19aD1-6

Mass-spectral Changes of Amino Acids Treated by Non-equilibrium Atmospheric Pressure Plasma
非平衡大気圧プラズマ照射によるアミノ酸の質量分析スペクトルの変化

[1]Hidehiko Fujita, [1]Takayuki Ohta, [2]Kenji Ishikawa, [2]Keigo Takeda, and [2]Masaru Hori
[1]藤田英彦, [1]太田貴之，[2]石川健治，[2]竹田圭吾，[2]堀 勝

[1]*Department of Electronics and Electrical Engineering, Meijo University*
1-501 Shiogamaguchi, Tempaku-ku, Nagoya 468-8502, Japan
名城大学大学院理工学研究科 〒468-8502 名古屋市天白区塩釜口1-501
[2]*Department of Electrical Engineering and Computer Science, Nagoya University*
Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan
名古屋大学大学院工学研究科 〒464-8603 名古屋市千種区不老町

Non-equilibrium atmospheric pressure plasma (NEAPP) has been recently used for various applications in medical or agricultural fields. Reactive oxygen species produced by the NEAPP reacts and changed with micro-organism. However detailed mechanisms of the ROS function have not been elucidated. Focused on amino acids, as main constituents of proteins and organisms as well as human body, this paper discusses the changes on amino acids observing with photoionization by the time-of-flight mass spectrometric system (TOF-MS) as a matrix-free method. The signal of arginine, as example of low-weight amino acid, was successfully obtained at m/z of 175.

1. Introduction

Researches have been carried out in medical and agricultural fields using non-equilibrium atmospheric pressure plasma (NEAPP).

Previously we have studied the inactivation of spore of *P. digitatum* spores and the induction of apoptosis to cancer cells [1-4]. From the electron spin resonance measurement, the signal of intracellular semiquinone, which is a free radical produced during the process of dehydrogenation, was observed [5]. It is important to understand extensively mechanism of functions of reactive oxygen species produced by NEAPP to the micro-organism.

The amino acids are the protein constituents of the human body. Figure 1 shows the structure of arginine (Arg), which is a basic amino acid having guanidine group [H_2N- (C = NH)-NH-], bonded to the side chain of the aliphatic hydrocarbon. The arginine is observed with large amount in the proteins generating an antioxidant and NO. Thus the Arg plays an important role for health maintenance; muscle strengthening, improvement of immune function, growth of hormone secretion, and so on. Oxidation of arginine, treated in solution by the plasma using atmospheric pressure He plasma jet, has been detected by electrospray ionization method. The oxidation product $(Arg+2O)H^+$ of Arg has measured [6]. Here we test to measure with photoionization technique and focus on elucidation of chemical modification on Arg of the micro-organism.

In this study, we have constructed the time-of-flight mass spectrometer (TOF-MS), for measurements of low-weight mass amino acids with high sensitivity. We detected the signal from Arg without derivatizing pretreatments, and will discuss the chemical modification of amino acid (arginine) treated by high-density Ar atmospheric pressure plasma.

2. Experiments

Figure 2 shows the schematic diagram of TOF-MS system. Nd:YAG laser (fourth harmonic generation: 266 nm, 30 Hz) was used as the ionization pulse laser. The laser intensity was adjusted by using a variable ND filter. The ionization chamber and the TOF chamber were kept at pressures below 10^{-7} Pa. The spectrometer consisted of reflectron to measure high resolution mass spectra. The detector was Micro-Channel Plate (MCP).

Procedure of sample preparation is illustrated in Figure 3. Aqueous solution of amino acid (10 mg / mL) was prepared by dissolution of 10 mg L-arginine hydrochloride (Sigma-Aldrich) in 1 mL ultrapure water (Millipore Direct-Q UV). The aqueous amino acid solution was dropped (0.5 μL × 5) on the substrate, and dried under air ambient.

Figure 4 shows a mass spectrum. The signal at m/z of 175 is clearly observed and can be identified as $(Arg+H)^+$ [7]. Commonly, amino acids are hardly ionizing, therefore deviating pretreatments is usually required. By using our constructed TOF-MS system [8], the signal of arginine was successfully observed at m/z of 175.

We constructed the system of TOF-MS to enable measurements of the low-weight amid acids. The TOF-MS system is utilized for elucidation of chemical modification on the amino acids under the atmospheric pressure plasma treatments.

References

[1] S. Iseki, K. Nakamura, M. Hayashi, H. Tanaka, H. Kondo, H. Kajiyama, H. Kano, F. Kikkawa, and M. Hori: Appl. Phys. Lett. **100** (2012) 113702.

[2] H. Tanaka, M. Mizuno, K. Ishikawa, K. Nakamura, H. Kajiyama, H. Kano, F. Kikkawa, and M. Hori: *Plasma Medicine* (Begel, NewYork, 2011) Vol.1, issue 3-4, p.265-277.

[3] H Hashizume, T. Ohta, J. Fengdong, K. Takeda, K. Ishikawa, M. Hori, and M. Ito: Appl. Phys. Lett. **103** (2013) 153708.

[4] M. Ito, T. Ohta, M. Hori: J. Korean Phys. Soc. **60** (2012) p. 937-943

[5] K. Ishikawa, H. Mizuno, H. Tanaka, K. Tamiya, H. Hashizume, T. Ohta, M. Ito, S. Iseki, K. Takeda, H. Kondo, M. Sekine, and M. Hori: Appl. Phys. Lett. **101**, (2012) 013704.

[6] E. Takai, T. Kitamura, J. Kuwabara, S. Ikawa, S. Yoshizawa, K. Shiraki, H. Kawasaki, R. Arakawa, and K. Kitano: J. Phys. D: Appl. Phys. **47** (2014) 285403.

[7] R. J. Beuhler, E. Flanigan, L. J. Greene, and L. Friedman: J. Am. Chem. Soc. **12** (1974) 3990.

[8] Pending on filing patent application.

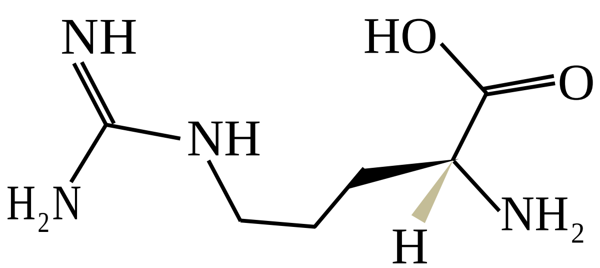

Fig.1. Structural formula of Arginine.

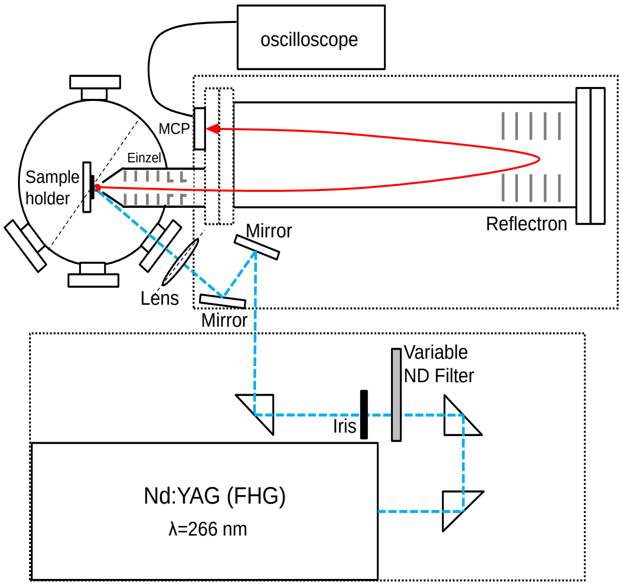

Fig.2. Experimental set up of TOF-MS system. (MCP: Micro-Channel Plate)

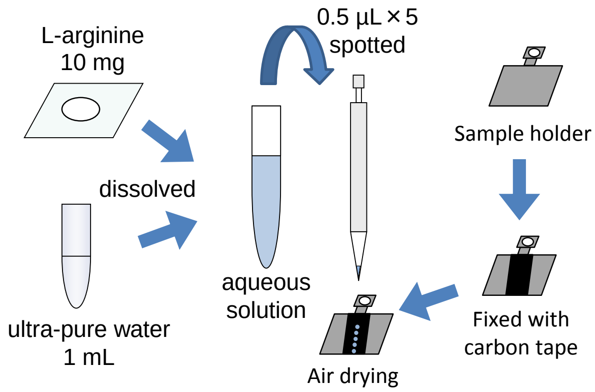

Fig.3. Sample preparation.

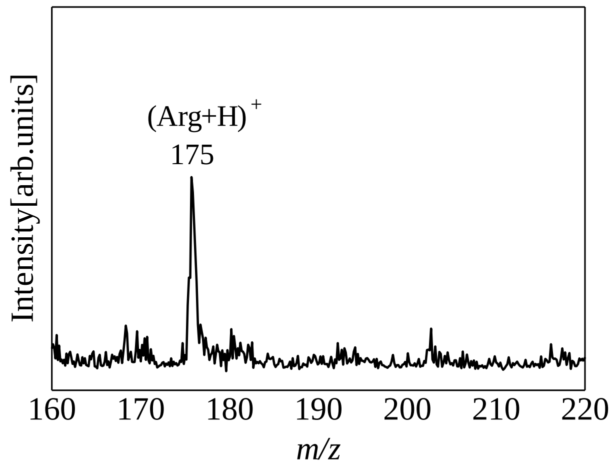

Fig.4. Mass spectrum of Arginine.