Chemical reaction of amino acids with plasma-generated dinitrogen pentoxide

Y. Oba, S. Sasaki, K. Takashima and T. Kaneko

Graduate School of Engineering, Tohoku University, Sendai, Japan.

Abstract: To understand the chemical reactions of the plasma-generated reactive species [*e.g.*, dinitrogen pentoxide (N_2O_5), ozone (O_3), and nitric oxides (NO_x)] with amino acids, 18 types of amino acids were exposed to the N_2O_5 gas and the products were analyzed. Among them, tyrosine and tryptophan showed clear coloration during the N_2O_5 treatment and nitrotyrosine and nitrotryptophan were detected. These findings could contribute to an understanding of chemical properties of the N_2O_5 gas.

Keywords: Dinitrogen pentoxide, Ozone, Amino acids

1. Introduction

Atmospheric-pressure plasma (APP) technology, enabling to convert air molecules into reactive oxygen/nitrogen species (RONS) with electricity, is of great interest and has been extensively investigated. In particular, ozone (O_3) produced by the APP technology has multifunctional abilities such as disinfection and deodorizing, and has already been into practical use.

Recently, we have developed a new air APP device/method that allows highly selective production of Dinitrogen pentoxide (N_2O_5) in situ exclusively from only air and electricity sources [1]. N_2O_5 is known as a strong nitrating and oxidizing compound that is highly reactive with H_2O and organic materials. Despite these unique properties, few example of applied study due to difficulties in synthesis and storage. This device/method can supply N_2O_5 gas on site without careful handling and have the potential to be used in bio-applications. As example of bio-applications, our research groups found an effect of the plasma-generated N_2O_5 gas on plant immunity activation [2]. However, there are few studies on N_2O_5 exposure to living organisms and the reaction mechanism of N_2O_5 gas with biomolecules is largely unknown.

In this study, 18 types of the protein-building amino acids (L-Tyr, L-Trp, L-Phe, L-Cys, L-Met, L-Val, L-Leu, L-Ile, L- Ala, L-Ser, L-Pro, L-Lys, L-Glu, L-His, L-Asp, Gly, L(+)-Arg, L(−)-Thr) were treated with the plasma-generated N_2O_5 gas, and changes in appearance were comprehensively investigated. In addition, derivative products of some amino acids were particularly analyzed by liquid chromatography-mass spectrometry (LC-MS) with photodiode array detector (PDA).

2. Experiment

Figure 1 shows the experimental method and procedure. N_2O_5 was synthesized by the device developed in the previous study [1]. It can be generated by mixing the gases from two independent plasma reactors; 1. low gas temperature (LT) plasma reactor for selective O_3 generation and 2. high gas temperature (HT) plasma reactor for selective NO/NO_2 generation. In addition, this plasma device can finely tune the composition of RONS in the exposure gas by independently controlling the discharge coupling power of the reactors. Thus, this device can supply not only N_2O_5-rich gas but also a reactive species admixture with N_2O_5 (*e.g.*, N_2O_5 + O_3, N_2O_5 + NO_2). N_2O_5 gas generated by the plasma device was always monitored using Fourier transform infrared spectroscopy (FT-IR) as shown in Fig. 1 and used for the amino acid treatments. Typical RONS density compositions of N_2O_5 mode and O_3 mode measured by FT-IR were shown in Table 1.

Amino acids powder were moistened 30 mg per 30 µL water before N_2O_5 treatment. After the RONS treatment, all samples were photographed, and some samples were further analysed using LC-MS with PDA.

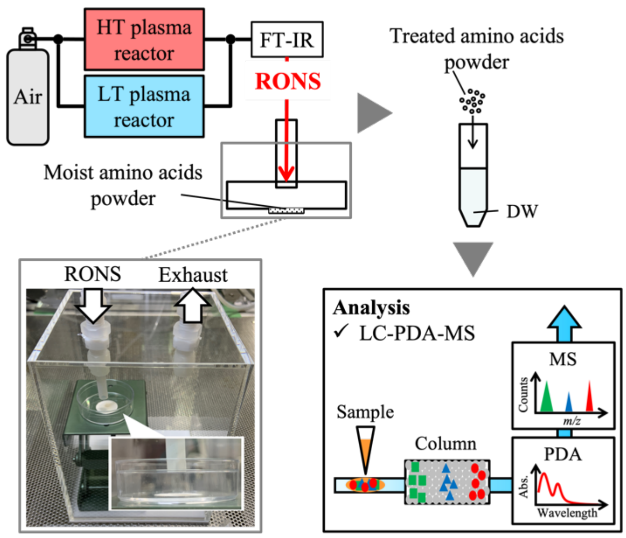

Fig. 1. Experimental setup.

Table 1. Typical RONS densities.

	O_3 (cm^{-3})	NO (cm^{-3})	NO_2 (cm^{-3})	N_2O_5 (cm^{-3})	HNO_3 (cm^{-3})
N_2O_5 mode	3.9×10^{14}	N.D	9.0×10^{14}	7.4×10^{15}	4.4×10^{14}
O_3 mode	1.9×10^{16}	N.D	N.D	1.9×10^{14}	3.6×10^{14}

3. Results and discussion

Figure 2 shows the photographs of 18 amino acids powder (a) before and (b) after the plasma-generated N_2O_5 gas treatment for 5 minutes. During the N_2O_5 gas exposure, only tyrosine and tryptophan showed clear coloration as shown in Fig. 2(b). Both tyrosine and tryptophan were gradually turning yellow-tinted on the surface, only tryptophan was finally colored orange.

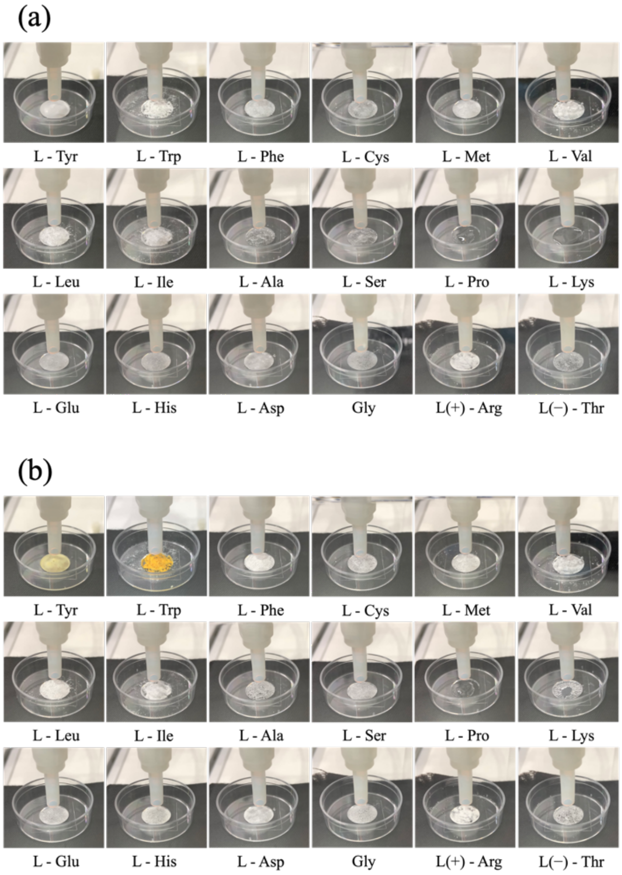

Fig. 2. Images of the moist amino acids powder (a) before and (b) after treatment of the plasma-generated N_2O_5 gas for 5 min.

Figure 3 shows the LC-PDA analysis of moist tyrosine powder treated by the N_2O_5 gas. From the LC chromatograms at 275 nm, in addition to the peak derived from tyrosine, a new peak appeared only when treated with N_2O_5. It was confirmed that this new peak was derived from 3-nitro-tyrosine, a nitride of tyrosine, using a standard reagent. Thus, the nitrating action of the plasma-generated N_2O_5 gas was demonstrated.

Figure 4 shows the LC-PDA analysis of moist tryptophan powder treated by the N_2O_5 gas. From the LC chromatograms at 330 nm, new peaks appeared only when treated with N_2O_5. One of new peaks was found to be derived from 5-nitro-DL-tryptophan and other peaks are estimated to be derived from nitrides or oxides. Further LC-MS analysis showed the products of new peaks have same nominal mass as 5-nitro-tryptophan, indicating that the yellow and orange compounds were nitro compounds (e.g., 6-nitro-tryptophan and 7-nitro-tryptophan). Thus, several types of nitro compounds might be produced by the N_2O_5 gas treatment.

In addition, analysis of the derivative products after N_2O_5 and $N_2O_5 + \alpha$ treatments showed that some of tyrosine and tryptophan derivatives (*e.g.*, L-dopa, dopachrome, kynurenine) were biologically active and could potentially be used as medical drugs.

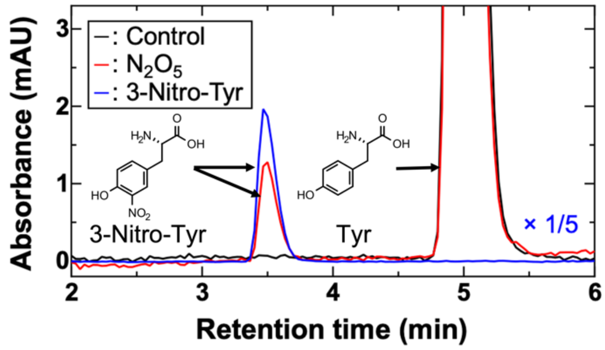

Fig. 3. LC chromatograms at 275 nm of solutions of moist tyrosine powders treated by the plasma-generated N_2O_5 gas and dry air. The concentration of 3-nitro-tyrosine is 25 μM.

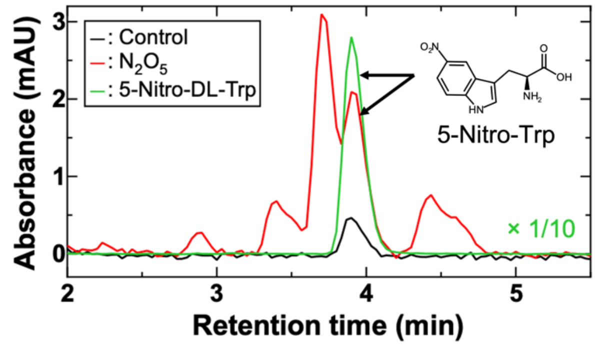

Fig. 4. LC chromatograms at 330 nm of solutions of moist tryptophan powders treated by the plasma-generated N_2O_5 gas and dry air. The concentration of 5-nitro-DL-tryptophan is 100 μM.

Acknowledgements

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4. References

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