Amino Acid Analyzer Basic Course

1. Principle and basics of amino acid analysis

Generally, organic compounds having -COOH (carboxyl group) and -NH 2 (amino group) in the molecular structure are called amino acids.

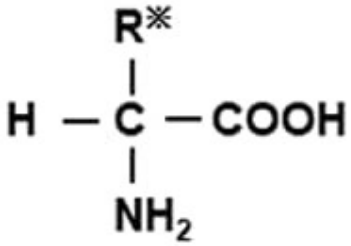


Fig. 1 General structural formula of amino acids

Individual organic groups are bonded to R. Characteristics of each amino acid differ depending on the nature of R.

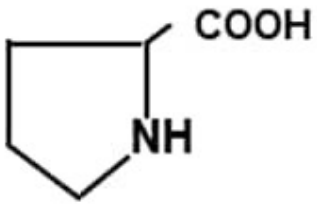


Fig. 2 Structure of Proline (Pro)

Proline has a ring structure. It shows specific optical properties different from other amino acids.

Amino acids are represented by three-letter code that are almost unified worldwide.

Table 1 Three-letter codes of amino acids

Asp	Aspartic acid	Val	Valine
Thr	Threonine	Met	Methionine
Ser	Serine	Ile	Isoleucine
Asn	Aspargine	Leu	Leucine
Glu	Glutamic acid	Tyr	Tyrosine
Gln	Glutamine	Phe	Phenylalanine
Pro	Proline	Lys	Lysine
Gly	Glycine	His	Histidine
Ala	Alanine	Trp	Tryptophan
Cys	Cystine	Arg	Arginine

In the field of amino acid analysis, Asparagine (Asn) and Glutamine (Gln) may be written as AspNH2 and GluNH2. Similarly, Cys may refer to Cysteine.

1) Standard amino acids (protein hydrolyzate amino acids)

The proteins that make up all animals are composed of amino acids. Amino acids that make up the proteins are called standard amino acids (protein hydrolyzate amino acids). As of today, 20 standard amino acids have been identified.

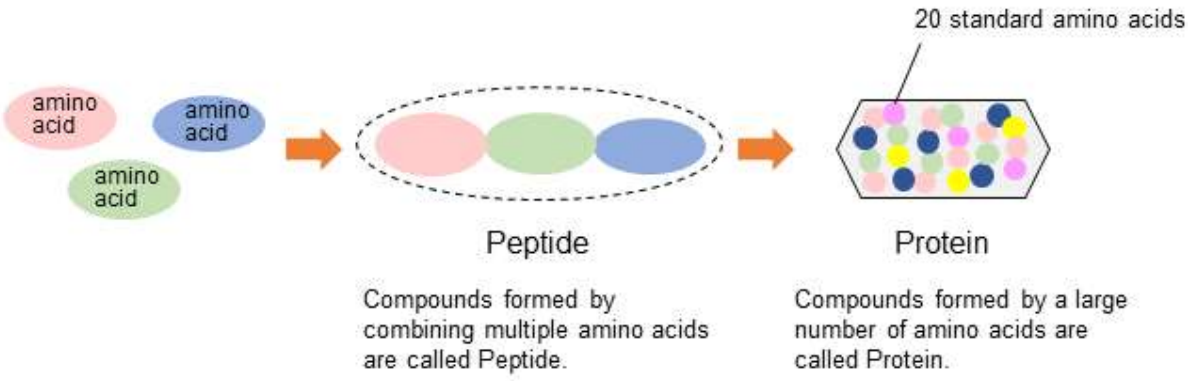
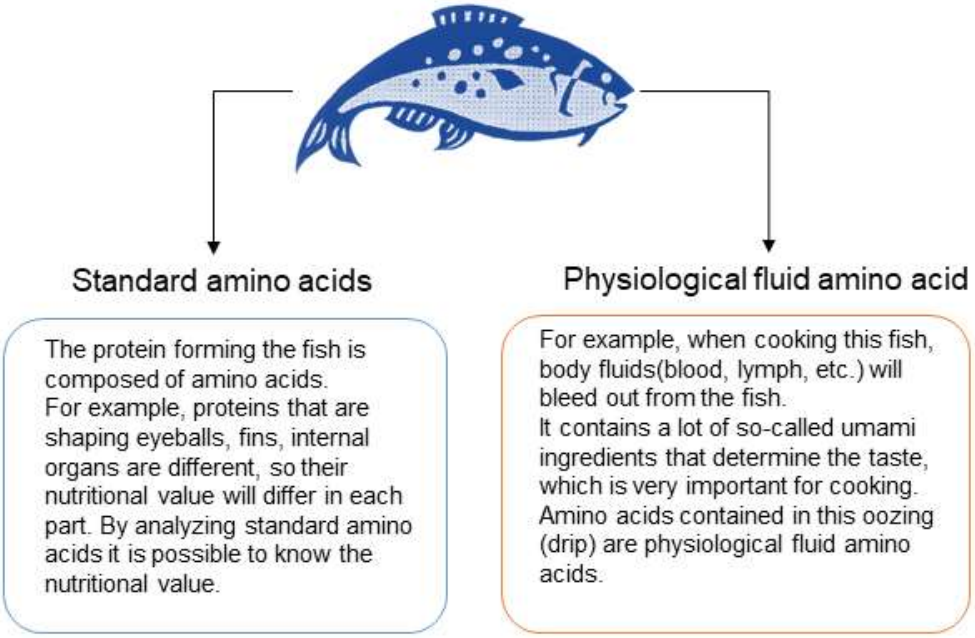


Fig. 3 Schematic diagram of amino acids, peptides, and proteins

2) Physiological fluid amino acids (free amino acids)

Basic of amino acids are 20 standard amino acids mentioned above, but if you take in them as food, it will be digested and will change to various substances. These amino acids including metabolites and precursors are called physiological fluid amino acids (free amino acids). Approximately 40 physiological fluid amino acids have been identified.



2. Method for analyzing amino acids

Most of amino acids are difficult to separate and detect because their hydrophilicity is high, and their UV absorption and fluorescence are low. With the post-column ninhydrin method, the Hitachi LA8080 Amino Acid Analyzer can afford separation and analysis of approximately 50 amino acids.

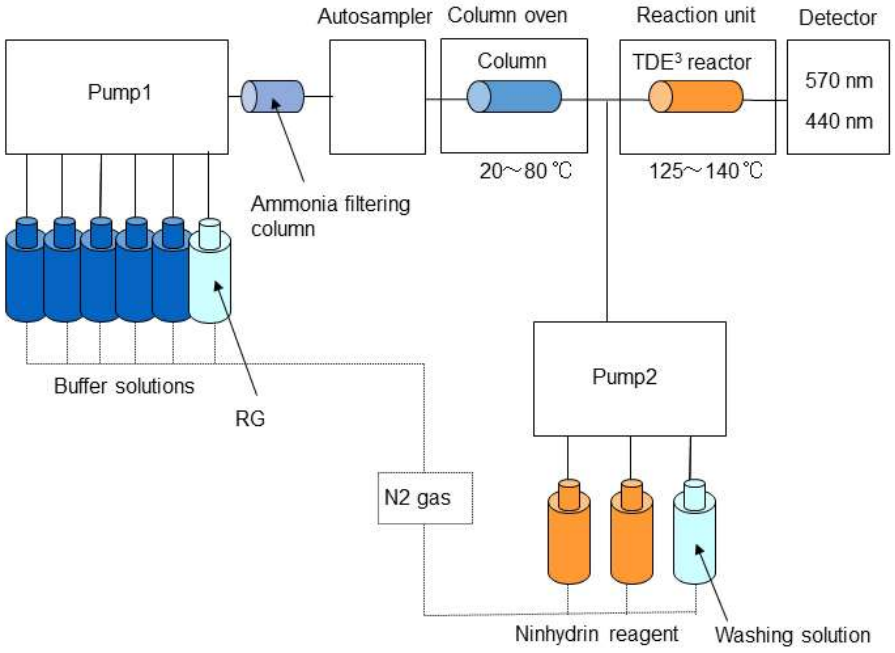
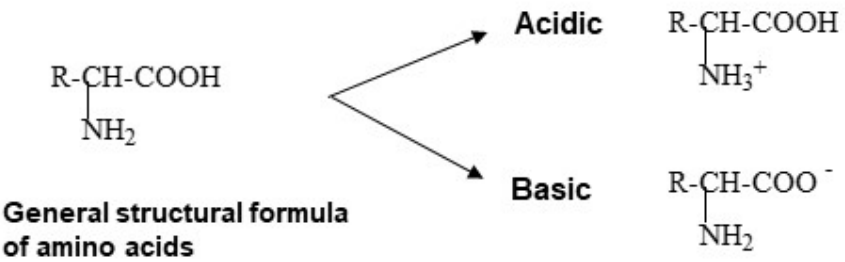


Fig. 4 Flow Diagram of LA8080

2-1. Cation exchange column and separation of amino acids



1. Amino acids are charged to + in the acidic solution, and electric attraction is generated again cation exchange resin.
2. In contrast, amino acids are charged to - in the basic solution, so that they repel and pass through the cation exchange resin.
   (Utilizing this chemical property, we use a sodium hydroxide solution (basic solution) for washing out residual substances in the column after analysis. It is called regeneration (RG) process.)
3. In amino acid analysis, separation is carried out by changing the pH of the eluent from acidic to basic.

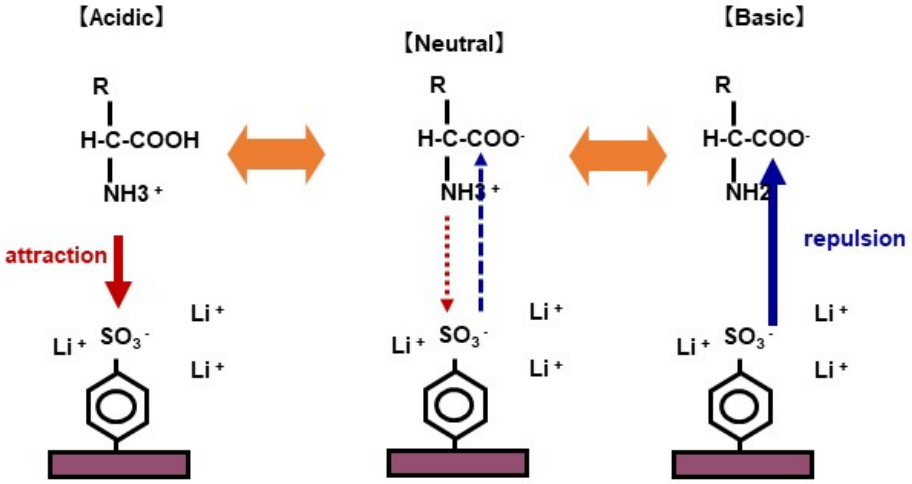


Fig. 5 Amino acid separation

The cation exchange column has a high chemical strength and can be washed with basic solutions. However, it doesn't have very high physical strength.

Sudden pressure fluctuations may cause degradation of the column, so you should pay attention to the pressure applied to the column.

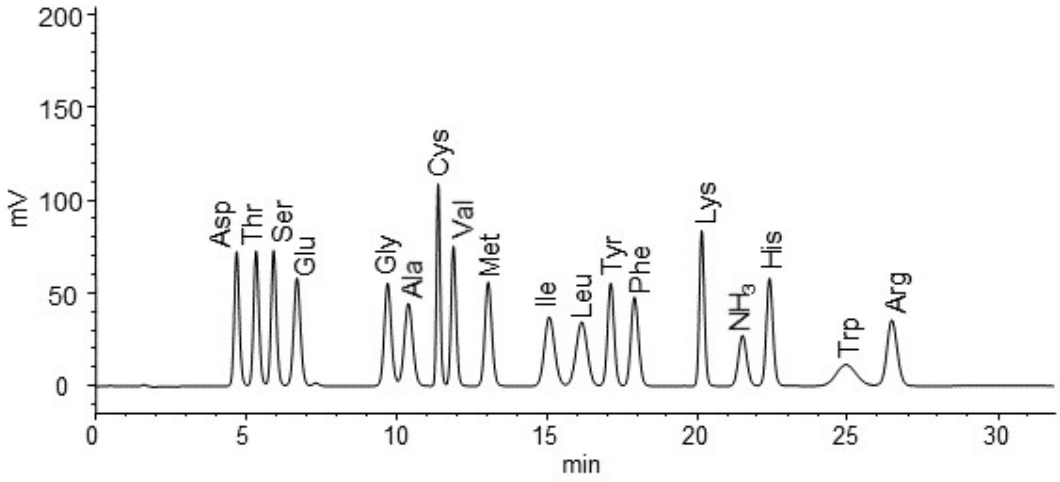


Fig. 6 Example of amino acids chromatogram

Table 2 Necessary elements for amino acid analysis buffer

Condition	Chemicals
pH	Citric acid

Ionic strength	NaCl, LiCl
buffer capacity	Sodium citrate, Lithiun citrate

Table 3 Other components of amino acid analysis buffer and purpose of addition

Reagents	Porpose
Ethanol	separation of Thr-Ser
Benzyl alcohol	separation of Trp
β-thiodiglycol	anti-oxidation of sulfur-containing amino acids
Brij-35	Pump pressure reduction
Caprylic acid	Anti-corruption

The buffer solutions can be purchased locally. For the detail, you can ask local sales.

You can also prepare the buffer solutions according to the "Buffers preparation" section of the instruction manual (main unit). The pH value described in the instruction manual is a reference value and adjustment of pH is not necessary.

You have to use special grade reagents or reagents graded for amino acid analysis.

2-2. Derivatization of amino acids

There are two types of derivatization: pre-column and post-column. The post-column derivatization method adopted for the Hitachi High-speed Amino Acid Analyzer LA8080 has advantages such as less influence of contaminants and better reproducibility.

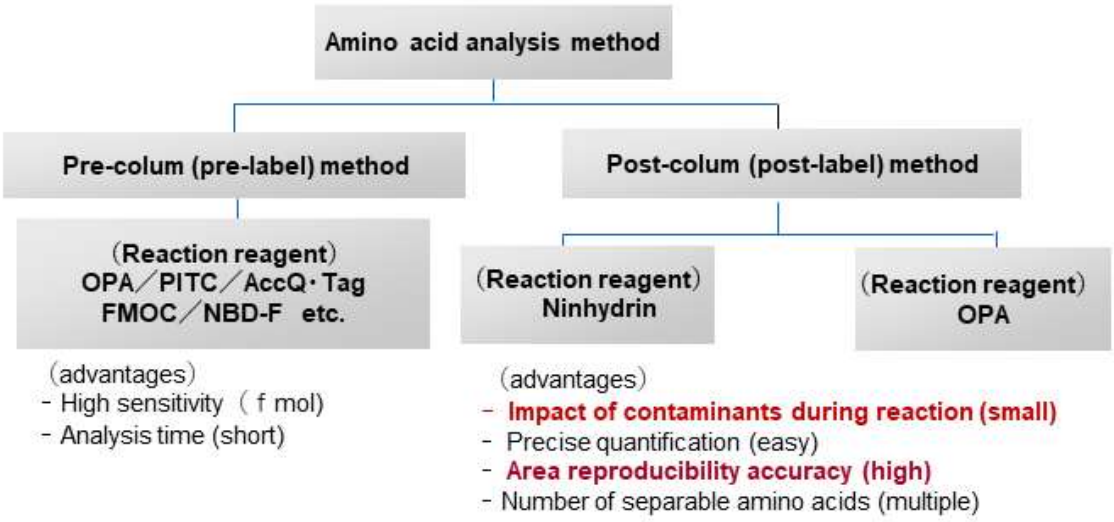


Fig. 7 Types of derivatization of amino acids

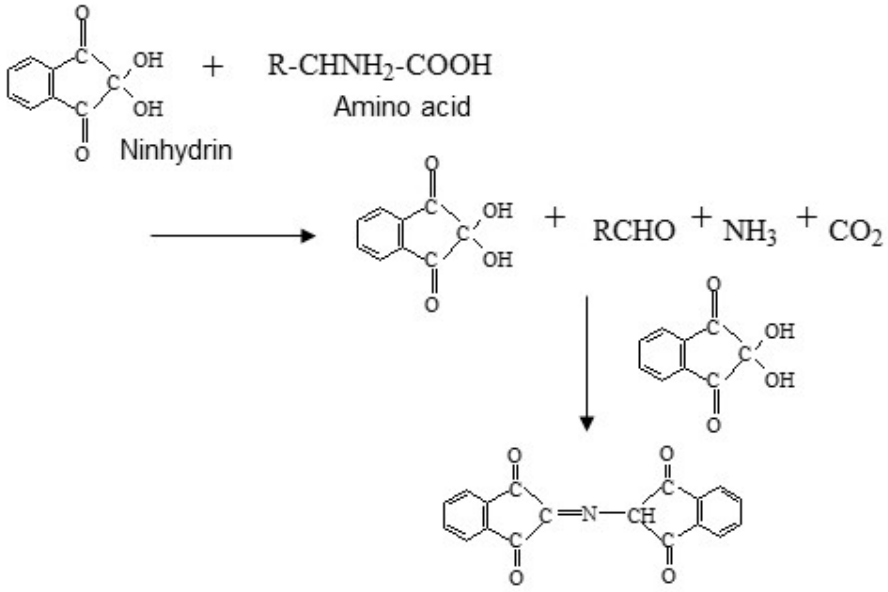
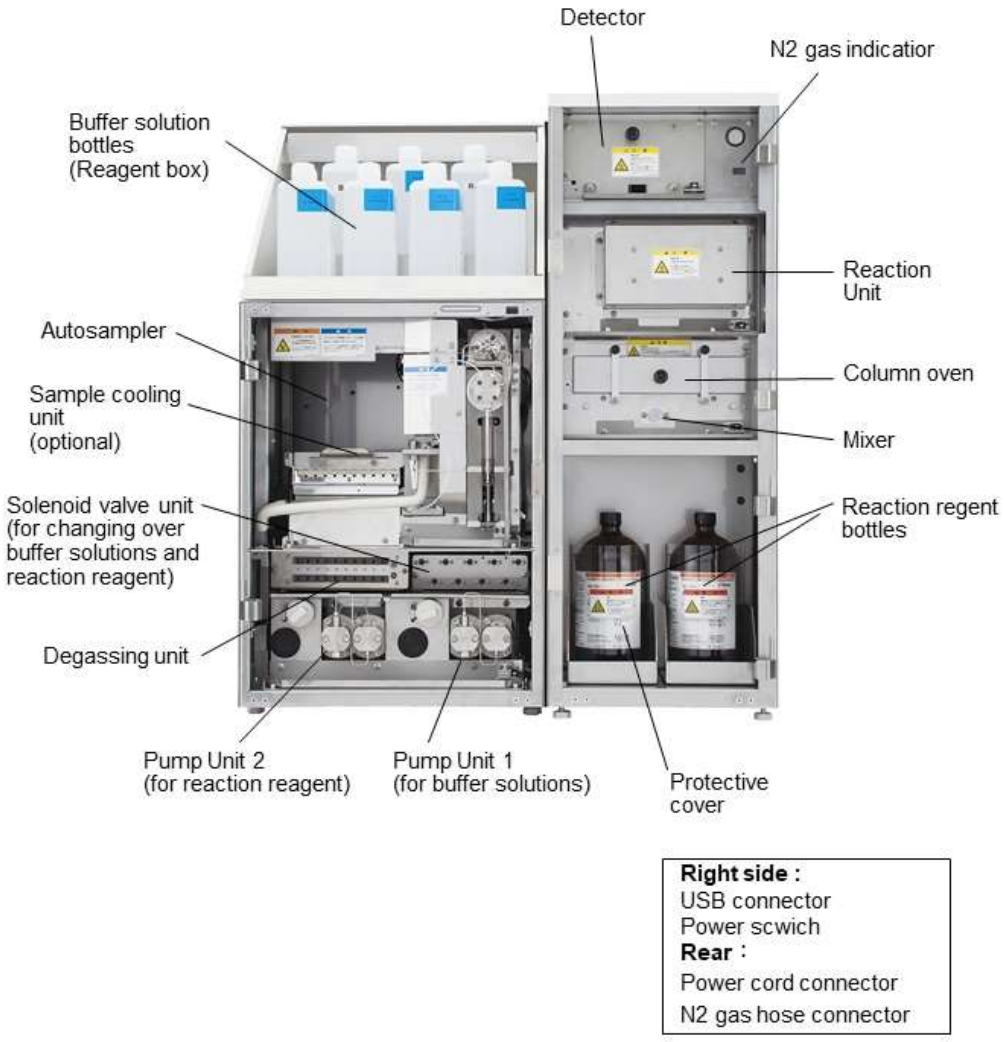


Fig. 8 Chemical reaction of amino acid and ninhydrin

Due to a ninhydrin reaction, a blue-violet substance (Ruhemann's purple) was produced and measured with the absorbance at 570 nm. Because proline and hydroxyproline produce yellow-red substances, they were measured with the absorbance at 440 nm.

3. Inside Front Doors



Related Products

- High-Speed Amino Acid Analyzer LA8080 AminoSAAYA

Source: Hitachi High Tech Science
https://www.hitachi-hightech.com/global/products/science/tech/ana/lc/aaa_basic_course/