[原著] A Study on the Improvement of Amino Acid Analysis by an OPA-Three-Reagent System

Ryukyu Med. J., 8(4): 155～159, 1985.

A Study on the Improvement of Amino Acid Analysis by an OPA-Three-Reagent System

Yukinori Eguchi, Hiroshi Takei and Yasutsugu Nakashima

Department of Biochemistry, School of Medicine,
University of the Ryukyus

Key words: Amino acid analysis, OPA- three-reagent system, High sensitivity, Stability

Abstract

By using an OPA-three-reagent system, in which Brij-35 is contained, better results were obtained on the peak areas of the amino acid cys, met, tyr and lys than those obtained from two reagent system.

Introduction

Recently it has become possible to perform amino acid analysis in a shorter time and with a higher sensitivity than before by the advent of a fluorescent reagent, orthophthalaldehyde (OPA) and by the progress of the high performance liquid chromatograhy (HPLC).[1)2)3)]

For the amino acid analysis with OPA, two kinds of solutions have been used so far; one is a NaClO solution and the other, a solution containing OPA, 2－mercaptoethanol and Brij-35.[4)]

However, there have been some problems with this system which must be solved such as smaller peaks than expected of cys, lys and tyr and instability of the reagent solutions.

The purpose of this experiment is to improve the lower sensitivity of cys, lys and tyr and to stabilize the reagent solutions to be used for a longer period of time by introducing a new system with three reagents.

Materials and Methods

A. HPLC Apparatus

A Hitachi 655 type apparatus was employed as HPLC system and a Hitachi 655－60 data processor, for calculation.

B. Analysis Condition

Hitachi #2619 packing material was employed. The column size was ϕ4 mm × 150mm, the column temperature, 60°C and the flow rate, 0.4 ml／min for separating amino acids. For the detection of amino acids, the flowing system as shown in Fig 1 was set up, each detecting reagent solution was run at the flow rate of 0.5 ml／min and the temperature was kept at 60°C.

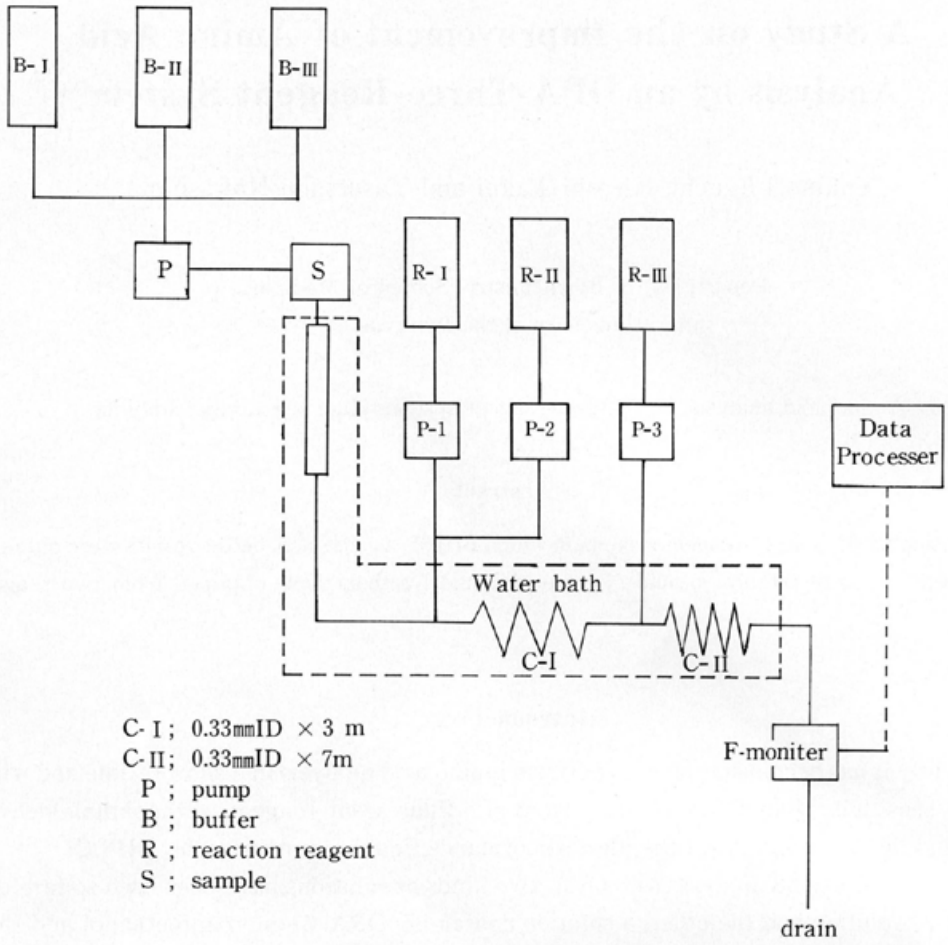

Fig. 1 Diagram of amino acid anlysis by OPA three reagent system

C. Reagents for Detecting Amino Acids

1. Buffer for Dissolving Each Reagent

A 0.23M boric acid-NaOH buffer (pH 10.4) was used for dissolving each detecting reagent.

2. Detecting Reagent R-I

10 ml of 10 % Brij-35 (Wakō Pure Chemical Industries, Ltd.) was dissolved in 1 liter of the buffer prepared above.

3. Detecting Reagent R-II

0.2 ml of sodium hypochlorite (Wakō, Practical Grade) was dissolved in 1 liter of the buffer described above in #1.

4. Detecting Reagent R-III

800 mg of OPA (Wakō, Biochemical Grade) was thoroughly dissolved in 10 ml of ethanol, to which 2ml of 2-mercaptoethanol and 10 ml of 10 % Brij-35 were added and the buffer prepared above in #1 was added to the final volume of 1 liter.

Results and Discussion

For amino acid analysis using the OPA method, a two-reagent system using a NaClO solution and an OPA solution is widely used at present. When using this system, it was observed that the peaks for cys, tyr and lys on the chart were smaller than those for the other amino acids.

The purpose of this study is to solve such problems and also to examine the stability of the reagent solutions used.

First of all, we were able to confirm from the present study that by adding Brij-35 to the NaClO solution, peaks with higher sensitivity for cys, met tyr and lys can be obtained. A diagram for such an amino acid analysis system is shown in Fig 1 and a chromatogram obtained from this system is shown in Fig 2. It is clearly shown in Fig 2 and in Table 1 that the peaks for the amino acids are more uniformly produced in this system than in the two-reagent system.

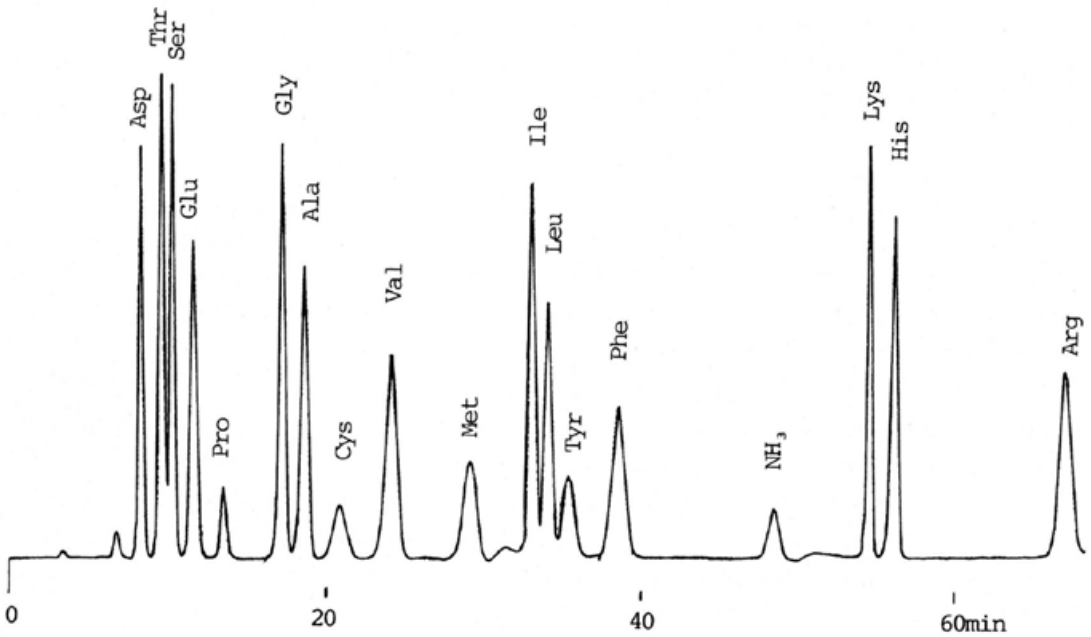

Fig. 2 Chromatogram of amino acid analysis by three reagent system

Table 1. Amino acid analysis by OPA system

	three-reagent system	two-reagent system*)
Asp	100.0	93.1
Thr	100.0	94.5
Ser	100.0	92.8
Glu	100.0	93.8
Pro	100.0	99.2
Gly	100.0	91.3
Ala	100.0	92.4
Cys	100.0	65.1
Val	100.0	95.1
Met	100.0	76.8
Ile	100.0	92.7
Leu	100.0	90.8
Tyr	100.0	73.7
Phe	100.0	92.0
Lys	100.0	80.4
His	100.0	93.4
Arg	100.0	100.2

Data indicate mean volumes of three times analysis
*) R-I used 0.23M boric acid-NaOH buffer (pH = 10.4)

By using the three-reagent system in which R-I is included, better results were obtained on the peak areas of the amino acids cys, met, tyr and lys. They are 1.54, 1.30, 1.36 and 1.24 times larger respectively than those obtained from the two-reagent system, which shows that the three-reagent system is more sensitive.

Better results were not obtained by using the two-reagent system in which Brij-35 was added to the OPA solution. Therefore, the three-reagent system in which R-I is included, is considered to be a more improved method.

Moreover, the authors have investigated the problem of the deterioration of the detecting reagents since the reagent solutions used in the OPA method are labile compared to those in the ninhydrin method, which decreases reproducibility and accuracy for quantitative analysis.

As seen in Table 2, by storing the reagent solution at room temperature, the effect of the deterioration of the reagents is noticed remarkably 6 days after preparation of the reagent solutions, which is considered to be caused primarily by the decrease of the net concentration of the NaClO in the R-II.

More stable reagents are available only by renewing the R-II buffer.

Furthermore, it is quite effective to store the reagent solutions in a cold room (6 °C) in order to avoid the deterioration of the reagents. This extends the time for usage of the reagents solution remarkably (Table 2).

Table 2. Effect of time and temperature on amino acid analysis by OPA-three-reagent system

	stored at room temperature					stored at 6 ° C	
	0 days	2days	4days	6davs	7days	7days	28days
Asp	100	100.6	102.7	103.7	98.6	97.4	95.7
Thr	100	100.4	102.2	101.2	99.5	99.8	109.9
Ser	100	101.6	103.3	104.7	101.3	96.3	108.1
Glu	100	101.5	103.3	103.8	100.1	100.4	95.4
Pro	100	97.6	92.4	88.8	61.5	111.3	104.6
Gly	100	103.4	106.2	105.0	103.6	99.3	109.5
Ala	100	101.8	102.3	102.2	99.9	98.0	95.0
Cys	100	77.7	66.5	68.3	63.3	108.2	102.1
Val	100	100.6	100.7	99.0	94.7	100.9	101.2
Met	100	122.9	118.1	116.3	110.2	96.2	107.2
Ile	100	102.8	104.3	100.4	91.8	101.9	99.9
Leu	100	104.5	106.8	104.6	93.9	100.6	91.3
Tyr	100	120.2	132.1	138.4	125.0	98.0	112.3
Phe	100	105.0	103.9	103.6	95.9	97.5	107.3
Lys	100	100.4	99.7	92.8	93.6	103.5	107.3
His	100	100.5	102.5	100.2	95.5	103.8	97.8
Arg	100	100.1	101.0	98.9	91.2	105.9	105.7

Data suggested mean volumes of three times analysis.

Thus by using the reagent solution which is stable for a long period of time after preparation it is possible to obtain reproducibility and accuracy of analysis comparable with the ninhydrin method.

Acknowledgment

The authors wish to thank Mr. M. Matsushita, president of Shaklee Japan K.K. for the convenience of lending them the HPLC Apparatus.

References

1) Roth, M., and Haampai, A. J. Chromatog. 83. 353 (1973)
2) Bohlen, P., and Mellet, M. Anal. Biochem. 94, 313 (1979)
3) Benson, J.R., and Hare, P.H. Proc. Natl. Acad. Sci. 72, 619 (1975)
4) Ishida, Y., Fujita, T., and Asai, K. J. Chromatog. 204, 143 (1981)