

AL-Mustaqbal University
College of Sciences
Department of Biochemistry Sciences

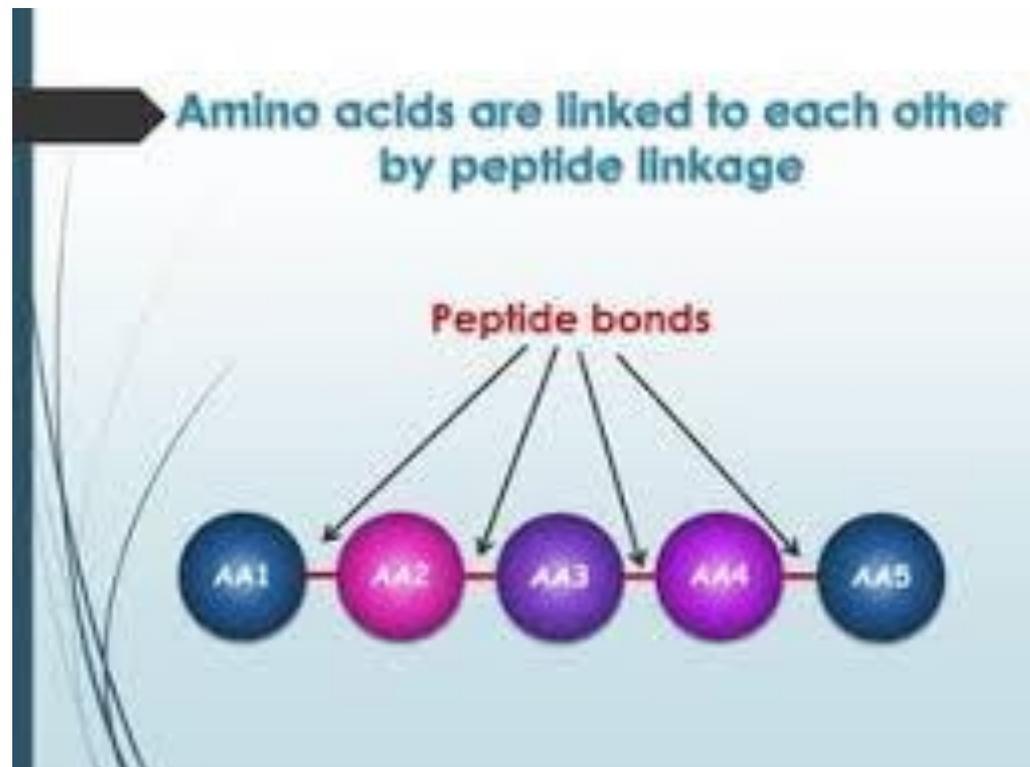


Lab. Biochemistry

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Proteins

are polymers of amino acids linked by covalent peptide bonds. Proteins ingested undergo digestion and get absorbed as amino acids into the portal vein and reaches liver and then to other tissues .

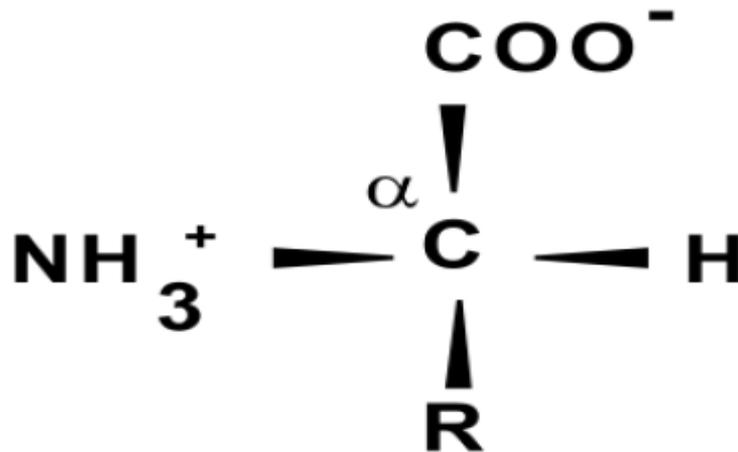


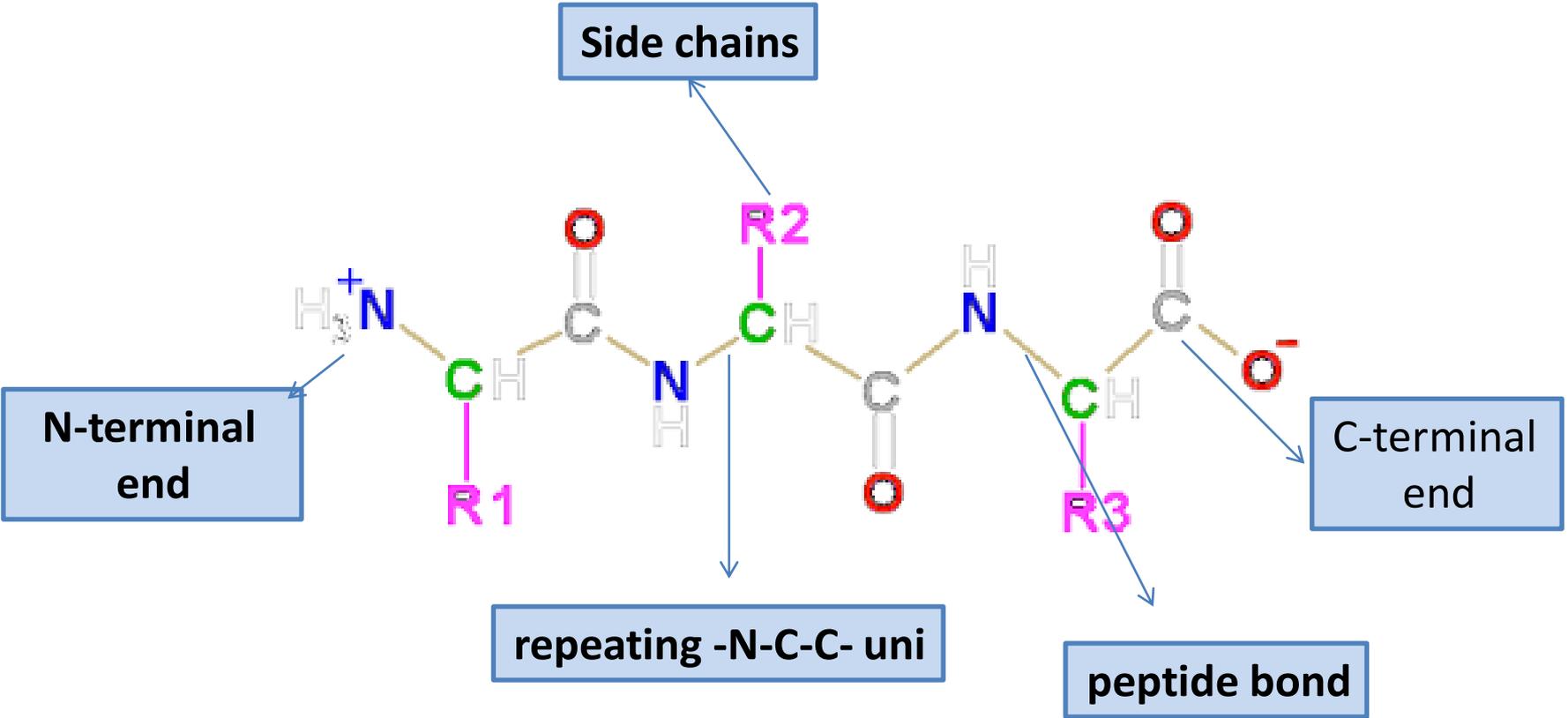
Amino acid structure

Each amino acid consists of : Central alpha carbon atoms that attached to:

1. An amino group
2. Carboxyl group
3. Hydrogen atom
4. Side chain (R group)

(All amino acids found in proteins have this basic structure, differing only in the **structure of the R-group** or the side chain.)





Classification of amino acids according source

1. Essential amino acids:

Are not synthesized by mammals and are therefore dietarily essential or Required in diet.

2. Non essential amino acids:

Amino acids which can be synthesized in the body are known as non-essential amino acids. (Not required in diet).

Obtained
from
Nutrition

Essential Amino Acids

*Leucine	Methionine
*Isoleucine	Phenylalanine
*valine	Threonine
Histidine	Tryptophan
Lysine	

Non-Essential Amino Acid

Alanine	Glutamine
Arginine	Glycine
Asparagine	Proline
Aspartic Acid	Serine
Cysteine	Tyrosine
Glutamic Acid	

Synthesized
by the
body

Amino acid in human body

Classification of proteins

Proteins are classified into

❖ **Fibrous proteins**

(offer mainly **structural function**) e.g: fibrinogen troponin, collagen, myosin and

❖ **Globular proteins**

(offer mainly **dynamic functions**), e.g. Hb, enzymes, peptide hormones, plasma proteins. Proteins are present in all types of body fluids.

Amino acids & Protein Analysis

During routine analytical laboratory work, two types of reactions are practiced.

1. Precipitation reactions

2. Color reactions

These reactions are useful for quantitative and qualitative studies of proteins.

By quantitative studies the concentration of the proteins are estimated.

Qualitative studies help to know the presence of proteins or specific amino acids present in the protein. They are useful mainly in the following situations

- 1. For the diagnosis of aminoacidurias**
- 2. For the nutritional assessment**
- 3. To detect the presence of proteins or amino acids in biological fluids or in fluids with unknown composition**

A. Identification tests for amino acids :

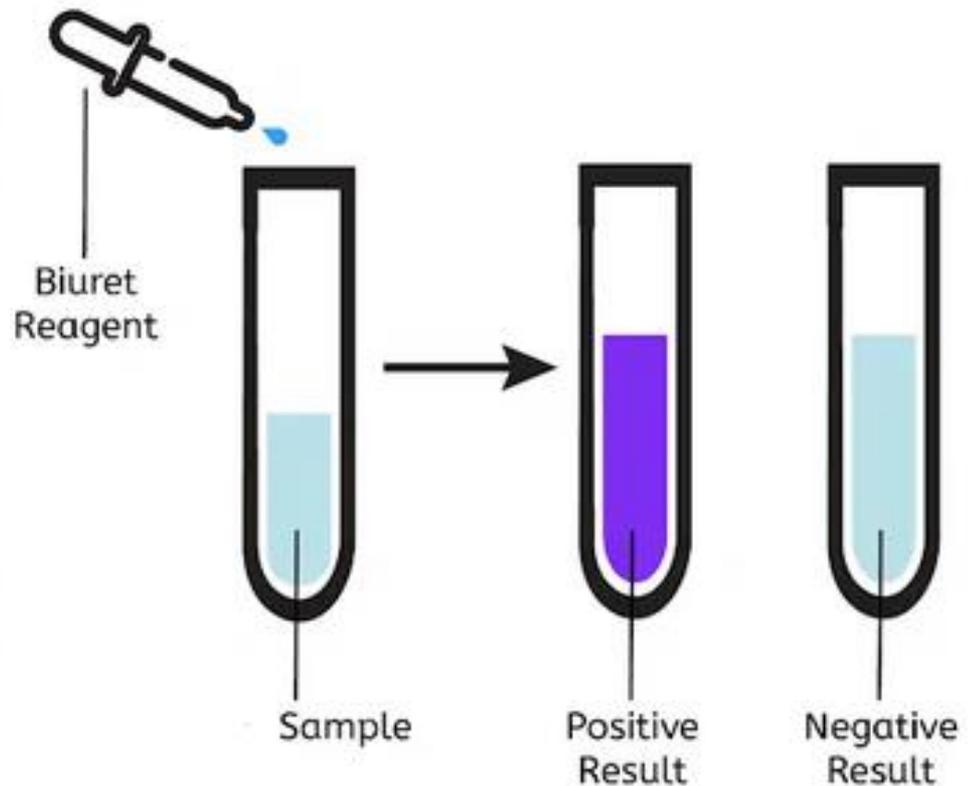
There number of test to detect the presence of amino acid ,These are largely depend on the nature of amino acids side chain usually.

Example of these tests are :

1. Ninhydrin test: for α -L amino acids
2. Xanthoproteic test: for Aromatic amino acids
3. Lead sulfite test: detection of amino acids containing sulfhydryl group (- SH)
4. Millon's test: for amino acids containing hydroxyphenyl group

B. Identification tests for proteins :

The presence of proteins in a solution is often detected by general tests, such as biuret or specific tests that depend on the presence of a specific proteins



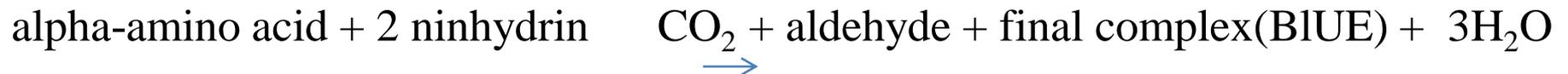
Ninhydrin Test

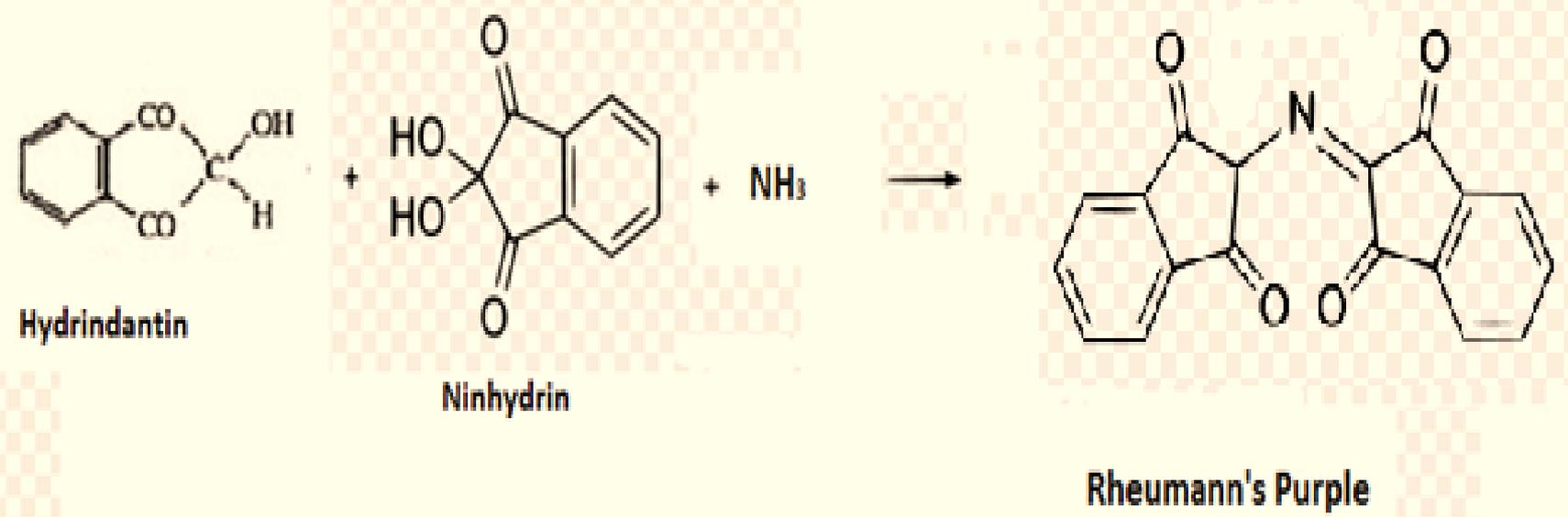
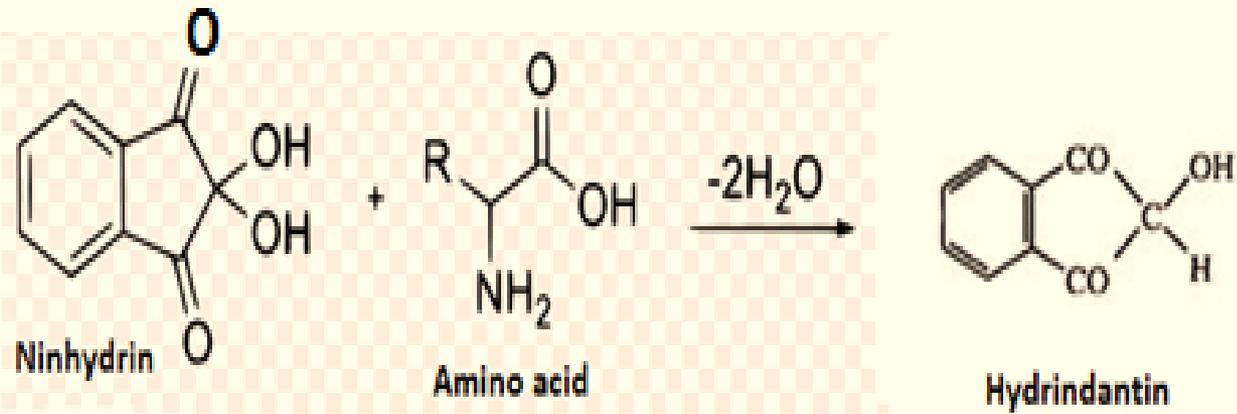
Principle:

1. Ninhydrin (triketohydrindene hydrate) degrades amino acids into aldehydes (on pH range 4), ammonia and CO₂ through a series of reactions

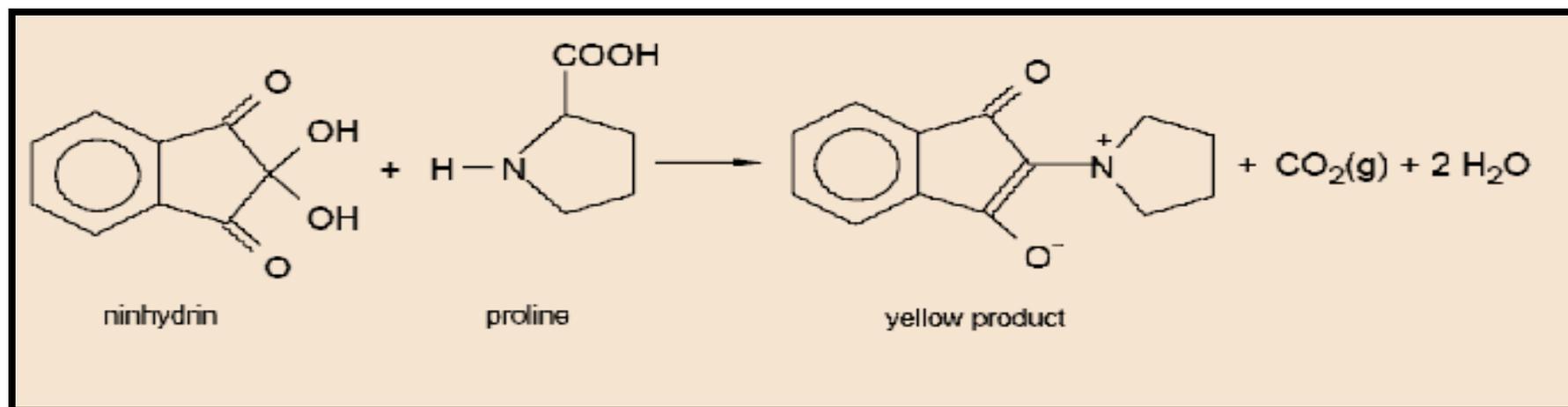
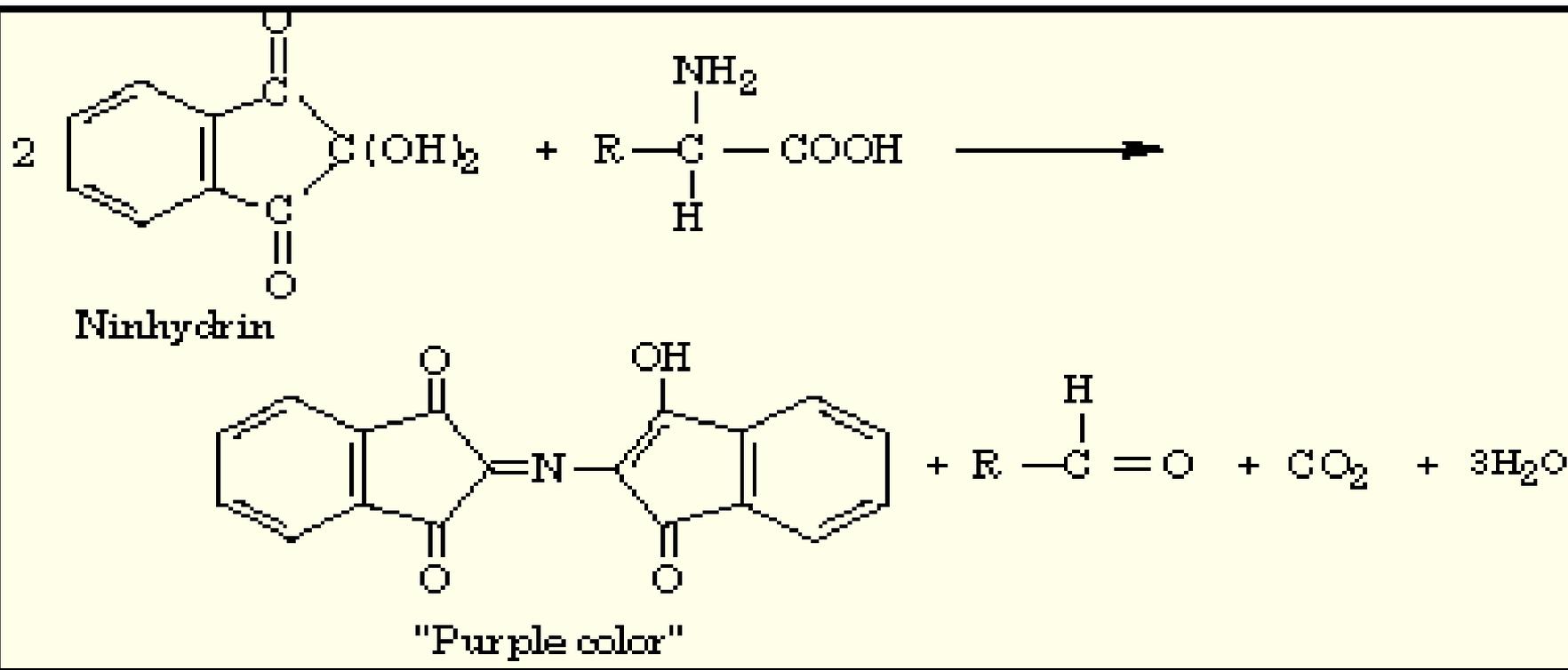
2. The reduction product obtained from ninhydrin then reacts with NH₃ and excess ninhydrin to produce an intensely blue or purple pigment, or color.

This reaction provides an extremely sensitive test for amino acids.





- All amino acids that have a free amino group will give positive result (purple color)
- The imino acids proline and hydroxyproline also react with ninhydrin, but they give a yellow colored complex instead of a purple one.
- Besides amino acids, other complex structures such as peptides, peptones and proteins also react positively when subjected to the ninhydrin reaction
- Ninhydrin ethanol reagent is flammable. Toxic, and irritant. Keep away from bunsen burner, flames , prevent eye, skin, clothing contact. Avoid inhaling the vapors or ingesting the reagent.



Procedure

To 1 mL solution add 5 drops of 0.5 % ninhydrine solution

Boil over a water bath for 2 min.

Allow to cool and observe the blue color formed.

Results: blue or yellow color in case of proline

No Color



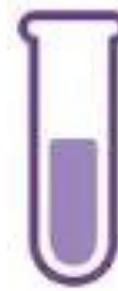
Negative Test
(No Amino acid)

Yellow Color



Indicate Presence
of Proline

Purple Color



Positive test (Amino
acid present)

Thank You

