# 22G06

# Biochemical Reaction of Amino Acid by Plasma-Induced Chemical Processing in Liquid

プラズマ誘起液中化学プロセスによるアミノ酸の生化学反応

Tsuyoshi Kitamura[1], Satoshi Ikawa[2], Hideya Kawasaki[1], Ryuichi Arakawa[1] and Katsuhisa Kitano[3]
北村　豪[1], 井川　聡[2], 川崎　英也[1], 荒川　隆一[1], 北野　勝久[3]

[1] *Faculty of Chemistry, Materials and Bioengineering, Kansai Univ., 3-3-35 Yamatecho, Suita, Osaka, 564-8680, Japan*
[2] *Technology Research Institute of Osaka Prefecture,* [3]*Eng. Osaka Univ.*
[1]関西大学化学生命工学部、〒111-222　吹田市中央区２－１
[2]大阪府立産業技術総合研究所、[3]大阪大学工学研究科

Plasma medicine is one of attractive new research areas, but fundamental reaction mechanism is still not made clear enough. In this study, we investigated the chemical effects of low-temperature atmospheric pressure plasma on amino acid in liquid using tyrosine and glutathione as a model. After plasma treatment, glutathione, which is known as an antioxidant agent, was confirmed to be changed into oxidized glutathione, which is chemically cross-linked by disulfide binding of bimolecular thiol group, from mass spectrometry. This research is an elementary step of plasma medicine to understand fundamental reactions, and would bring many thought of important knowledge about biochemical reaction between plasma and biomacromolecule.

## 1. Introduction

In recent years, novel application researches of atmospheric pressure plasmas such as medical application or synthesis of biomaterial have been conducted intensively [1].

For the purpose of plasma disinfection of human body, Ikawa and Kitano have successfully found that efficient bactericidal activity can be achieved if the solution is sufficiently acidic [2]. It is interesting to note that there is a critical pH value of about 4.7 for the bactericidal effects, below which the bacteria are efficiently inactivated and above which the bacteria are hardly affected by the plasma application. It has been found experimentally that the presence of superoxide anion radicals ($O_2^-\cdot$) in the solution is essential for bacterial inactivation by the plasma application [3]. The critical pH value may be associated with pKa of the dissociation equilibrium between $O_2^-\cdot$ and hydroperoxy radicals ($HOO\cdot$), which is known to be approximately 4.8.

Other experimental results indicated that not physical reaction but biochemical modification induced inactivation. To progress like this basic research to applications, it is necessary to make clear the reaction mechanism to biomacromolecures. In this paper, plasma treated amino acid in solution by using LF plasmajet [4,5], which is one of the atmospheric low temperature plasma, is analyzed to evaluate the interaction between plasma and amino acid by using mass spectrometry.

Tyrosine is one of amino acids which a component of protein and play an important role for neural signaling. In vivo, it is known that tyrosine has a strong reactivity with peroxynitrite ($ONOO^-$). $ONOO^-$ is one of reactive oxygen species generated from the reaction of $NO\cdot$ with $O_2^-\cdot$. $ONOO^-$ can induce nitration of protein and nitrotyrosine residue of protein is seen around blood vessel of arteriosclerosis and ischemia, and inflamed area [6].

Glutathione is known as a typical antioxidant and is easily oxidized under oxidant stress to protect living cells. Most of glutathione within a cell usually exists as a reduced form and the ratio of reduced glutathione to oxidized glutathione is useful as indicator of cellular toxicity [7].

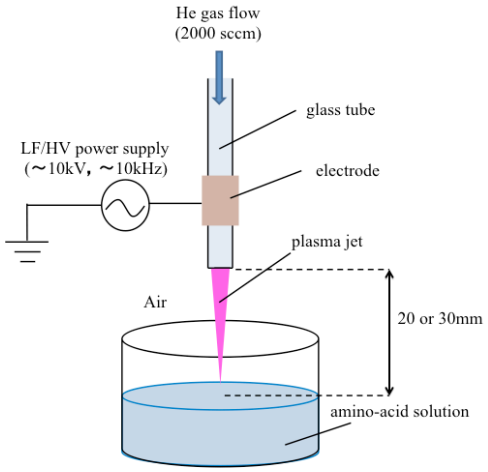



Fig.1. Schematic diagram of the plasma exposure system. LF/HV pulses are applied to the single electrode around the quartz glass tube.

## 2. Experimental Setup

A schematic illustration of the experimental setup is shown in Fig. 1. The system consists of a dielectric tube through which He gas flows (2000sccm) and a single electrode to which LF high-voltage pulses (~10kV, ~10kHz) are applied. Amino acid in pure water was prepared at 1mM. The distance between front end of the glass tube and the solution surface was set to 20mm (plasma and water were contact) or 30mm (non-contact). After plasma treatment, the solution was diluted by the solvent (pure water：acetonitrile = 1：1) to 10uM, and then analyzed by electro spray ionization (ESI) mass spectrometry (MS).

## 3. Experimental Results

### 3-1.Plasma Treatment of Tyrosine

As the result of plasma treatment under the condition where plasma and solution were contact, mass spectrum of tyrosine (Fig.2) was changed as shown in Fig.3. While protonated tyrosine molecule ion (m/z=182) was seen before treatment, new peaks of ion species were at m/z=198, 214, 227, 243 after treatment. Analysis result of these shows that 198 is one oxygen atom addition ion (+16), 214 is two oxygen addition ion, 227 is two oxygen and one nitrogen addition ion, 243 is three oxygen and one nitrogen addition ion. This result represents that plasma treatment induces not only oxidation but also nitration.

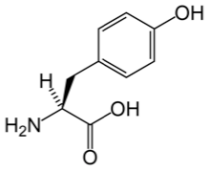



Fig.2. Chemical structural formula of L-Tyrosine (Mw 181.19 g/mol).

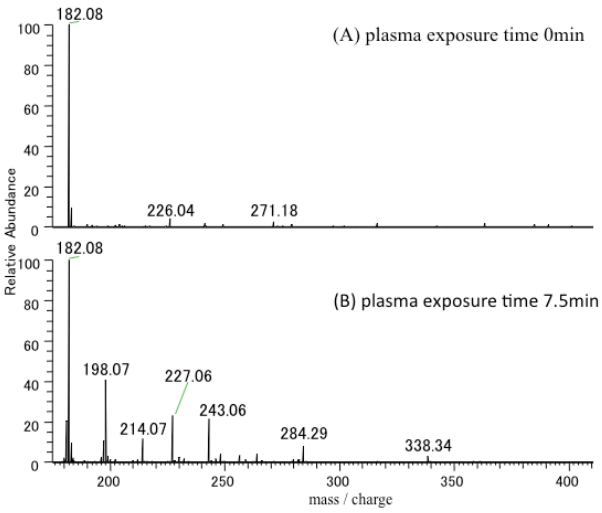
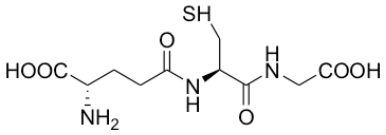



Fig.3. Positive ions mass spectrometry of tyrosine (A) plasma exposure time 0min (B) plasma exposure time 7.5min.

### 3-2. Plasma Treatment of Glutathione

Same experiment was conducted against the solution of glutathione (Fig.4(a)) under plasma contact condition and mass spectrum was obtained in negative ion mode. Before plasma treatment, the peaks of deprotonated glutathione molecule ion (m/z=306) and deprotonated dimer molecule of glutathione (m/z=613) were seen. After treatment, peaks of m/z=354, 611 were newly seen. They were identified to three oxygen addition ion (m/z=354) and oxidized form glutathione (m/z=611). The latter is brought by cross-linking reaction at disulfide binding of bimolecular thiol groups (Fig.4(b)). Here, the production of oxidized glutathione was seen in slightly different way under non-contact condition.

(a)




(b)

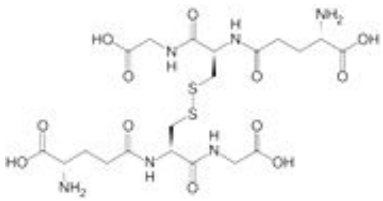



Fig.4. Glutathione in (a) reduced and (b) oxidized form.

## 4. Summary

It is important subject to figure out the reactions of plasma to biomacromolecule considering plasma induced chemical processing in liquid. In this paper, we evaluated the interaction between plasma and amino acid solution by using mass spectrometry. It was found that chemical modification was brought to both tyrosine and glutathione. Especially in the case of plasma process in solution, like this biochemical evaluation is important because kinetic momentum of plasma produced active species is practically nought at collisional atmospheric pressure.

## References


[1] 北野勝久、現代化学、7 月号、p25-31、(2009).
[2] 井川聡、日本国特許第 4408957 号.
[3] S. Ikawa, Plasma Process. Polym., 7, pp.33, (2010).
[4] 北野勝久、応用物理学会誌、４月号、(2008).
[5] 北野勝久、日本国特許第 4677530 号.
[6] Beckman, Chem. Hoppe-Seyler 1994, 375, 81-88.
[7] A. Pastore, Clinical Chemistry 47 (2001) No.8.