

- A general test to detect the presence of α-L-amino acids.

Principle:

In the pH range of 4-8, Ninhydrin (triketohydrindene hydrate) degrades amino acids into aldehydes, ammonia and CO_2 through a series of reactions and the ninhydrin partially reduced to form hydrindantin (1). Then more Ninhydrin condenses with ammonia and hydrindantin to produce an intensely blue or purple pigment (diketohydrin), sometimes called ruhemann's purple (2). The color varies slightly from acid to acid. All amino acids that have a free amino group will give a purple color, whereas imino acids (not free amino group) like proline and hydroxyproline give a yellow colored complex, because the N is not available for the reaction as it is locked in the ring structure, therefore no ammonia is produced. All primary amines and ammonia react similarly and produce blue/purple product but without the liberation of carbon dioxide.[6]

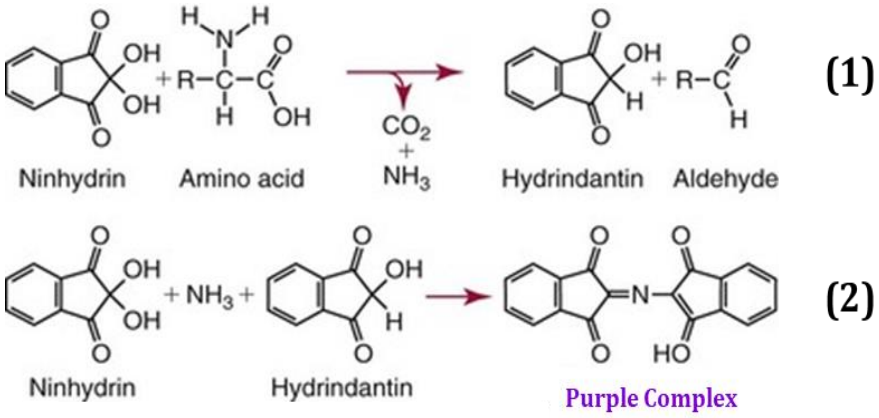


Figure 4. Series of reaction in Ninhydrin test.[7]

Materials:

Chemical

Glycine, tryptophan, proline, distal water, 0.2% Ninhydrin reagent*.

*__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.

Equipment and Glassware

Test tubes, rack, pipette, pipette pump, water bath.

Protocol:

1. Label four tubes (1 - 3), then add 1 ml of each amino acid (glycine, tryptophan and proline).
2. Add 1 ml of ninhydrin solution.
3. Boil the mixture over a water bath for 2 min.
4. Allow to cool and observe the blue-purple color formed.
5. Record your results.

Results:

Tube	Observation
Glycine	
Tryptophan	
Proline	

Experiment (3). Xanthoproteic test:

Aim:

- To differentiate between aromatic amino acids which give positive results and other amino acids.

Principle:

In the presence of concentrated nitric acid (HNO_3), the aromatic phenyl ring is nitrated to give yellow colored nitro-derivatives (nitration reaction). At alkaline pH, the color changes to orange due to the ionization of the phenolic group. 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. (6)

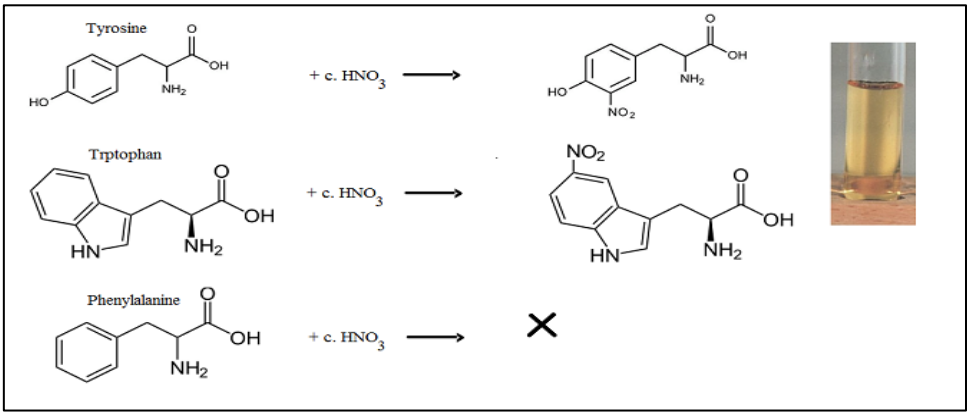


Figure 5. Formation of yellow color as result of nitration reaction in Xanthoproteic test. (8)

Materials:

Chemical

Tyrosine, tryptophan, phenylalanine, phenol, distal water, con. HNO_3 *, 10M NaOH.

*__Caution:__ Concentrated HNO3 is a toxic, corrosive substance that can cause severe burns and discolour your skin. Prevent eye, skin and cloth contact. Avoid inhaling vapors and ingesting the compound. Gloves and safety glasses are a must; the test is to be performed in a fume hood.

Equipment and Glassware

Test tubes, rack, pipette, pipette pump, water bath.

Protocol:

1. Label four tubes (1 - 4), then add 1 ml of each amino acid solutions (tyrosine, tryptophan and phenylalanine) and phenol solution to those test tubes each alone.
2. Add 1 ml of concentrated HNO_3.
3. Then record your result
4. Now COOL THOROUGHLY under the tap and CAUTIOSLY add 5 drops of 10M NaOH to make the solution strongly alkaline.
5. Record your results.

Results:

Tube	Observation	
	+ HNO_3	+NaOH
Tyrosine		
Tryptophan		
Phenylalanine		
Phenol		

Experiment (4). Millon's test:

Aim:

- Detection of amino acid containing phenol group, i.e. tyrosine.

Principle:

Phenolic amino acids such as tyrosine and phenols response to this test. First, the phenol group of tyrosine is nitrated by nitric acid, then 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. Millon's reagent made by dissolving metallic mercury in nitric acid and diluting with water. [6,9]

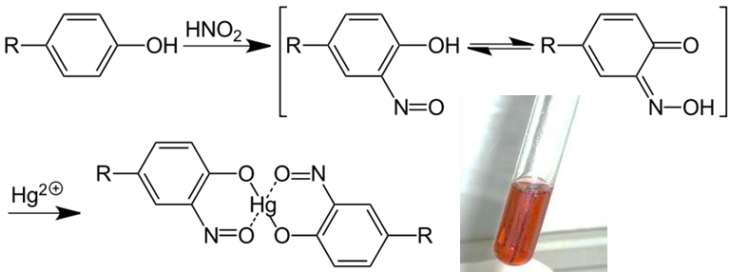


Figure 6. Formation of the red complex in Millon's test.(9)

Experiment (5). Sakaguchi test:

Aim:

- Detection of amino acid containing gauanidium group, i.e. arginine.

Principle:

Under alkaline condition, α- naphthol (1-hydroxy naphthalene) reacts with a mono-substituted guanidine compound like (R-NH-C= $(NH_2)^{2+}$-NH_2) arginine, which upon treatment with hypobromite or hypochlorite as an oxidize agent, produces a characteristic red color as a positive result. (6)

Materials:

Chemical

Glycine, arginine, distal water, 10% NaOH, α-naphthol in 10% ethanol, 5% sodium hypobromate.

Equipment and Glassware

Test tubes, rack, pipette, pipette pump, water bath.

Protocol:

1. Label 2 test tube and put in each one 2 ml of the amino acid solution.
2. Add to each tube 2ml of NaOH solution. Mix well
3. Add to each tube 5 drops of α-naphthol solution. Mix well.
4. Add to each tube 5 drops of sodium hypobromite solution.
5. Record your result.

Results:

Tube	Observation
Glycine	
Arginine	

Experiment (6). Lead-sulphite test:

Aim:

- Detection of amino acid containing sulphur, i.e. cysteine.

Principle:

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 black lead sulphide (PbS) precipitate, using lead acetate solution $Pb(CH_3COO)_2$. [6]

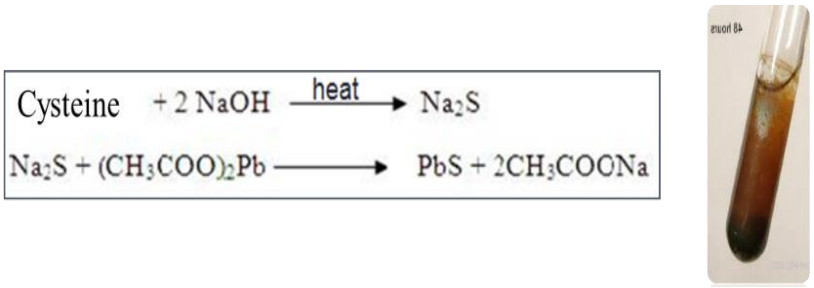


Figure 7. Formation of black precipitate lead sulphite test.[6]

References:

1. http://www.biology.arizona.edu/biochemistry/problem_sets/aa/aa.html
2. https://socratic.org/questions/how-does-ph-affect-amino-acid-structure
3. Chatterjea MN, Shinde R. Chemistry of proteins and amino acods. Textbook of Medical Biochemistry, 8th edn. JP Medical Ltd; 2011. p.81.
4. http://www.biologydiscussion.com/amino-acids/properties-of-amino-acids-biochemistry/71284
5. https://www.chemguide.co.uk/organicprops/aminoacids/background.html
6. http://vlab.amrita.edu/?sub=3&brch=63&sim=1094&cnt=1
7. http://vlab.amrita.edu/?sub=3&brch=63&sim=1094&cnt=1
8. http://www.onlinebiologynotes.com/xanthoproteic-test-objective-principle-reagents-procedure-and-result/
9. Walsh, Edward O'Farrell (1961). An Introduction to Biochemistry. London: The English Universities Press Ltd. pp. 406–407.