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Protein Inactivation in Solution by Plasma-Induced Chemical Processing
プラズマ誘起液中化学プロセスによるタンパク質の失活

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Plasma medicine is one of the attractive new research areas, but fundamental understanding related to plasma modification of biomacromolecules in aqueous solution remains elusive. We investigated the chemical effects of low-temperature atmospheric pressure plasma on protein in solution using lysozyme as a model. Plasma treatment decreased enzymatic activity, which is attributed to the increased molecular weight of lysozyme with chemical modification. These effects arise neither from UV light nor from heat load, suggesting the attack of reactive species on lysozyme. This is a fundamental step for elucidating chemical reactions by plasma for biomedical applications.

1. Introduction

Plasma processing at atmospheric pressure has attracted a great deal of attention because of its advantageous features, such as its obviation of complicated and expensive vacuum systems, its capacity for adequate and controllable gas temperature, its high concentrations of chemically reactive species, and its flexible operation. Such attributes of atmospheric pressure plasma have been applied as ionizer of mass spectrometry, elimination of organic materials, tooth bleaching, and sterilization. Although various biomedical applications of the atmospheric pressure plasma have been developed, fundamental understanding of interaction between plasma and biomacromolecules has remained insufficient.

Our plasma system, using a low-frequency (LF) high voltage (HV) power supply and a single-HV electrode, which is designated as an LF plasma jet, can easily generate low-temperature atmospheric pressure plasma (LTAP) at low cost. The advantage of the LF plasma jet became possible to sterilize bacteria in solution without heat load, current, and ultraviolet (UV) light. [1] To apply the LF plasma jet to medical applications, this investigation of enzymatic activity and molecular weight would be important for understanding fundamental reactions of complicated biological reactions.

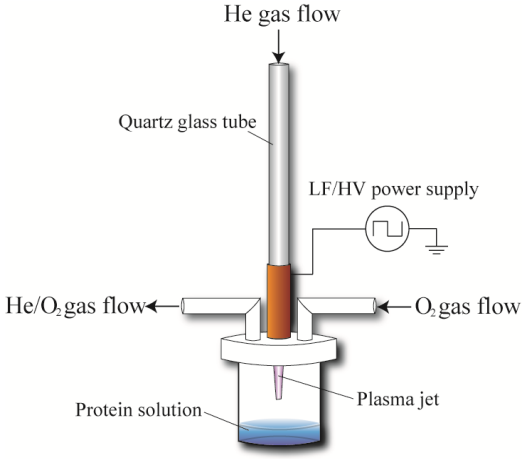

Fig. 1. Schematic diagram of the plasma generation system. LF/HV pulses are applied to the electrode, which is wound around the quartz glass tube. The device is He-based low-temperature atmospheric plasma. Plasma is generated in He gas flowing (500 sccm) in the quartz glass tube. Supplying of O_2 gas of 150 sccm to the chamber maintains the O_2 concentration in the chamber atmosphere.

2. Experimental Section

Experimental setup is shown in figure 1, comprising the plasma generator and sample chamber. The LF plasma jet was generated from the end of the quartz glass tube, in which helium (He) gas flowed, by the application of alternating HV pulses (from -3.5 to +5.0 kV with the frequency of 13.9 kHz) to the electrode, which was a small metal sheet wound around the tube. He plasma with low gas temperature was generated with an elongated shape. This plasma jet was generated inside the airtight chamber from its center port. In the chamber, various active oxygen species produced from oxygen (O_2) gas were supplied to the solution. The ambient gas of the chamber can be controlled with the other O_2 gas supply port connected to the side of the plasma jet port. The sample of 0.3 ml containing 0.1 mg/ml lysozyme in 10 mM phosphate buffer (pH 7.4) was applied to the solution in the vessel. To exchange the ambient gas,

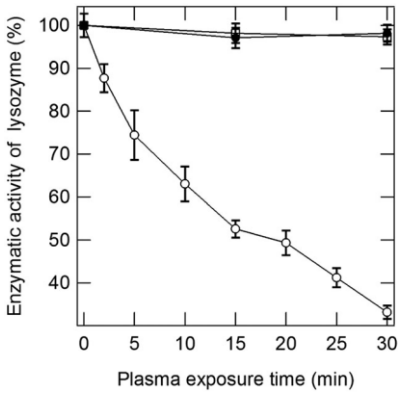

Fig. 2. Residual activity of lysozyme treated by an LF plasma jet (open circles), hot air treatment with heat (closed circles), and plasma treatment through a quartz glass plate with UV (open squares).

gases were flowed for 3 min before the plasma generation. After the plasma treatment, samples were corrected with pure water to compensate for evaporation.

3. Experimental Results

Experimental result is shown in figure 2 presents the time course of residual activity of lysozyme after treatment of the LF plasma jet for the respective periods. The residual activity decreased with time, about one-third after the plasma treatment for 30 min.. To confirm the cause of inactivation, two control experiments were performed; hot-air treatment and plasma treatment through the quartz glass plate. Briefly, hot-air treatment was performed as the control experiment with air at 80 °C instead of the LF plasma jet blown on the samples. The plasma treatment through a quartz glass plate was performed as the control experiment that the LF plasma jet irradiated on the samples through a UV-permeability glass vial to confirm the effect of UV alone. As expected, the enzymatic activity of lysozyme did not change in these control experiments (Fig. 2). These data indicate that the decrease in the residual activity results from chemical modification by plasma treatment.

To investigate the chemical reaction on the protein, mass spectrometry (MS) was used. Figure 3(a) shows data of matrix-assisted laser desorption/ionization time of flight MS of the samples after or before plasma treatment for 30 min.. The most abundant peak of native lysozyme was m/z 7153, which corresponds to the doubly protonated molecule; the singly protonated molecule showed a peak at a maximum of m/z 14306. A minor species at m/z 28612 was detected, which corresponds to the dimer with one protonation. However, the peaks of the MS

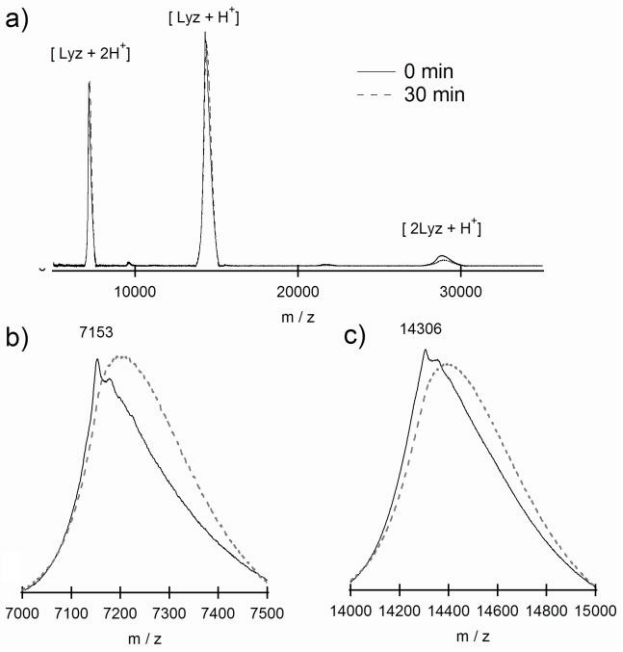

Fig. 3. MS spectrum of lysozyme treated by LF plasma jet for 0 or 30 min between m/z (a) 5000-35000, (b) 7000-7500 and (c) 14000-15000.

spectrum after the plasma treatment for 30 min were shifted to high molecular weight; plasma-treated lysozyme increased the mass of about 90 (Fig. 3(c)). These results indicate that the LF plasma jet modifies some amino acid side chains in lysozyme.

4. Discussion

We found that the LF plasma jet on lysozyme decreased enzymatic activity and increased molecular weight, which are caused neither by UV light nor heat from plasma. The data suggest that reactive species generated by the LF plasma jet affect on lysozyme. A possible mechanism of the LF plasma jet on protein is biochemical reaction caused by hydroxyl radical, superoxide anion radical, hydroperoxy radical and nitric oxide, which results in the chemical modifications of chemically reactive side-chain of the amino acids, such as cysteine, aromatic rings of phenylalanine, tyrosine, and tryptophan. Further experiments will be performed to clarify the chemical and physiological influences of the LF plasma jet on biological molecules. These data imply the interesting application of the LF plasma jet on protein for biomedical applications.

References

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