Note

Application of Glass Fiber Paper Chromatography to
Measurement of ^{15}N Content of Amino Acids[†]

Masayuki OHMORI and Kazuko OHMORI*

Laboratory of Marine Biochemistry, Ocean Research Institute, University of Tokyo

1-15-1, Minamidai, Nakano-ku, Tokyo 164

*Faculty of Domestic Science, Showa Women's University

1-7-57, Taishido, Setagaya-ku, Tokyo 156

Received August 2, 1982

Key Words: glass fiber paper chromatography, nitrogen-15-amino acid

A method for separation of amino acids using glass fiber paper chromatography is described. Each amino acid spot was cut out and converted to N_2 gas by Dumas' combustion. The ^{15}N content of the gas sample was determined by emission spectrometry.

1. Introduction

Application of emission spectrometry to ^{15}N determination has improved the research on nitrogen metabolism in plants^{1),2)}. Kano, et al.³⁾ reported a method for microdetermination of ^{15}N -amino acids separated by silica gel thin layer chromatography. We have recently developed a new procedure for emission spectrometric analysis by which a small amount of nitrogen can be determined⁴⁾. Based upon this procedure we describe here a simple method for ^{15}N microdetermination of cellular amino acids using a newly developed glass fiber paper chromatography. We have found that a glass fiber filter paper can be used for the separation of amino acids in place of the filter paper usually used and that, since this glass fiber paper contains no organic substances, amino acids separated on the paper can directly be subjected to Dumas' combustion to yield $^{15}\text{N}_2$ for analysis by emission

spectrometry. This procedure eliminates the complexity of steps in sample preparation for ^{15}N determination.

2. Experimental

Amino acids were separated using a glass fiber filter paper (Reeve Angel 984H, $20\text{ cm} \times 20\text{ cm}$) two-dimensionally at room temperature. The solvent systems consisted of phenol : chloroform : water (6 : 2 : 1, v/v/v) and water-saturated *n*-butyl alcohol : formic acid (19 : 1, v/v). After development, amino acid spots were visualized by spraying a ninhydrin solution containing 0.02% ninhydrin (w/v) in an ethyl alcohol-acetic acid mixture (500 : 1, v/v).

For correction of the nitrogen contamination from the paper, a blank paper was also developed two-dimensionally. Determination of the amount of ^{15}N in each amino acid spot was carried out as follows:

Fig. 1 Preparation process of $^{15}\text{N}_2$ sample. For details see text.

[†] ガラス繊維ろ紙クロマトグラフィーを用いたアミノ酸の ^{15}N 含量測定。大森正之, 大森和子*: 東京大学海洋研究所海洋生化学部門, 164 東京都中野区南台1-15-1, *昭和女子大学 家政学部, 156 東京都世田谷区太子堂1-7-57。

(1) Each spot of amino acids on the paper was cut out together with tracing papers overlapped on both sides of the glass fiber paper.

(2) The same areas as the sample spots were cut out from blank paper.

(3) The cuttings of the glass fiber paper were dried *in vacuo* overnight and put into a Pyrex glass tube with several pieces of CuO (ca. 70 mg, Kishida Chemical Co., Osaka) as illustrated in Fig. 1a. To minimize nitrogen contamination, the glass tubes and CuO had been baked at 250°C for over 10 h prior to use.

(4) The sample-containing tube was subjected to modification to make a thin neck 5 cm from the bottom (Fig. 1b).

(5) The tube was then attached to a vacuum apparatus, evacuated to 10^{-5} Torr and sealed (Fig. 1c,d).

(6) The sample tube was put into an electric furnace and subjected to Dumas' digestion at 520°C overnight.

(7) After cooling to room temperature, the tube was inserted into a glass-made breaker (Fig. 1e) which was attached to a gas preparation system as described previously¹¹. Then $^{15}\text{N}_2$ gas was introduced into the emission tube and the ^{15}N content was measured using a JASCO NIA I emission spectrometer.

3. Results and Discussion

Figure 2 shows the separation pattern of standard amino acids by two-dimensional glass fiber paper chromatography. Special efforts were made to separate glutamate, glutamine and aspartate because these amino acids play important roles in ammonium assimilation in plants²¹. Some other solvent systems such as *n*-butyl alcohol: ethyl acetate: water, *n*-butyl alcohol: pyrophosphate buffer, *n*-butyl alcohol: acetic acid: chloroform and *m*-cresol: phenol: borate buffer: chloroform were tested, however, no successful results were obtained.

Contamination of nitrogenous substances from developing solvents, ninhydrin solution and air caused a serious error in ^{15}N microdetermination⁵¹. In this method about 0.02 μg atomN per cm^2 of the glass fiber paper was detected as contaminated nitrogen. This problem was overcome by measuring the nitrogen content of the

Fig. 2 Two-dimensional glass fiber paper chromatogram of standard amino acids. 0.1 μmol per amino acid was applied. I; phenol: chloroform: water (6:2:1, v/v/v), II; water-saturated *n*-butyl alcohol: formic acid (19:1, v/v). Asp: aspartate, Glu: glutamate, Ser: serine, Ala: alanine, Gln: glutamine, His: histidine.

Table 1 Observed and corrected values of ^{15}N content in ^{15}N -glutamate spotted on glass fiber paper

Amount of glutamate spotted* (μmol)	Observed value (atom%)	Corrected value** (atom%)
0.2	7.82	10.50
0.5	9.38	10.35
1.0	10.00	10.38

* ^{15}N -glutamate (10 atom%, Hikari Kogyo Co., Tokyo) was used.

** The ^{15}N atomic abundance of the sample(x) was calculated by the following equation:

$$x = \frac{1}{a-b}(ay-bz)$$

where a is the amount of nitrogen of the amino acid spot including contamination, b is the contaminated nitrogen included in an equal area of blank paper, y is the observed value of ^{15}N atom% of the sample, and z is the ^{15}N atom% of the contaminated nitrogen (usually 0.36 atom%). The amount of nitrogen of the amino acid spot and the blank were measured manometrically according to the method previously reported¹¹.

blank paper which was also developed two-dimensionally without any sample. Table 1 shows that the observed value of ^{15}N abundance

tends to decrease with the decrease amount of applied ^{15}N -glutamate. This indicates that contaminated ^{14}N significantly lowers the apparent ^{15}N abundance when the amount of sample nitrogen is very small. However, corrected value of ^{15}N abundance agreed well with each other. The memory effect has been found to be negligible⁴⁾. Pathway of inorganic nitrogen assimilation in blue-green algae is now being investigated using this technique.

Acknowledgments

The authors wish to thank Prof. A. Hattori of this laboratory for his valuable discussion

and Mr. K. Sugai for his skillful glass work. This work was supported by grants Nos. 434034 and 56540401 from the Ministry of Education, Science and Culture, Japan.

References

- 1) R.D. Hauck and J.M. Bremner: *Advan. Agronomy*, **28**, 219 (1976)
- 2) B.J. Mifflin and P.J. Lea: *Ann. Rev. Plant Physiol.*, **28**, 299 (1977)
- 3) H. Kano, et al.: *Anal. Biochem.*, **67**, 327 (1975)
- 4) M. Ohmori, et al.: *ibid.*, **111**, 83 (1981)
- 5) O. Ito, et al.: *Radioisotopes*, **25**, 448 (1976)