Application Note

430021H

Analysis of Protein Hydrolysate Amino Acids using OPA Post-column Derivatization by Quaternary Low Pressure Gradient System

Introduction

Amino acid analysis has been applied to several categories such as food, medicine, protein science and metabolome study, and is also an important measurement term as basic technology. Newly developed amino acid analysis system using low pressure gradient unit (OPA post column derivatization) enables to obtain stable baseline and excellent repeatability as well as to analyze with good separation in a short time.

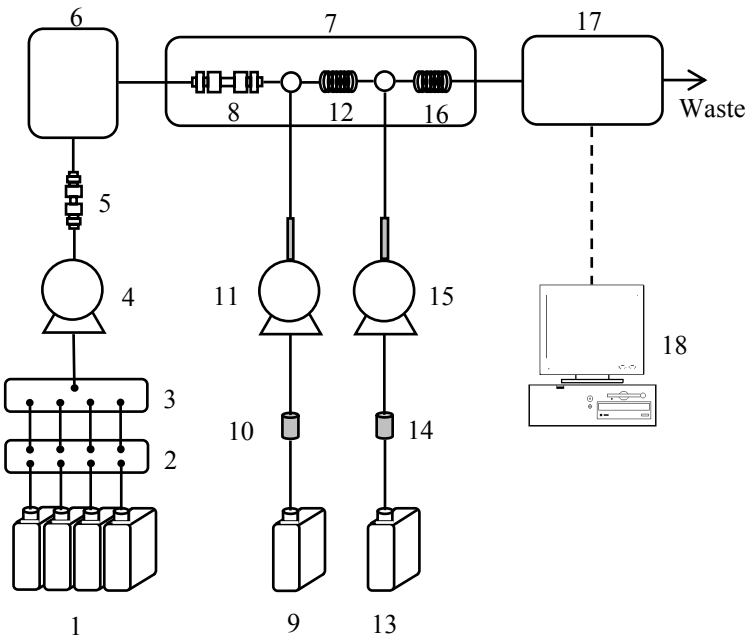
In this paper, the analysis results of food analysis and amino acid composition analysis of protein using this new amino acid analysis system are reported.

Keyword: Amino acids to organize proteins , Quaternary low pressure gradient, OPA, Post column derivatization, AA-pak, Na-LG, Fluorescence detector

Experimental

Equipment		Conditions	
Eluent Pump:	PU-2089	Column:	AApak Na-LG (6.0 mmID x 50 mmL, 5 µm)
Reagent Pump:	PU-2085 x 2	Ammonia filter:	AECpak Na-LG (4.6 mmID x 35 mmL)
Column oven:	CO-2065	Eluent:	Amino Buffer Na-LG (1st ~ 4th)
Autosampler:	AS-2057	Reagent:	Amino Reagent Na-LG (Hypo, OPA)
Detector:	FP-2025	Eluent flow rate:	0.5 mL/min
		Reagent flow rate:	0.5 mL/min each
		Column temp.:	60ºC
		Reaction temp.:	60ºC
		Wavelength:	Ex. 345 nm, Em. 455 nm, Gain x10
		Injection volume:	10 µL
		Standard sample:	20 amino acids 50 nmol/mL each in 0.2 N citric buffer (pH 2.2)

[Schematic diagram]



1: Eluent (Amino Buffer Na-LG (1st ~ 4th))
2: Degasser
3: Low pressure gradient unit
4: Eluent pump
5: Ammonia filter (AECpak Na-LG)
6: Autosampler
7: Column oven
8: Column (AApak Na-LG)
9: Reagent 1 (Amino Reagent Na-LG Hypo)
10: Air trap 1
11: Reagent pump 1 (Hypo)
12: Reaction coil 1
13: Reagent 2 (Amino Reagent Na-LG OPA)
14: Air trap 2
15: Reagent pump 2 (OPA)
16: Reaction coil 2
17: Fluorescence detector
18: Chromatography data system (ChromNAV)

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Results and discussion

Fig. 1 shows the chromatogram of standard mixture of 20 kinds of amino acids. The sample was well separated within 45 minutes (1 cycle 60 min) .

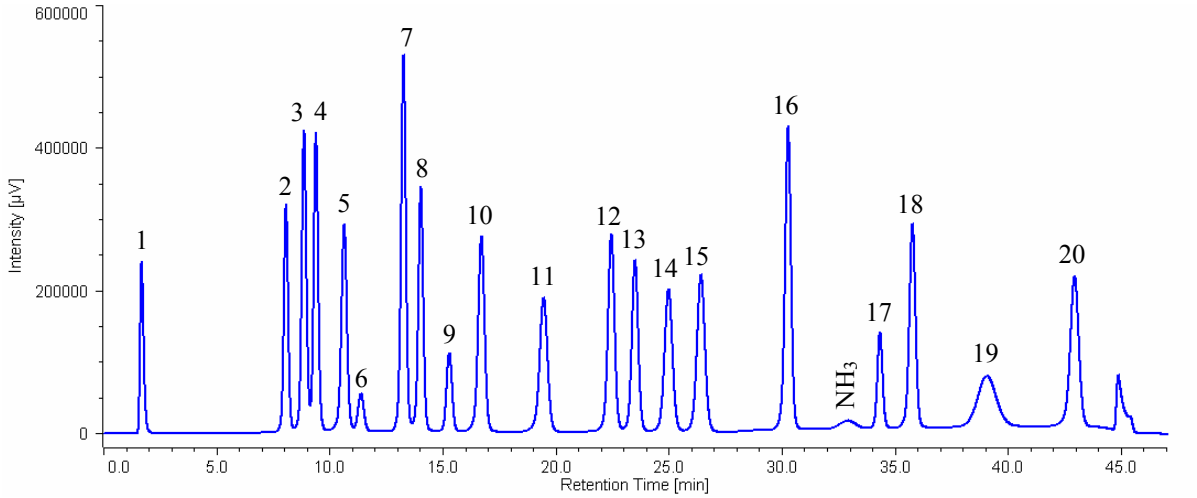

Fig. 1. Chromatogram of amino acid standard mixture (500 pmol each)
1: Cysteic acid, 2: Asparatic acid, 3: Threonine, 4: Serine, 5: Glutamic acid, 6: Proline, 7: Glycine, 8: Alanine
9: Cystine, 10: Valine, 11: Methionine, 12: Isoleucine, 13: Leucine, 14: Tyrosine, 15: Phenylalanine, 16: GABA
17: Lysine, 18: Histidine, 19: Tryptophan, 20: Arginine

Fig. 2 shows the chromatogram of sports drink and Fig. 3, the chromatogram of white wine containing GABA.

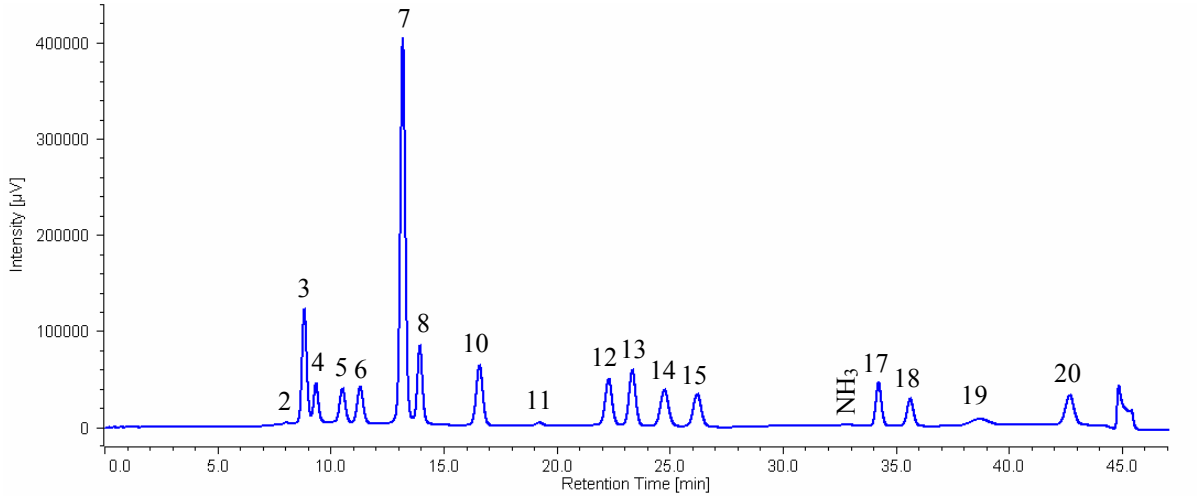

Fig. 2. Chromatogram of sports drink
2: Asparatic acid, 3: Threonine, 4: Serine, 5: Glutamic acid, 6: Proline, 7: Glycine, 8: Alanine, 10: Valine,
11: Methionine, 12: Isoleucine, 13: Leucine, 14: Tyrosine, 15: Phenylalanine, 17: Lysine, 18: Histidine,
19: Tryptophan, 20: Arginine

Sample preparation: Sports drink was 150-fold diluted by 0.2 N citric acid buffer (pH 2.2) and then filtrated using 0.45 μm membrane filter.

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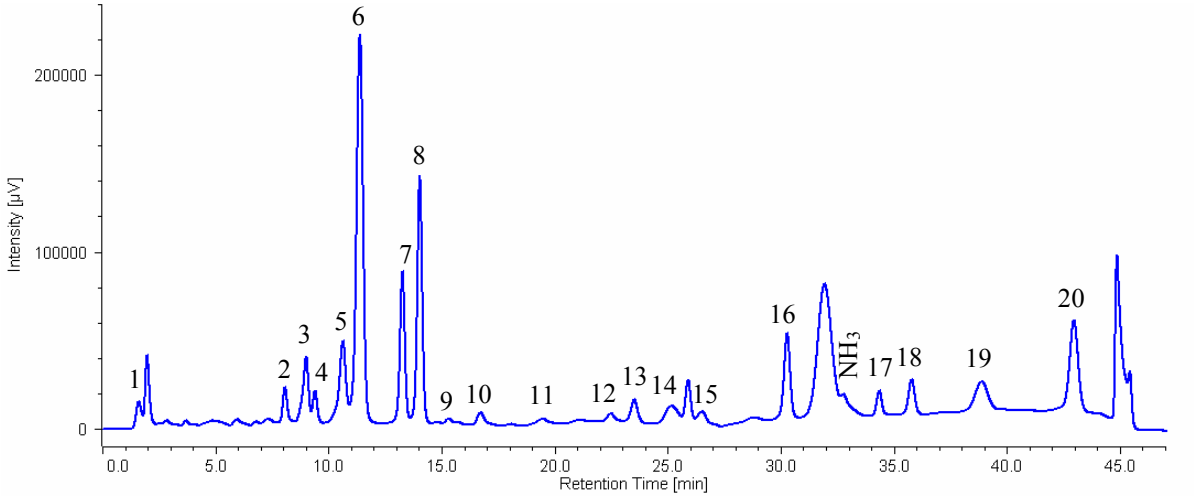

Fig. 3. Chromatogram of white wine containing GABA
1: Cysteic acid, 2: Asparatic acid, 3: Threonine, 4: Serine, 5: Glutamic acid, 6: Proline, 7: Glycine, 8: Alanine
9: Cystine, 10: Valine, 11: Methionine, 12: Isoleucine, 13: Leucine, 14: Tyrosine, 15: Phenylalanine, 16: GABA
17: Lysine, 18: Histidine, 19: Tryptophan, 20: Arginine
Sample preparation: White wine including GABA was 10-fold diluted by 0.2 N citric acid buffer (pH 2.2) and then filtrated using 0.45 μm membrane filter.

Fig. 4 shows the chromatogram of hydrolyzed myoglobin (horse skeletal muscle). Table 1 shows the results of amino acid composition comparing with theoretical value (referred to Japan Biochemistry Data Book l, Tokyo Kagaku Dojin). It was confirmed that the amino acid composition calculated from this measurement was in good agreement with the theoretical value.

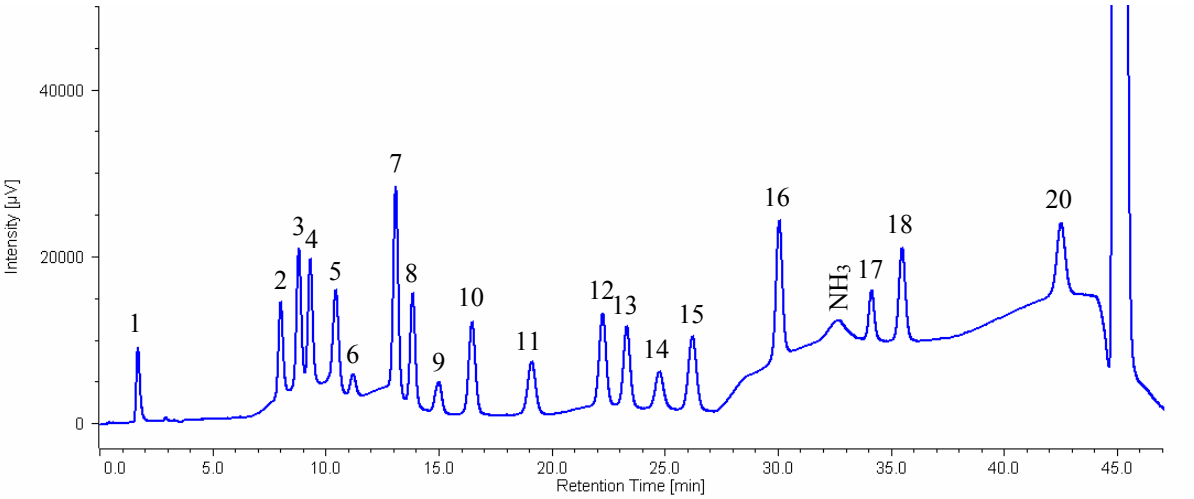

Fig. 4 Chromatogram of hydrolyzed myoglobin (horse skeletal muscle)
1: Cysteic acid, 2: Asparatic acid, 3: Threonine, 4: Serine, 5: Glutamic acid, 6: Proline, 7: Glycine, 8: Alanine
9: Cystine, 10: Valine, 11: Methionine, 12: Isoleucine, 13: Leucine, 14: Tyrosine, 15: Phenylalanine, 16: GABA
17: Lysine, 18: Histidine, 20: Arginine

Sample preparation:

1. Myoglobin was diluted to 200 μg/mL by ultrapure water.
2. The solution was filled in 20 μL sample tube and then dried up by centrifugal evaporator.
3. Sample tube was set in vessel for hydrolysis and 0.3 mL of hydrochloric acid was added to the vessel.
4. The sample was heated for 24 hours under vacuumed condition.
5. The sample was hydrolyzed and the residual chlorine was removed using vacuum pump.
6. 500 μL of 0.2 N citric acid buffer (pH 2.2) was added to the sample and agitated.

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Table 1 Comparison of amino acid composition of myoglobin between measured and theoretical value.

Amino acids	Measured values	Theoretical values
Asp	10.2	10
Thr	7.2	6
Ser	5.4	6
Glu	16.2	18
Pro	4.7	4
Gly	13.5	13
Ala	15.4	17
Val	7.0	8
Met	2.2	2
Ile	8.6	8
Leu	18.3	17
Tyr	2.8	2
Phe	7.4	7
Lys	19.5	19
His	11.9	12
Arg	2.7	2

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